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Psychedelics

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ABBREVIATIONS: 2C-I, 2-(4-iodo-2,5-dimethoxyphenyl)aminoethane; 2C-T-7, 2,5-dimethoxy-4-*n*-propylthiophenethylamine; 5-CSRTT, five-choice serial reaction time task; 5-HT, 5-hydroxytryptamine (serotonin); 5-HTP, L-5-hydroxytryptophan; 5-MeO-DIPT, 5-methoxy-*N*,*N*-diisopropyltryptamine; 5-MeO-DMT, 5-methoxy-*N*,*N*-dimethyltryptamine; 5D-ASC, five-dimensional altered states of consciousness; 8-OH-DPAT, 7-(dipropylamino)-1,2,3,4-tetrahydronaphthalene; A-381393, 2-[4-(3,4-dimethylphenyl)piperazin-1-ylmethyl]-1*H*-benzoimidazole; AA, arachidonic acid; ABT-724, 2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1*H*-benzimidazole); ACC, anterior cingulate cortex; ACh, acetylcholine; ACP-103, *N*-(4-fluorophenylmethyl)-*N*-(1-methylpiperidin-4-yl)-*N*′-(4-(2-methylpropyloxy)phenylmethyl)carbamide; AD, Alzheimer's disease; AMRS, Adjective Mood Rating Scale; AMS, α-methylserotonin; APZ, Abnormal Mental States; ASC, altered state of consciousness; ASR, acoustic startle response; BDI, Beck Depression Inventory; BDNF, brain-derived neurotrophic factor; BOL, BOL-148, 2-bromo-lysergic acid-*N*,*N*-diethylamide; BOLD, blood oxygen level–dependent; BP554, 1-[3-(3,4-methylenedioxyphenoxy)propyl]-4-phenyl-piperazine; BPD, borderline personality disorder; BPM, behavioral pattern monitor; CBF, cerebral blood flow; CHO, Chinese hamster ovary; CIMBI-5, *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine; Cimbi-36, *N*-(2-[^{11}C]methoxybenzyl)-2,5-dimethoxy-4-bromophenethylamine; CMRglu, cerebral metabolic rate of glucose; CNS, central nervous system; CRPD, cancer-related psychosocial distress; CSTC, cortico–striato–thalamocortical; CT, complete transection; DH, double hemisection; DMN, default mode network; DMT, *N*,*N*-dimethyltryptamine; DOB, 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane; DOC, 1-(4-chloro-2,5-dimethoxyphenyl)-2-aminopropane; DOI, 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane; DOM, 2,5-dimethoxy-4-methylamphetamine; DPT, *N*,*N*-dipropyltryptamine; DSA, dichoptic stimulus alternation; *DSM-IV*, *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition*; EEG, electroencephalography; EGF, epidermal growth factor; EGFP, enhanced green fluorescent protein; EMD 281014, 7-[[4-[2-(4-fluorophenyl)ethyl]-1-piperazinyl]carbonyl]-1*H*-indole-3-carbonitrile hydrochloride; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; ERK, extracellular signal-regulated kinase; FAIR, Frankfurt Attention Inventory; FDG, fludeoxyglucose; fMRI, functional magnetic resonance imaging; FR, fixed ratio; GAD, glutamate decarboxylase; GPCR, G protein–coupled receptor; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; H-reflex, Hoffman reflex; HAM-A, Hamilton Rating Scale for Anxiety; HAM-D, Hamilton Rating Scale for Depression; HEK-293, human embryonic kidney 293; HPPD, hallucinogen persisting perception disorder; HTR, head twitch response; i3, intracellular loop 3; IL, interleukin; IOP, intraocular pressure; IP, inositol phosphate; ISI, interstimulus interval; JHU, Johns Hopkins University; KO, knockout; L-745,870, 3-[[4-(4-chlorophenyl)piperazin-1-yl]methyl]-1-1*H*-pyrrolo[2,3-*b*]pyridine; LC, locus coeruleus; LFCO, low frequency cortical oscillation; LFP, local field potential; LOC, lateral occipital complex; LSD, (5*R*,8*R*)-(+)-lysergic acid-*N*,*N*-diethylamide; LY293558, (3*S*,4a*R*,6*R*,8a*R*)-6-[2-(2*H*-tetrazol-5-yl) ethyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid; LTP, long-term potentiation; LY294002, 2-morpholin-4-yl-8-phenylchromen-4-one; LY341495, 2-[(1*S*,2*S*)-2-carboxycyclopropyl]-3-(9*H*-xanthen-9-yl)-D-alanine; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY379268 [(1*R*, 4*R*,5*S*,6*R*)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid; LY566332, *N*-(4′-cyano-biphenyl-3-yl)-*N*-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride; M100907, (*R*)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol; MAO, monoamine oxidase; MAPK, mitogen-activated protein kinase; MDD, major depressive disorder; MDL100907, (*R*)-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperinemethanol; MDL11939, α-phenyl-1-(2-phenylethyl)-4-piperidine methanol; MDMA, 3,4-methylenedioxymethamphetamine; MEF, mouse embryonic fibroblast; MEG, magnetoencephalography; MEK, mitogen-activated protein kinase kinase; mGluR, metabotropic glutamate receptor; MK-212, 6-chloro-2-(1-piperazinyl)pyrazine; MK-801, (5*R*,10*S*)-(−)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cylcohepten-5,10-imine; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; mPFC, medial prefrontal cortex; MUA, multiunit activity; NE, norepinephrine; NMDA, *N*-methyl-D-aspartate; NMS, *N*-methylspiperone; NSDUH, National Survey on Drug Use and Health; NT-3, neurotrophin-3; NYU, New York University; OCD, obsessive-compulsive disorder; OFC, orbitofrontal cortex; OVA, ovalbumin; PAG, periaqueductal gray matter; PCC, posterior cingulate cortex; PCP, phencyclidine; PCR, polymerase chain reaction; PD, Parkinson's disease; PD98059, 2-(2-amino-3-methoxyphenyl)-4*H*-1-benzopyran-4-one; PET, positron emission tomography; PFC, prefrontal cortex; phMRI, pharmacological magnetic resonance imaging; PI, phosphoinositide; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PMA, phorbol ester; PP2, 4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo [3,4-*d*]pyrimidine; PPI, prepulse inhibition; PTSD, post-traumatic stress disorder; RA, rheumatoid arthritis; REM, rapid eye movement; Ro25-6981, (α*R*,β*S*)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol; Ro60-0175, (*S*)-6-chloro-5-fluoro-1*H*-indole-2-propanamine; ROI, region of interest; RS102221, *N*-{5-[5-(2,4-dioxo-1,3,8-triazaspiro[4.5]dec-8-yl)pentanoyl]-2,4-dimethoxyphenyl}-4-(trifluoromethyl)benzenesulfonamide; RSC, retrosplenial cortex; RSK, ribosomal S6 kinase; RT, reverse transcription; SB-206553, 3,5-dihydro-5-methyl-*N*-3-pyridinylbenzo[1,2-*b*:4,5-*b*′]dipyrrole-1(2*H*)-carboxamide hydrochloride; SB-242084, 6-chloro-5-methyl-*N*-{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl}indoline-1-carboxamide; SB-243213, 2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-6-(trifluoromethyl)-1*H*-indole-1-carboxamide; SCH23390, 7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol; SCI, spinal cord injury; SER-082 [(+)-*cis* 4, 5,7a,8,9,10,11,11a-octahydro-7*H*-10-methylindolo[1,7-*bc*][2,6]-naphthyridine; sEPSC, spontaneous excitatory postsynaptic current; SERT, serotonin transporter; SL0101, 3-[(3,4-di-*O*-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]-5,7-dihydro-2-(4-hydroxyphenyl)-4*H*-1benzopyran-4-one; SNP, single nucleotide polymorphism; SPECT, single-photon emission computed tomography; SSRI, selective serotonin reuptake inhibitor; STAI, State-Trait Anxiety Inventory; TCB-2, 4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine; TNF, tumor necrosis factor; TPN, task positive network; U0126, 1,4-diamino-2,3-dicyano-1,4-*bis*[2-aminophenylthio]butadiene; U73122, 1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; VEGF, vascular endothelial growth factor; VI, variable interval; VP, ventral pallidum; VTA, ventral tegmental area; WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide; WAY-161503 [(*R*)-8, 9-dichloro-2,3,4,4a-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one; WT, wild type; YFP, yellow fluorescent protein.

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***Abstract*——Psychedelics (serotonergic hallucinogens) are powerful psychoactive substances that alter perception and mood and affect numerous cognitive processes. They are generally considered physiologically safe and do not lead to dependence or addiction. Their origin predates written history, and they were employed by early cultures in many sociocultural and ritual contexts. After the virtually contemporaneous discovery of (5*R*,8*R*)-(+)-lysergic acid-*N*,*N*-diethylamide (LSD)-25 and the identification of serotonin in the brain, early research focused intensively on the possibility that LSD and other psychedelics had a serotonergic basis for their action. Today there is a consensus that psychedelics are agonists or partial agonists at brain serotonin 5-hydroxytryptamine 2A receptors, with particular importance on those expressed on apical dendrites of neocortical pyramidal cells in layer V. Several useful rodent models have been developed over the years to help unravel the neurochemical correlates of serotonin 5-hydroxytryptamine 2A receptor activation in the brain, and a variety of imaging techniques have been employed to identify key brain areas that are directly affected by psychedelics. Recent and exciting developments in the field have occurred in clinical research, where several double-blind placebo-controlled phase 2 studies of psilocybin-assisted psychotherapy in patients with cancer-related psychosocial distress have demonstrated unprecedented positive relief of anxiety and depression. Two small pilot studies of psilocybin-assisted psychotherapy also have shown positive benefit in treating both alcohol and nicotine addiction. Recently, blood oxygen level–dependent functional magnetic resonance imaging and magnetoencephalography have been employed for in vivo brain imaging in humans after administration of a psychedelic, and results indicate that intravenously administered psilocybin and LSD produce decreases in oscillatory power in areas of the brain's default mode network.**

I. Introduction

I was delighted when the editors invited me to write a review on "psychedelics," perhaps a watershed moment, representing a shift in opinion that has been developing for more than 3 decades with respect to research and understanding of psychedelics. When I began my graduate studies in 1969, it was politically correct in scientific circles to refer to these substances only as *psychotomimetics*, a negative term suggesting that they fostered a mental state resembling psychosis (Hoffer, 1967). Later, as it was realized that these compounds did not provide very realistic models of psychosis or mental illness, it became more correct to refer to them as *hallucinogens*, again a pejorative term suggesting that they principally produce hallucinations. Yet that is not what they do in most users at ordinary doses, so this term likewise is not particularly descriptive or useful, although it is still widely used and seems to remain the preferred name for these substances in most scientific writing. In addition, the term *hallucinogen* is often used as a rather broad category to include all kinds of psychoactive molecules, including cannabinoids, "ecstasy," dissociative agents, and others.

This review will focus exclusively on the so-called classic serotonergic hallucinogens (psychedelics), which are substances that exert their effects primarily by an agonist (or partial agonist) action on brain serotonin 5-hydroxytryptamine (5-HT) 2A receptors, as discussed later. The discussion will not consider cannabinoids, dissociatives such as ketamine, salvinorin A (a specific opioid κ agonist), or entactogens such as 3,4-methylenedioxymethamphetamine (MDMA). In certain contexts, all of these and some related agents have been swept into the catchall category "hallucinogens." Although they all can produce profound changes in consciousness, they have a different mechanism of action and will not be discussed unless there is a specific reason to do so.

The name *psychedelics* for these substances was coined by Humphrey Osmond in 1957, connoting that they have a mind-manifesting capability, revealing useful or beneficial properties of the mind (Osmond, 1957). This name has been popular among the lay public for more than 5 decades, but it has generally been frowned upon by the scientific community because it implies that these substances have useful properties. The notion that psychedelics can have beneficial effects has thus far not been embraced in most medical or scientific circles; indeed, federal funding agencies (e.g., the National Institutes of Health National Institute

on Drug Abuse and the National Institute of Mental Health) have no mission to support research on potentially useful properties of psychedelics. Yet this term has remained popular with the public and even appears to be gaining popularity. As I intend to show in this discussion, however, the idea that psychedelics may have useful properties is not at all farfetched, and very recent clinical studies have reinforced the belief by many that psychedelics are well worth studying from a number of different perspectives. Indeed, one of the most striking developments in this field has been the initiation and successful completion of a variety of clinical studies of psychedelics in the past 15 years, most of which have been targeted to specific medical indications. As will be discussed later, the results have been, in the main, remarkably positive.

It should be kept in mind that the relative dearth of research on psychedelics in the past half century did not result from a lack of scientific interest, but rather occurred as a consequence of political forces that manifested principally in the United States in the 1960s and 1970s (Grinspoon and Bakalar, 1979). Use of (5*R*,8*R*)-(+)-lysergic acid-*N*,*N*-diethylamide (LSD) and marijuana by so-called hippies who demonstrated against the Vietnam War during the 1960s created great consternation among authorities and legislative bodies, both at the federal and state levels. Antiwar attitudes and rejection of conventional social norms by adolescents and college students were often perceived by the mainstream culture to be a consequence of drug use; hence, these substances were often believed to be "perverting" the minds of our youth. Furthermore, the outspoken Harvard University professor and firebrand Timothy Leary encouraged young people to "turn on, tune in, and drop out," essentially coaching them to take drugs, discover their true selves, and abandon convention. Such messages did not play well with the mainstream culture, all while the mass media fanned the flames of public hysteria with greatly exaggerated reports of drug-induced insanity, chromosomal damage, attempts to fly, and so forth.

Strict laws were quickly passed. After the passage of the Controlled Substances Act of 1970, LSD and other psychedelics known at the time were placed into the most restrictive category of drugs, Schedule 1. This classification made them virtually impossible to study clinically and effectively ended any significant research into the pharmacology and medical value of psychedelics for more than 3 decades. Nevertheless, there can be no doubt that psychedelics played a substantial role in defining the youth culture of the 1960s and 1970s, with books and essays too numerous to cite being written on this topic. It is believed that more than 30 million people have used LSD, psilocybin, or mescaline (Krebs and Johansen, 2013). One suspects that had LSD never been discovered, the world might look very different today than it does now, for better or worse, depending on one's perspective.

Despite the recreational use of psychedelics, a quote from a book by Grinspoon and Bakalar (1979 Pg 192) needs to be kept in mind:

> Many people remember vaguely that LSD and other psychedelic drugs were once used experimentally in psychiatry, but few realize how much and how long they were used. This was not a quickly rejected and forgotten fad. Between 1950 and the mid-1960s there were more than a thousand clinical papers discussing 40,000 patients, several dozen books, and six international conferences on psychedelic drug therapy. It aroused the interest of many psychiatrists who were in no sense cultural rebels or especially radical in their attitudes.

One very important scientific consequence of the discovery of LSD also is often overlooked. The powerful psychologic effect of LSD was accidently discovered in 1943 (Hofmann, 1979a), followed only a decade later in 1953 by the detection of serotonin in the mammalian brain (Twarog and Page, 1953). The presence of the tryptamine moiety within LSD was also quickly seen to be the scaffold for the chemical structure of serotonin (Fig. 1).

This recognition led to a proposal only 1 year later by Woolley and Shaw (1954) that "mental disturbances caused by lysergic acid diethylamide were to be attributed to an interference with the action of serotonin in the brain." Therefore, one could reasonably argue that the whole field of serotonin neuroscience, and especially the role of serotonin in brain function, was catalyzed by the discovery of LSD! By way of illustration, in 1952, there were only 10 publications in the National Library of Medicine concerning serotonin, nearly all of them dealing with some aspect of its ability to constrict blood vessels. Only 8 years later, in 1960, there were 300 publications on serotonin, 35 of which were now focused on studies of serotonin in the brain. For comparison, in 1960, there were only 197 publications about norepinephrine (NE)/noradrenaline, a neurotransmitter that had been discovered and studied in the mid-1940s. Green (2008) provides an interesting overview of the 1950–1970 period of intense research activity after the discovery of serotonin in the brain.

There have been numerous recent reviews on this topic, usually titled as hallucinogens, and the reader is encouraged to consult these works for further details

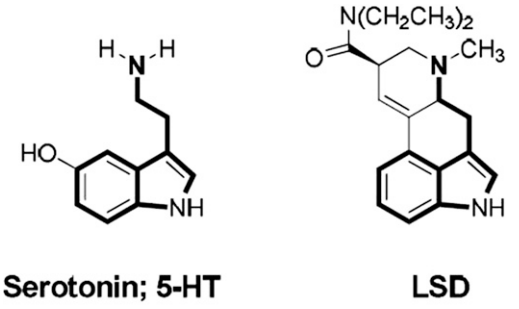


Fig. 1. Chemical structures of serotonin and LSD.

(Nichols, 2004; Nichols and Chemel, 2006; Fantegrossi et al., 2008a; Green, 2008; Passie et al., 2008; Winter, 2009; Griffiths and Grob, 2010; Vollenweider and Kometer, 2010; Brandt and Passie, 2012; Beck and Bonnet, 2013; Halberstadt and Geyer, 2013b; Baumeister et al., 2014; Halberstadt, 2014; Tylš et al., 2014). I wrote a comprehensive review on the subject in 2004, so the literature considered for this review will focus primarily, but not exclusively, on the years from 2004 to the present.

A. Historical Use

Psychedelics are a class of drug that cannot be fully understood without reference to a number of other fields of research, including anthropology, ethnopharmacology, psychiatry, psychology, sociology, and others. This review will focus mostly on pharmacology, both preclinical and clinical, but on occasion reference will be made to aspects of some of those other areas.

Psychedelics may be the oldest class of psychopharmacological agents known to man. Important examples of these substances include a substance used in ancient India known as *Soma*, which was highly revered and is frequently mentioned in the Rigveda, with numerous Vedic hymns written in praise of *Soma* (Wasson and Ingalls, 1971). In the ancient village of Eleusis, outside Athens, for more than 2000 years there was an annual all-night secret ceremony that is believed to have involved ingestion of a hallucinogenic brew known as *κψκεον* (Wasson et al., 1978). We know almost nothing about the ceremony other than that profound insights about life could be achieved, and it was apparently a treasured once-in-a-lifetime opportunity for any Greek citizen who had not been convicted of murder.

Psilocybin mushrooms were used by the Aztec shaman in healing and in a variety of religious and divinatory rituals. These mushrooms were known as *teonanacatl*, meaning "god's flesh" (Ott and Bigwood, 1978; Schultes and Hofmann, 1979). The use of various psychoactive plant materials and substances was common in pre-Columbian Mesoamerican societies, including the Olmec, Zapotec, Maya, and Aztec cultures (Carod-Artal, 2015). In the Bradshaw rock art in the Kimberly region of Australia and in the Sandawe rock art in the Kolo region of Eastern Tanzania, one finds uniquely shared images such as the "mushroom head" symbol of psilocybin use, suggesting that the two cultures were linked and had shamanic practices that used psychoactive mushrooms (Pettigrew, 2011).

Peyote (*Lophophora williamsii*) is a small cactus native to the American Southwest and Northern Mexico that has been used for millennia and is consumed as a sacrament during services of the Native American Church. Two peyote samples from a cave on the Rio Grande River in Texas were analyzed and subjected to radiocarbon dating. The average age of the samples, both of which contained mescaline, dated to 3780–3660 BCE (El-Seedi et al., 2005). This evidence supports the use of peyote by Native North Americans as long ago as 5700 years (Bruhn et al., 2002). Classic psychedelics that have been extensively studied include LSD, shown earlier, mescaline, psilocybin, and *N,N*-dimethyltryptamine (DMT) (Fig. 2).

Ayahuasca, also known as yagé or hoasca, has a long history of use by natives in the Amazon valley of South America (Dobkin de Rios, 1971; Schultes and Hofmann, 1979). Ayahuasca is a decoction prepared from an admixture of two plants: the pounded bark from *Banisteriopsis caapi* vines and leaves from *Psychotria viridis*. The latter contains the hallucinogen DMT, a Schedule 1 controlled substance under U.S. law, and it is generally considered that the psychoactive effects of ayahuasca can be attributed to its DMT content. Although DMT is not orally active, *B. caapi* contains β-carboline alkaloids that inhibit the liver monoamine oxidase (MAO) that normally breaks down DMT; thus, ayahuasca is taken orally as a "tea." Its use has been incorporated as a sacrament into the religious practices of two syncretic Brazilian churches [União do Vegetal (UDV) and the Santo Daime] that have branches in the United States, with the U.S. Supreme Court rendering a 2006 decision to allow the use of ayahuasca by the UDV under the Religious Freedom Restoration Act.

B. What Are Psychedelics?

In view of the widespread historical use of psychedelics as sacraments in a variety of other cultures, Jaffe's (1990) definition for the class of psychedelics can perhaps be appreciated: "…the feature that distinguishes the psychedelic agents from other classes of

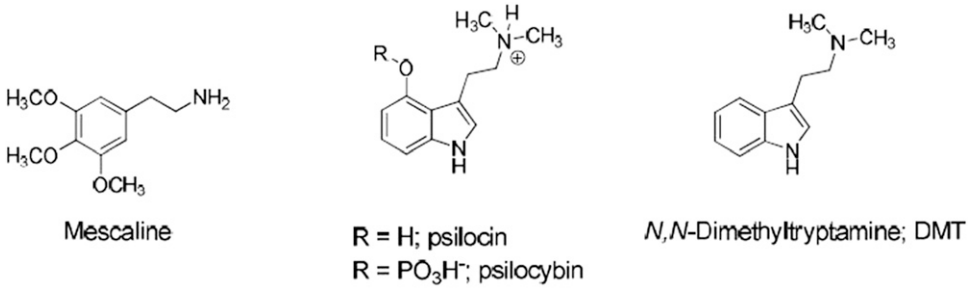


Fig. 2. Chemical structures of classic psychadelics mescaline, psilocybin, and DMT.

drug is their capacity reliably to induce states of altered perception, thought, and feeling that are not experienced otherwise except in dreams or at times of religious exaltation." All pharmacologists will recognize that this definition for a class of psychoactive drugs is indeed quite unique!

One of the pioneers of LSD research, the late Daniel X. Freedman, noted that "…one basic dimension of behavior… compellingly revealed in LSD states is 'portentousness'— the capacity of the mind to see more than it can tell, to experience more than it can explicate, to believe in and be impressed with more than it can rationally justify, to experience boundlessness and 'boundaryless' events, from the banal to the profound" (Freedman, 1968). Freedman's observation is completely consistent with Jaffe's definition.

The use of psychedelics as a central feature of many religious practices, as well as the profound and unique psychopharmacological effects suggested by Jaffe's definition and the observations of Freedman, surely makes us aware that psychedelics are an exceptional category of mind-altering substances. Indeed, this knowledge prompted Ruck et al. (1979) to coin the word *entheogen* as a replacement for the terms hallucinogen and psychedelic, both of which they felt had negative connotations. Entheogen is derived from the Greek roots *entheos*, meaning "God (theos) within," and *genesthe*, meaning "to generate." The word entheogen thus essentially refers to a substance or material that generates God or the divine within someone. Although the term entheogen is now seeing fairly wide acceptance within the culture of those who use these substances "recreationally," a search of the term in the National Library of Medicine finds only five hits. Although it seems unlikely that the term entheogen will be adopted within the formal scientific community, the reader should realize that in some circles entheogen is generally synonymous with psychedelic. Nonetheless, it should be appreciated that the effects produced by psychedelics are highly dependent on the set (mental expectation) of the user and the setting (environment). A set and setting designed to facilitate a mystical experience will increase the probability of such an occurrence, whereas an unstructured or party-type setting is less likely to lead to a positive outcome.

Studerus et al. (2012) investigated the importance of 24 predictor variables on the acute response to psilocybin and confirmed that nonpharmacological factors play an important role in the effects of psilocybin. Variables examined included age, sex, education, personality traits, drug pre-experience, mental state before drug intake, experimental setting, and drug dose. Their analysis was based on pooled data from 23 controlled experimental studies involving 261 healthy volunteers who had participated in 409 psilocybin administrations over a 19-year period in the authors' laboratory. Multiple linear mixed-effects models were fitted for each of 15 response variables. Drug dose was shown to be the most important predictor for all measured response variables, but several nonpharmacological variables significantly contributed to the effects of psilocybin. Specifically, having a high score in the personality trait of absorption, being in an emotionally excitable and active state immediately before drug intake, and having experienced few psychologic problems in past weeks were most strongly associated with pleasant and mystical-type experiences. High emotional excitability, young age, and an experimental setting involving positron emission tomography (PET) most strongly predicted unpleasant and/or anxious reactions to psilocybin. Interestingly, in addition to confirming that nonpharmacological variables play an important role in the effects of psilocybin, an experimental setting involving PET most strongly predicted unpleasant and/or anxious reactions to psilocybin.

Two instruments have been most widely used to assess the subjective effects of hallucinogenic drugs. The first of these, the Hallucinogen Rating Scale (HRS) was developed by Strassman et al. (1994) during their studies of the intravenous administration of DMT. The HRS was first drafted based on interviews with 19 experienced DMT users and was modified during the early stages of their study. The final version, used for their double-blind study, contained 126 individual items. HRS items were placed into six conceptually coherent clusters: somaesthesia (interoceptive, visceral, and cutaneous/tactile effects), affect (emotional/affective responses), perception (visual, auditory, gustatory, and olfactory experiences), cognition (alteration in thought processes or content), volition (a change in capacity to interact willfully with themselves, the environment, or certain aspects of the experience), and intensity (strength of the various aspects of the experience). Subjects were asked to recall their experiences from the immediately preceding session. Most questions were scored on a 0–4 scale (0, not at all; 1, slightly; 2, moderately; 3, quite a bit; and 4, extremely).

The second assessment instrument widely used to quantify the subjective effects of hallucinogens is the Abnormal Mental States (APZ) questionnaire, first developed by Dittrich (1994, 1998) to measure altered states of consciousness (ASCs). In a series of 11 experiments using different induction methods in 393 healthy subjects, the hypothesis was tested that ASCs have major dimensions in common, irrespective of their mode of induction. The original version contained 158 items covering a range of phenomena potentially occurring during an ASC. The common denominator of ASCs was described by three primary oblique dimensions, designated as "oceanic boundlessness" (OSE, later as OBN), "dread of ego dissolution" (AIA, and later as DED), and "visionary restructuralization" (VUS, later as VRS). The APZ questionnaire became the international validated standard for the assessment of ASCs

and the name was further revised to the OAV scales (Bodmer et al., 1994) and later to the five-dimensional altered states of consciousness (5D-ASC) (Dittrich et al., 2006). Details of the development of the original APZ questionnaire and its further refinements were summarized by Studerus et al. (2010). To overcome a variety of methodological limitations, Studerus et al. (2010) carried out a psychometric evaluation of the OAV and 5D-ASC in a relatively large sample from 43 pooled experimental studies of healthy subjects who had received psilocybin, ketamine, or MDMA. The total sample was composed of 591 drug sessions. After comprehensive and detailed statistical analyses, the authors found that the original OAV scales were multidimensional constructs, and arrived at an improved final model that had 11 factors: experience of unity, spiritual experience, blissful state, insightfulness, disembodiment, impaired control and cognition, anxiety, complex imagery, elementary imagery, audiovisual synesthesia, and changed meaning of percepts. Correlations between the 11 factors and their association with the original OAV scales were presented. The new lower-order scales were proposed to be better suited to assess drug-induced ASCs.

Mechanistically, psychedelics have agonist or partial agonist activity at brain serotonin 5-HT_{2A} receptors. This molecular characterization will be elaborated in some detail later in this review, and aspects of the anatomic and functional importance of this receptor type will also be extensively explored. Discussions over the years with many colleagues working in the pharmaceutical industry have informed me that if upon screening a potential new drug is found to have serotonin 5-HT_{2A} agonist activity, it nearly always signals the end to any further development of that molecule.

A recent study by Turton et al. (2014) reported on the subjective experience of intravenous psilocybin administered during a functional magnetic resonance imaging (fMRI) examination. Fifteen volunteers were administered an intravenous infusion of either placebo or 2 mg psilocybin and were blinded as to whether they would receive placebo or drug for a particular experiment. Drug infusion began 6 minutes after the start of a scan in a magnetic resonance imaging scanner. Subjects completed a visual analog scale rating the intensity of the drug experience at the start of the scan and prior to drug infusion, 5 minutes postinfusion, and 12 minutes postinfusion. All subjects subsequently were interviewed about the drug effects, and interviews were analyzed using interpretative phenomenological analysis, a qualitative method. All subjects reported that the onset of drug effect was very rapid and intense, with a duration of effect lasting 10–15 minutes. The investigators identified nine broad categories of phenomenology. Altered somatosensory, visual, auditory, and proprioceptive sensations were reported, with 14 of 15 subjects describing perceptual changes as the primary effect of the drug. Thirteen subjects reported changes in perception of time, either speeding up or slowing down. The report describes a variety of effects on cognition, mood, memory, and spiritual or mystical experiences. Overall, subjects found the experience difficult to describe, yet most found it pleasant and positive.

In a new study by Schmid et al. (2015), LSD (200 μg) was administered orally to 16 healthy subjects in a double-blind, randomized, placebo-controlled, crossover study. LSD produced a pronounced alteration in waking consciousness that lasted for 12 hours and included visual hallucinations, audio-visual synesthesia, and positively experienced derealization and depersonalization phenomena. Compared with placebo, LSD increased subjective well-being, happiness, closeness to others, openness, and trust. Increases in blood pressure, heart rate, body temperature, pupil size, plasma cortisol, prolactin, oxytocin, and epinephrine also were measured. On the 5D-ASC scale, LSD produced higher scores than did psilocybin or DMT. The authors also described subjective effects on mood that were similar to those reported for MDMA that might be useful in psychotherapy. No severe acute adverse effects were observed and the effects subsided completely within 72 hours.

In a very recent study by Carhart-Harris et al. (2015a), LSD was shown to enhance responsiveness to suggestion. Their study was prompted by very early reports indicating that LSD increased suggestibility (Sjobergand Hollister, 1965; Middlefell, 1967). Thus, Carhart-Harris et al. (2015a) administered LSD (40–80 μg, i.v.) to 10 healthy volunteers in a within-subject placebo-controlled design. Suggestibility and cued mental imagery were assessed using the Creative Imagination Scale and a mental imagery test. The two instruments were administered between 110 and 140 minutes after drug infusion, at the peak of the drug effect. Subjects scored significantly higher on the Creative Imagination Scale, but not the mental imagery test after LSD administration, compared with placebo. The magnitude of suggestibility enhancement was positively correlated with the subject's baseline trait conscientiousness. This enhanced suggestibility may have implications for the use of LSD as an adjunct to psychotherapy, but it also indicates that individuals with a high trait conscientiousness are particularly sensitive to the suggestibility-enhancement effect of LSD.

It has been axiomatic among users of psychedelics that music takes on an intensified and more enjoyable quality under the effects of LSD or other psychedelics, yet no modern placebo-controlled study had ever been carried out to confirm that widely held belief. Early studies at the Maryland Psychiatric Research Center found that music was a very effective stimulus and complement to the effect of LSD (Bonny and Pahnke,

1972). Gaston and Eagle (1970) also reported that the presence of music was much preferable to its absence in alcoholic patients undergoing treatment using LSD therapy, both by patient preference and treatment results. Numerous other studies had been reported on the value of music in the context of various shamanic and therapeutic approaches (see references in Kaelen et al., 2015). Very recently, therefore, Kaelen et al. (2015) tested the hypothesis that the emotional response to music is enhanced by LSD. Ten participants were administered placebo or various doses of LSD, including 40, 50, 70, or 80 μg, infused intravenously over 3 minutes. They then listened to five different instrumental music tracks on each of two study days: a placebo day followed by an LSD day, separated by 5–7 days. Music tracks were chosen that produced the highest liking and lowest familiarity by a separate sample of nine participants. The question "How emotionally affected were you by the music?" was asked immediately after each track and served as the study's primary outcome. The nine-category Geneva Emotional Music Scale (Zentner et al., 2008) was also used. Mean scores for all music stimuli in response to the question "how emotionally affected were you by the music?" were significantly higher for the LSD condition than for placebo. In addition, all nine factors on the Geneva Emotional Music Scale were scored higher after LSD administration than after placebo. Emotions related to transcendence also were enhanced by LSD. Specific emotions showing the strongest enhancement included wonder, transcendence, tenderness, and power. Furthermore, there was a significant positive relationship between ratings of the intensity of the LSD effect and emotional arousal to music. The results of Zentner et al. (2008) support the hypothesis that LSD enhances music-evoked emotion.

C. Psychedelics Can Engender Ecstatic States with Persistent Positive Personality Change

The experimental study of mystical or ecstatic states engendered by psychedelics perhaps began with the so-called "Good Friday experiment," carried out at Boston University's Marsh Chapel in 1962 by Walter Pahnke as his research for the Ph.D. in religion and society at the Harvard Divinity School. Pahnke (1963) examined the similarities and differences between experiences described by mystics and those induced by psilocybin. On Good Friday in 1962, 20 Christian theological student volunteers attended a 2.5-hour religious service in Boston University's Marsh Chapel. The setting and preparation of the subjects was designed to optimize a spiritual or mystical experience. In a double-blind procedure, subjects were given either an oral dose of 30 mg psilocybin, or a 200-mg placebo dose of nicotinic acid, administered in identical capsules. Based on responses to a variety of instruments and questionnaires, subjects who received psilocybin had experiences that were indistinguishable from those experienced by mystics. The experiences were powerful and personally meaningful. Doblin (1991) reported a follow-up to the Pahnke study in 1989 and was able to locate and interview 19 of the original 20 experimental participants. All of the psilocybin subjects felt that the experience had significantly affected their lives in a positive way and they expressed appreciation for having participated in the experiment.

An extension of the Good Friday experiment was recently carried out by Griffiths et al. (2006). The investigators used rigorous double-blind clinical methods to evaluate acute and longer-term effects of psilocybin (30 mg/70 kg) compared with an active comparator compound (40 mg/70 kg methylphenidate). A complex design was used to obscure which treatments were administered from the study participants and the monitors, and the study was designed to minimize adverse effects. Thirty-six healthy volunteers were enrolled, all of whom indicated some participation in regular religious or spiritual activities. Instruments used to assess effects 7 hours after drug administration were the HRS, the Addiction Research Center Inventory, the States of Consciousness questionnaire, and the Mysticism Scale. Seven to 8 weeks after each session, and before any additional session, subjects completed the Persisting Effects Questionnaire, the Mysticism Scale–Lifetime, the Spiritual Transcendence Scale, the NEO Personality Inventory, and the Positive and Negative Affect Schedule Expanded Form. Based on measures of mystical experience, 22 of the 36 volunteers had a complete mystical experience after psilocybin administration, whereas only 4 did after the methylphenidate (placebo) sessions. Based on ratings of personal meaningfulness and spiritual experience, 67% of the volunteers rated the psilocybin experience to be either the single most meaningful experience of their lives or among the top five most meaningful experiences in their lives. Based on community observer ratings, psilocybin sessions were associated with significant positive changes in the volunteer's behavior and attitudes.

Thus, when psilocybin was administered under structured conditions to well prepared volunteers, it occasioned experiences that had marked similarities to classic mystical experiences, imparting to the participants substantial personal meaning and spiritual significance. The investigators point out that the high value some subjects placed on the psilocybin experience may in part explain the long-term historical use of psychedelics within some cultures for divinatory or religious purposes. Griffiths et al. (2006) conclude with the statement that, "The ability to prospectively occasion mystical experiences should permit rigorous scientific investigations about their causes and consequences."

Griffiths et al. (2008) subsequently conducted a 14-month follow-up of the subjects from their earlier

study. Subjects were asked to identify in which session they experienced the "most pronounced changes in your ordinary mental process." It was found that the 14-month retrospective follow-up ratings for the psilocybin session did not differ significantly from the immediate postsession ratings. Compared with methylphenidate, the psilocybin experience produced significant increases in ratings of positive attitudes, mood, social effects, and behavior at 14 months of follow-up. At the 14-month follow-up, 58% of the 36 volunteers still rated the psilocybin experience as among the five most personally meaningful experiences of their lives and 67% rated it as among the five most spiritually significant experiences of their lives.

A second study using a similar protocol with 18 volunteers examined dose effects of psilocybin, using 0, 5, 10, 20, and 30 mg/70 kg (Griffiths et al., 2011). The percentage of subjects who met the criteria for having a complete mystical-type experience increased with dose. Overall, 72.2% of volunteers had complete mystical experiences at either or both doses of 20 and 30 mg/70 kg. Positive ratings about life, attitudes about self, mood, social effects, and behavior also increased as a function of dose. Ratings at the 14-month follow-up were undiminished compared with ratings at 1 month after the sessions.

MacLean et al. (2011) analyzed the data from the two double-blind controlled studies of psilocybin reported by Griffiths et al. (2006, 2011). In particular, their goal was to use the NEO Personality Inventory to analyze possible personality changes that might have occurred after the high-dose psilocybin sessions in those studies. It is generally believed that personality traits are relatively enduring and that an individual's personality is predominantly stable across the lifespan. Yet evidence also exists that significant life events may dramatically change adult personality (see references in MacLean et al., 2011). The most widely accepted model of personality structure is the five-factor model, which describes five broad domains of personality: neuroticism, extroversion, openness, agreeableness, and conscientiousness (see references in MacLean et al., 2011). The authors suggest that numerous subjective claims of long-term changes after hallucinogen use appear to align with the personality trait of "openness," which encompasses aesthetic appreciation and sensitivity, imagination and fantasy, and broad-minded tolerance of others' viewpoints and values. Thus, they hypothesize that the mystical experiences reported in the studies by Griffiths et al. might lead to enduring increases in openness. Analysis of personality was assessed 1 to 2 months after a high-dose psilocybin session and again 16 months later to determine the persistence of any personality change(s). Consistent with their hypothesis, a mystical experience after psilocybin administration was significantly correlated with increases in openness. No such effect was seen after methylphenidate treatment. In addition, there were no significant changes in any of the other four personality factors after psilocybin administration. At the 16-month follow-up, openness levels still remained significantly elevated. The authors note that "This is the first study to demonstrate changes in personality in healthy adults after an experimentally manipulated discrete event."

Studerus et al. (2011) pooled raw data from eight double-blind placebo-controlled experimental psilocybin studies conducted between 1999 and 2008. The data were analyzed for acute, short- and long-term subjective effects of psilocybin in 110 healthy human subjects who had received between one and four doses of 45–315 μg/kg psilocybin. Studerus et al. (2011) reported that nearly 40% of the participants in their laboratory studies of psilocybin claimed positive long-term changes in aesthetic experience and in their relationship with the environment (i.e., nature) after their psilocybin sessions. At 8–16 months after psilocybin sessions, more than 60% of subjects rated the experience as "very enriching," and more than 90% described it as enriching to at least a medium degree. These effects occurred despite the fact that no attempt was made in their experiments to optimize conditions for a spiritual or mystical experience, which contrasts with the setting and preparations used in the two Griffiths studies cited above.

Bouso et al. (2012) compared 127 regular ayahuasca users with 115 actively religious controls who did not use ayahuasca. Baseline measurements were taken of general psychologic well-being, mental health, and cognition and the groups were then compared 1 year later to determine whether regular ayahuasca use had an effect on these measurements. Regular ayahuasca users showed lower scores on all psychopathology scales as assessed by the Symptom Checklist 90–Revised, as well as on measures of harm avoidance and self-directedness. Participants scored higher on a measure of psychosocial well-being and performed better on the Stroop test (an indicator of resistance to emotional interference) and the Wisconsin Card Sorting Task (a measure of working memory). No evidence of psychologic maladjustment, mental health deterioration, or cognitive impairment was found in the ayahuasca-using group.

Lerner and Lyvers (2006) compared users of psychedelic drugs with users of nonpsychedelic drugs and nonillicit drug–using social drinkers. Samples were drawn from Israel and Australia. Compared with the other two groups, psychedelic drug users scored significantly higher on mystical beliefs (e.g., oneness with God and the universe), life values of spirituality, and concern for others, and scored lower on the value of financial prosperity, irrespective of culture of origin.

Lyvers and Meester (2012) carried out a website survey of 337 adults who used a variety of drugs,

including psychedelics. Only about 25% reported "spiritual" motives for using psychedelics, yet use of high doses of LSD and psilocybin was significantly correlated in a dose-related manner with scores on two well known indices of mystical experiences; use of MDMA, cannabis, cocaine, opiates, or alcohol was not. Thus, even when taken recreationally, psychedelics have the potential to induce mystical experiences.

Quite interestingly, the underlying neuronal basis for mystical/spiritual experiences has recently been the subject of scientific investigation. Kometer et al. (2015) studied the neuronal basis of spiritual experiences and insightfulness after administration of psilocybin to human subjects. They conducted a double-blind, placebo-controlled study and administered psilocybin (170 or 215 μg/kg, p.o.) to 50 healthy human volunteers. Electroencephalography (EEG) data were recorded from 64 scalp electrodes. Exact low-resolution brain electromagnetic tomography was applied to compute the three-dimensional intracerebral current density values of the scalp-recorded EEG rhythms. They used lagged phase synchronization, a new measure that can capture nonlinear neuronal relationships to assess dynamic functional connectivity (Pascual-Marqui et al., 2011).

The 11-dimension 5D-ASC questionnaire was used to quantify the subjective psychologic effects of psilocybin, with a particular interest in the spiritual experience subscale, and the related subscales of experience of unity, comprising experience of oneness with the environment and the self, insightfulness, measuring profound insights into life and existence, blissful state, measuring experiences of pleasure, inner peace, and love. Voxel-wise product-moment correlations between current source density in the psilocybin condition and the 11-dimension 5D-ASC subscale scores were computed by regression analysis.

Psilocybin significantly increased scores of all subscales on the 5D-ASC but significantly decreased current source density of oscillations in all frequency bands up to 20 Hz (eyes-closed condition) or up to 30 Hz (eyes-open condition). There was a significant and consistent psilocybin-induced reduction of current source density across low frequency bands ($<$20 Hz) in the posterior cingulate cortex (PCC) and the retrosplenial cortex (RSC). Psilocybin decreased the current source density of neuronal 1.5- to 2.0-Hz oscillations within a neural network comprising the PCC, anterior cingulate cortex (ACC), and parahippocampal regions. The intensity of psilocybin-induced spiritual experience and insightfulness correlated with the lagged phase synchronization of 1.5-to 2.0-Hz δ oscillations between the RSC, parahippocampus, and lateral orbitofrontal area.

The extent of lagged phase synchronization within a network of deep cortical structures strongly and positively correlated with score on the insightfulness subscale of the 5D-ASC and spiritual experiences subscales of the 5D-ASC during the eyes-closed condition. Lagged phase synchronization of 1.5- to 4-Hz δ oscillations within a network comprising the RSC, parahippocampus, and lateral orbitofrontal area was associated with spiritual experiences and insightfulness.

These findings of Kometer et al. (2015) provide further evidence that decreased ongoing oscillations below 20 Hz, particularly θ/α oscillations, may be a common mechanism of action of psychedelics. The decrease in lower frequency oscillations was found to be localized within an extended network that included the PCC, RSC, ACC, and parahippocampal regions, a network that strongly overlaps with the default mode network (DMN). Thus, psilocybin may modulate default mode functions by decreasing ongoing lower frequency oscillations within this network.

Lower frequency oscillations, particularly in the α range, mediate rhythmic cortical inhibition of neuronal ensembles (see references in Kometer et al., 2015). The marked decrease in lower frequency oscillations observed in this study may indicate that psilocybin induces a shift of the resting excitation/inhibition balance toward excitation, which would be expected to disrupt the ordinary temporal structure of neuronal processes within the extended DMN.

Lagged phase synchronization was strongly associated with the psilocybin-induced state of consciousness, supporting the view that neural integration, rather than activity, underlies the state of consciousness. Scores of the spiritual experiences subscale of the 5D-ASC questionnaire were also associated with increased lagged phase synchronization of δ oscillations between parahippocampal regions and the RSC. Kometer et al., (2015) speculate that the neuronal network processes they identified may constitute a crucial pathway that can be modulated by serotonergic receptors to regulate mental health, a conclusion that would be consistent with some of the potential positive mental health outcomes discussed earlier in this section.

II. Safety of Psychedelics

For decades, the media have largely portrayed psychedelics as extremely dangerous drugs; in fact, the classic serotoninergic psychedelics are generally considered very physiologically safe, certainly compared with opiates and psychostimulants. Jaffe (1985) stated, "In man, deaths attributable to direct effects of LSD are unknown," and this statement remains true even today. Nonetheless, despite the relative physiologic safety of psychedelics, they can lead to serious psychologic consequences. In addition, as will be discussed later, some of the newer highly potent synthetic phenethylamine hallucinogens have proven to be unexpectedly toxic. This section will detail studies indicating that psychedelics can be safely used under supervision, and that few documented serious adverse effects occur even

after recreational use. The discussion will then turn to some generally recognized adverse reactions, followed by case reports of some more serious and even fatal reactions to psychedelics. It should be emphasized that these latter fatalities, which are rare, have occurred after use of newer synthetic phenethylamine compounds, and not as a result of ingestion of LSD, psilocybin, mescaline, or DMT.

Analysis of early published reports on adverse reactions and long-term negative sequelae induced by classic psychedelics failed to identify significant adverse events; if long-term adverse effects from repeated use did occur, they were subtle or nonsignificant (Strassman, 1984; Halpern and Pope, 1999). Their reviews were based on reports from supervised clinical studies using pure drugs, so the same conclusions might not apply to recreational use of drugs with unknown identities or purity.

These substances do not lead to addiction or dependence and are not considered to be reinforcing (O'Brien, 2001). This is understandable when one realizes that the serotonergic hallucinogens do not have direct effects on brain dopaminergic systems, a pharmacology that appears essential for nearly all drugs that can engender dependence. Attempts to train animals to self-administer hallucinogens, an animal model that can predict abuse liability, have generally been unsuccessful.

Using 2001–2004 data drawn from the National Survey on Drug Use and Health (NSDUH), Krebs and Johansen (2013) recently evaluated possible associations between lifetime use of psychedelics and current mental health in the U.S. adult population. In a large sample of respondents, 13.4% reported lifetime psychedelic use. No significant associations were found between lifetime use of any psychedelic or past-year use of LSD and increased rate of any mental health outcome. Surprisingly, in several cases, use of psychedelics was associated with a lower mental health problem rate.

A statewide survey of the adult population in Colorado sought to determine whether psychedelic use was correlated with the lifetime risk of panic attacks (Bonn-Miller et al., 2007). No association was found between psychedelic use and panic attacks, but psychedelic abuse and dependence were significantly related to an increased lifetime risk of panic attacks. It should be noted that in this study, however, phencyclidine (PCP) was included in their survey as a psychedelic, and this substance, in contrast with the classic serotonergic psychedelics, can cause dependence.

Peyote (*L. williamsii*) is a small cactus that grows in the Southwestern United States and Northern Mexico. It contains the psychedelic compound mescaline and has been used for centuries by Native American populations in rituals and ceremonies. Mescaline is also found in the San Pedro and Peruvian Torch cacti, and these have also been used ceremonially. Although peyote is classified as a Schedule 1 controlled substance, members of the Native American Church have a legal exemption to use it in their religious services. Halpern et al. (2005) compared 61 Navajo Native American Church members who regularly ingested peyote with 79 individuals reporting minimal use of peyote, alcohol, or other substances. Cognitive function was assessed using the Rand Mental Health Inventory and 10 standard neuropsychological tests of memory and attentional/executive functions. The peyote-using group showed no significant deficits on the Rand Mental Health Inventory or on any of the 10 other tests used. For the peyote-using group, total lifetime peyote exposure was not associated with neuropsychological performance.

By contrast, recreational use of peyote has led to adverse events, although peyote exposures reported to poison control centers are relatively rare compared with other drugs of abuse. In 2007, for example, only 116 peyote or mescaline exposures were reported to U.S. poison control centers out of more than 2.4 million total drug exposures (Bronstein et al., 2008). Carstairs and Cantrell (2010) retrospectively reviewed the California Poison Control System electronic database between the years 1997 and 2008 for reports of cases involving adverse reactions to peyote or mescaline ingestion when it was the sole intoxicating agent. A total of 31 cases were identified that met their inclusion criteria. Life-threatening symptoms did not occur, and most exposures were associated with only mild to moderate clinical effects, which most commonly included tachycardia and central nervous system (CNS) effects. Symptoms typically resolved within 24 hours or less and did not usually require anything more than supportive measures or sedation. One case of a prolonged peyote-induced psychosis was reported by Lu et al. (2004), in which the psychosis resolved after sleep. The case involved a 54-year-old Native American man with no prior history of psychosis. He drank peyote juice during a healing ceremony and within a few hours became convinced that he was hunted by animal spirits. He was unable to sleep for 2 weeks, at which time he developed visual and auditory hallucinations of the spirits and became increasingly depressed. He was persuaded to enter a hospital, where he received trazodone to help him sleep. He fell asleep and slept for 15 hours, which led to complete resolution of his psychotic symptoms. The authors speculated that his psychosis was a result of his prolonged sleep deprivation.

Hasler et al. (2004) studied eight subjects given either placebo or 45, 115, 215, or 315 μg/kg psilocybin (a very low, low medium, or high dose, respectively). Instruments used to assess psilocybin effects included the 5D-ASC, the Frankfurt Attention Inventory (FAIR), and the Adjective Mood Rating Scale (AMRS). Several physiologic and plasma hormones were also measured. Psilocybin dose-dependently increased all measures on the 5D-ASC. The medium and high doses of psilocybin led to a 50% reduction in performance on the FAIR test.

The only scores that were increased on the AMRS were "general inactivation," "emotional excitability," and "dreaminess." Hasler et al. (2004) found no evidence that psilocybin is hazardous with respect to somatic health.

Bouso et al. (2015) used magnetic resonance imaging to examine potential differences in cortical thickness in 22 Spanish regular users of ayahuasca, compared with 22 matched controls. Inclusion criterion for ayahuasca users was that they had used it at least 50 times in the 2 previous years. Subjects also were assessed using three neuropsychological tests, including the two-back test to assess working memory, the Wisconsin Card Sorting Test to assess executive function, and a switching task to assess set shifting. Personality was also assessed by self-report using the Spanish version of the Temperament and Character Inventory–Revised questionnaire. Ayahuasca users scored significantly better than controls on several variables derived from the neuropsychological tests. No increased psychopathology or worse neuropsychological performance was observed in the ayahuasca group, consistent with findings reported earlier by Grob et al. (1996) for ayahuasca users who were members of the Brazilian church, the UDV. Indeed, ayahuasca users scored significantly better than controls on "harm avoidance," and its subscale "anticipatory worry," and significantly higher on "self-transcendence." Cortical thinning was found for six brain areas in the ayahuasca group: the middle frontal gyrus, the inferior frontal gyrus, the precuneus, the superior frontal gyrus, and the PCC. By contrast, cortical thickening was seen in the precentral gyrus and the ACC. Correlation analysis revealed that lifetime use of ayahuasca was inversely correlated to cortical thickness in the PCC.

A. General Issues of Safety and Mental Health in Psychedelic Users

Although there is a general public perception that psychedelic drugs are dangerous, from a physiologic standpoint they are in fact one of the safest known classes of CNS drugs. They do not cause addiction, and no overdose deaths have occurred after ingestion of typical doses of LSD, psilocybin, or mescaline. Cohen (1967) and Jaffe (1985) have both stated that death due to direct LSD toxicity is unknown. Indeed, recreational users who have consumed massive doses of LSD have survived. For example, eight individuals who believed they had cocaine accidentally insufflated an extremely high dose of LSD. Their plasma LSD levels were reported as between 1000 and 7000 μg/100 ml (recall that a typical total oral dose of LSD might be 100–200 μg). These individuals all became comatose, with hyperthermia, vomiting, light gastric bleeding, and respiratory problems. With hospital treatment, however, all eight survived and without apparent residual effects (Klock et al., 1974).

Although the classic psychedelics have not been directly responsible for causing death, the judgment of users is certainly impaired while under the influence of these drugs. This is a particular concern when hallucinogens are used in unsupervised settings. Users may believe that they are invincible or possess superpowers and may do things they would not normally consider, such as believing they can fly (Reynolds and Jindrich, 1985), jumping from buildings (Keeler and Reifler, 1967), or incurring severe ocular damage by prolonged staring at the sun (Schatz and Mendelblatt, 1973; Fuller, 1976).

Studerus et al. (2010) analyzed acute, short-, and long-term subjective effects of psilocybin in healthy humans. Again, using pooled raw data from eight double-blind placebo-controlled experimental studies conducted between 1999 and 2008, their analysis included 110 healthy subjects who had received between one and four oral doses of psilocybin (45–315 μg/kg body weight). Psilocybin dose-dependently induced profound changes in mood, perception, thought, and self-experience, but most subjects described the experience as pleasurable, enriching, and nonthreatening. Acute adverse reactions were characterized by strong dysphoria and/or anxiety/panic, but occurred only at the two highest doses of psilocybin in a relatively small number of subjects. All acute adverse drug reactions were successfully managed through interpersonal support and did not require psychopharmacological intervention. Follow-up questionnaires indicated no subsequent drug abuse, persisting perception disorders, prolonged psychosis, or other long-term impairment of functioning in any of the subjects. The results indicate that the administration of modest psilocybin doses to healthy, high-functioning, and well prepared subjects in the context of a carefully monitored research environment carries an acceptable level of risk.

The recent resurgence of interest in the clinical uses of psychedelics led Johnson et al. (2008) to propose appropriate procedures for using them in clinical practice. The guidelines they outline have certain parallels with ritual uses of hallucinogens by older indigenous cultures. In particular, Johnson et al. (2008) cite the need for structured use (expressed as ritual in indigenous use) and restrictions on use, including the need for guidance and appreciation of the powerful psychologic effects of hallucinogens (expressed as reverence in indigenous use). Psychedelic administration in humans results in a unique profile of effects and potential adverse reactions that need to be appropriately addressed to maximize safety. The primary safety concerns with psychedelics are largely psychologic rather than physiologic in nature. Somatic effects vary but are relatively insignificant, even at doses that elicit powerful psychologic effects. The proposed guidelines extend and complement the recommendations of Fischman and Johanson (1998) for high-dose hallucinogen research. The guidelines include 1) the presence of two "monitors" with some medical knowledge, knowledge of

ASCs, and a degree of clinical sensitivity; 2) a physical environment that is safe, aesthetically pleasing, and comfortable; 3) careful subject preparation, including several meetings to establish rapport and trust with the monitors; 4) a detailed consent form and explanations of the study procedures, detailed discussions about the range of potential experiences, and time of onset and duration of the effects; and 5) an available physician in the event of an untoward medical reaction. Anyone contemplating carrying out a clinical research program with a psychedelic is strongly encouraged to read the detailed guidelines presented by Johnson et al. (2008).

Krebs and Johansen (2013) evaluated any association between lifetime use of psychedelics and current mental health in the adult population. Data were analyzed for 2001–2004 for 130,152 randomly selected NSDUH respondents; 21,967 respondents (13.4% weighted) reported lifetime psychedelic use. The authors found no significant association between lifetime use of any psychedelic and increased rate of any mental health outcomes. Indeed, they discovered that psychedelic use was associated with a lower rate of mental health problems in several cases. Johansen and Krebs (2015) subsequently analyzed a new data set of 135,095 randomly selected U.S. adults that included 19,299 users of psychedelics. Data were from the NSDUH for 2008–2011. As in their earlier study, the authors found no significant associations between lifetime use of psychedelics and increased likelihood of past-year serious psychologic distress, mental health treatment, depression, anxiety, or suicidal thoughts, plans, or attempts. Johansen and Krebs (2015) failed to find any evidence that use of psychedelics is an independent risk factor for mental health problems. Indeed, they report that lifetime use of psychedelics was associated with *decreased* inpatient psychiatric treatment.

Hendricks et al. (2014) analyzed data from 2002–2007 for 25,622 individuals charged with a felony in the Southeastern United States and under community corrections supervision in the Treatment Accountability for Safer Communities program, which is a case management intervention program for individuals with a history of substance involvement. The authors examined relationships between any hallucinogen use disorder (versus no hallucinogen use disorder) and all available sociodemographic and psychosocial variables. They report that any hallucinogen use disorder was associated with a decreased probability of supervision failure. They note the contrast with any cannabis, cocaine, alcohol, opiate, or amphetamine use disorder, each of which was associated with an *increased* probability of supervision failure. Their results suggest that hallucinogens may promote alcohol and other drug abstinence and prosocial behavior in a population with high rates of recidivism.

In a more recent report by Hendricks et al. (2015), the authors evaluated any relationship between use of a classic psychedelic and psychologic distress and suicidality among more than 190,000 U.S. respondents pooled from the NSDUH for 2008–2012. Lifetime use of a psychedelic was associated with significantly reduced odds of past-month psychologic distress, past-year suicidal thinking, past-year suicidal planning, or past-year suicide attempt. By contrast, lifetime use of other illicit drugs was associated with an *increased* likelihood of these outcomes. The authors suggest that classic psychedelics may hold promise in the prevention of suicide. These findings are consistent with the surveys of Krebs and Johansen (2013) and Johansen and Krebs (2015); in all three studies, the authors suggest that their data are not compatible with the highly restricted legal status of psychedelics and that more extensive clinical research is warranted.

B. Adverse Reactions

Use of high doses of psychedelics can lead to vascular problems because the 5-HT_{2A} receptor is associated with vascular smooth muscle contraction, platelet aggregation, thrombus formation, and coronary artery spasms (Nagatomo et al., 2004). Acute vasoconstriction caused by serotonin is usually shared by activation of 5-HT_{1B} and 5-HT_{2A} receptors; however, in intracranial arteries, only the 5-HT_{1B} receptor mediates constriction (Kaumann and Levy, 2006). Both 5-HT_{2A} and 5-HT_{1B} receptors can mediate coronary artery spasm. 5-HT_{2A} receptors also constrict the portal venous system, including esophageal collaterals in cirrhosis. Data from studies by Ootsuka et al. (2004) suggest that spinal 5-HT_{2A} receptors contribute to sympathetically induced cutaneous vasoconstriction regulated by the raphe/parapyramidal neurons in the brainstem.

Balíková (2005) reports a fatal and nonfatal overdose after ingestion of the psychedelic phenethylamine 2,5-dimethoxy-4-bromoamphetamine (DOB) by two male individuals. Gas chromatography–mass spectrometry was used to detect the presence of DOB in both gastric and urine samples of the two men. Although one subject survived, the other suffered convulsions and metabolic acidosis and died 6 days after admission.

Psilocybin, when administered in a controlled setting, has frequently been reported to cause transient, delayed headache, with incidence, duration, and severity increased in a dose-related manner (Johnson et al., 2012). Bickel et al. (2005) reported the case of a 25-year-old hepatitis C–infected man, who presented with severe rhabdomyolysis and acute renal failure after *Psilocybe* mushroom ingestion. He later developed encephalopathy with cortical blindness. Respiratory and cardiovascular support, mechanical ventilation, continuous venovenous hemodialysis, and corticosteroid treatment led to improvement and the patient recovered completely over several months.

Psilocin was identified in the urine of a subject who was investigated for driving under the influence

(Tiscione and Miller, 2006). The subject apparently did not exhibit any response to the crash of his automobile, seemingly unaware of the severity of his situation or immediate surroundings.

Although very rare, there have been reports of rhabdomyolysis after ingestion of LSD (Berrens et al., 2010). A newer tryptamine, 5-methoxy-*N,N*-diisopropyltryptamine ("foxy") also produced rhabdomyolysis and transient acute renal failure in an otherwise healthy 23-year-old man (Alatrash et al., 2006).

Although many ergot alkaloids are known to produce vasospasm, especially after chronic use, LSD has rarely been associated with this adverse effect. Nevertheless, Raval et al. (2008) reported on a 19-year-old woman who experienced severe lower-extremity ischemia related to a single use of LSD 3 days prior to presentation. After intra-arterial nitroglycerin and verapamil failed, balloon percutaneous transluminal angioplasty therapy led to rapid clinical improvement in lower-extremity perfusion. As of the date of the report, the patient had not required a major amputation.

Sunness (2004) described a 15-year-old female patient with a 2-year history of afterimages and photophobia after a history of drug use that included LSD, marijuana, and other illicit drugs. She had discontinued LSD 1 year prior to examination. Although the author connected her visual problems with her prior LSD use, it is not at all clear from the report that her LSD use was the cause of her visual problem.

Bernhard and Ulrich (2009) reported a case of cortical blindness in a 15-year-old girl. She had headache and nausea 5 days after taking LSD and suddenly developed complete blindness in both eyes. The blindness persisted for 48 hours. Over the next 3 months, the subject had three more episodes of complete blindness that lasted 12–36 hours, with no visual disturbances between episodes. The authors suggested that the temporary blindness might be a correlate of "flashbacks" caused by LSD.

Toxicity also has been noted for several of the so-called designer drugs. For example, Jovel et al. (2014) reported the case of a healthy young male individual who ingested 5-methoxy-*N,N*-diallytryptamine, one of the emerging new tryptamine-type research chemicals. The patient was admitted with extreme agitation, tachycardia, diaphoresis, and combativeness that required physical restraint and intravenous sedation, but the patient did recover.

Andreasen et al. (2009) reported a fatality involving the potent synthetic psychedelic phenethylamine compound 1-(8-bromobenzo[1,2-*b*; 4,5-*b*′]difuran-4-yl)-2-aminopropane, known commonly as Bromo-Dragonfly. An 18-year-old woman was found dead after ingesting 1 ml of a "hallucinogenic liquid." She and her boyfriend had ingested it between 10 and 11 PM on the previous evening and then they both fell asleep. On awakening at 5 AM the next morning, the woman's boyfriend discovered that she was dead. Autopsy findings 3 days after her death included edema of the lungs, slight edema of the brain, spleen enlargement, irritation of the mucous membrane in the stomach, and ischemic changes in the kidneys. Her femoral blood concentration of the drug was 4.7 μg/kg. The bottle containing the hallucinogenic liquid was recovered and analyzed by ultraperformance liquid chromatography time-of-flight mass spectrometry, high-performance liquid chromatography diode array detection, ^{1}H nuclear magnetic resonance, and ^{13}C nuclear magnetic resonance and found to contain a solution of almost pure Bromo-Dragonfly. Based on the solution concentration and the amount of solution consumed, it was estimated that she had ingested approximately 700 μg. Although that would seem to be a relatively small dose, no other drugs were discovered in her system, including the absence of ethanol.

It is often difficult to establish whether the drug is pure or has been coingested with other unknown drugs of unknown purity. For example, Ovaska et al. (2008) reported a case of "sympathomimetic toxicity" in a patient who was reported to have ingested 2,5-dimethoxy-4-chloroamphetamine (DOC), yet toxicological screening showed the patient had ingested both DOC and MDMA.

Data for 2005 to 2006 from the Texas Poison Control Centers were reviewed for mushroom exposures (Barbee et al., 2009). There were a total of 742 exposures, which were all acute and intentional. Of those, 59 individuals were admitted to a hospital, with 17 requiring admission to a critical care unit. Nonetheless, only 10 of the admissions that were identified involved psilocybin. Of all of the admissions, major toxic reactions were uncommon, with no deaths reported.

C. Hallucinogen Persisting Perception Disorder

One adverse effect of hallucinogen use, particularly associated with LSD use, is hallucinogen persisting perception disorder (HPPD). This term has displaced an earlier somewhat more nonspecific one known as "flashbacks," which was a re-experiencing of one or more of the perceptual effects induced by a hallucinogen at some later time, after the acute drug effects had worn off. HPPD is composed of afterimages, perception of movement in peripheral visual fields, blurring of small patterns, halo effects, and macro- and micropsia long after the drug has been used.

The *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* (*DSM-IV*), *Text Revision* lists the following three criteria for HPPD: A) re-experiencing, after the use of a hallucinogen, of one or more of the perceptual symptoms that were experienced while intoxicated with the hallucinogen; B) the symptoms in criterion A cause clinically significant distress or impairment in social, occupational, or other important areas of functioning; and C) the symptoms are not

due to a general medical condition and are not better accounted for by another mental disorder.

Halpern and Pope (2003) noted that when LSD was used in a therapeutic or research setting, HPPD appeared less frequently than when LSD was used recreationally. The authors concluded, however, that some individuals, especially users of LSD, can experience a long-lasting HPPD syndrome with symptoms of "persistent perceptual abnormalities reminiscent of acute intoxication." Nevertheless, the incidence of HPPD is very small given the many tens of millions of persons who have taken LSD, most often in a recreational setting. Litjens et al. (2014) provided a recent comprehensive review on the subject of HPPD. The actual incidence of HPPD is not known and depends on the prevalence of use in different countries, but epidemiologic information is scarce.

Hermle et al. (2008) reviewed MEDLINE data for 1997–2007, searching for reports of hallucinogen-induced psychosis, flashbacks, and HPPD. The authors reported that adolescent intoxication with psychedelic drugs rarely produced acute psychotic syndromes, further stating that "The clinical relevance of flashback phenomena as a post-hallucinogenic psychiatric disorder has to be disputed."

Although LSD was most widely used and therefore has led to the greatest number of HPPD cases, it is clear that other hallucinogens also can evoke the syndrome. For example, Espiard et al. (2005) reported HPPD in an 18-year-old man after mixed intoxication with psilocybin and cannabis. The symptoms persisted for more than 8 months. Ikeda et al. (2005) reported flashbacks after use of 5-methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) by a 35-year-old man without a previous psychiatric history. He had used the substance six or seven times over 5 months but discontinued it after he had a bad trip, with anxiety, palpitations, auditory oversensitiveness, and visual distortions. Treatment with oral risperidone ameliorated his symptoms. Another case study described a 33-year-old woman who developed HPPD after LSD use for a year. Although treatment with antidepressants and risperidone did not ameliorate her symptoms, treatment with the antiseizure drug lamotrigine almost completely abolished her visual disturbances (Hermle et al., 2012).

D. N-(2-methoxybenzyl)-2,5-dimethoxy-4-substituted phenethylamines (NBOMe) Compounds

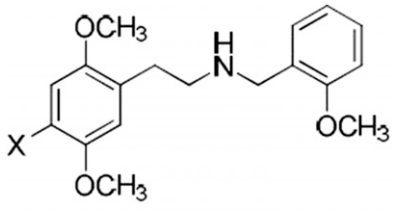
Although the classic serotonergic hallucinogens are not recognized to be particularly toxic, a new class of substituted phenethylamines with toxic properties has recently become very popular as recreational drugs (Nikolaou et al., 2014). Unfortunately, there are now several reports of hospitalizations and fatalities attributed to these compounds (Poklis et al., 2013, 2014; Rose et al., 2013; Nikolaou et al., 2014; Tang et al., 2014; Walterscheid et al., 2014), but it is not clear whether deaths resulted from ingestion of lethal amounts of pure bulk drug or whether the drug has some inherent toxicity that is not normally associated with other psychedelics.

Suzuki et al. (2015) provided a comprehensive literature review of toxicities associated with NBOMe ingestion. The most common adverse reactions were agitation (including aggressiveness), tachycardia, and hypertension, with seizures reported in 40% of the patients. In the 20 individual cases they reviewed, 3 (15%) were fatalities.

The most potent of these new recreational chemicals are shown in Fig. 3, with potency increasing going from X = H to X = I.

For purposes of law enforcement the iodo compound (X = I; 25I-NBOMe) is presently considered by the U.S. Drug Enforcement Administration to be an analog of 2C-I [2-(4-iodo-2,5-dimethoxy)aminoethane], which is currently a Schedule 1 controlled substance. The procedure to classify 25I-NBOMe as a Schedule 1 substance has been initiated and it has been placed temporarily into Schedule 1 (Drug Enforcement Administration, 2013). Global interest in these compounds and closely related analogs has attracted increasing interest. For example, the European Monitoring Centre for Drugs and Drug Addiction has received a range of notifications from European Union member states about analytically confirmed nonfatal and fatal intoxications associated with 25I-NBOMe. This was followed by a risk assessment conducted by the European Monitoring Centre for Drugs and Drug Addiction Scientific Committee to assess health and social risks associated with the iodo analog (European Monitoring Centre for Drugs and Drug Addiction, 2014). In addition, the World Health Organization's Expert Committee on Drug Dependence reviewed the status of a range of new substances for its 36th meeting in June 2014, which included 25I-NBOMe and its 4-bromo and 4-chloro analogs (World Health Organization, 2014).

In the mouse head-twitch assay, 25I-NBOMe and a related analog were extremely potent in inducing this behavior, which was blocked by preadministration of the selective 5-HT_{2A} antagonist M100907 [(*R*)-(+)-*α*-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol] (Halberstadt and Geyer, 2014). As discussed in the section on mouse models later in this



25H-NBOMe, X = H
25C-NBOMe, X = Cl
25B-NBOMe, X = Br
25I -NBOMe, X = I

Fig. 3. NBOMe compounds.

review, the mouse head twitch has shown a high correlation with human psychedelic activity.

A relatively large series of 48 NBOMe-type compounds has been evaluated for affinity and function at 5-HT_2 family receptors (Hansen et al., 2014). Their work was directed toward development of potential radioligands for in vivo PET imaging of 5-HT_{2A} receptors that would be selective over 5-HT_{2C} receptors. One compound was discovered that had approximately 100-fold selectivity for both affinity and function at the 5-HT_{2A} versus 5-HT_{2C} receptor. Their high affinity and relative selectivity for the 5-HT_{2A} receptor has made some of these compounds useful as agonist ligands for in vivo PET imaging (Ettrup et al., 2010; Finnema et al., 2014).

Curiously, the NBOMe-type compounds do not appear to be orally active and are typically administered bucally, or by nasal insufflation. Their potency is so high that they are often distributed on blotter papers and marketed as being LSD. Users place the blotters against their gums to effect absorption. One hypothesis put forward to explain the lack of oral activity for these highly active compounds is a significant first-pass metabolic effect (Leth-Petersen et al., 2014). In that study, the microsomal stability of 11 phenethylamines and their *N*-benzylated congeners was studied using human liver microsomes. It was found that the *N*-benzylated compounds had a much higher intrinsic clearance than did the simple phenethylamines, and the authors hypothesized that their low hepatic stability was the reason for their lack of oral activity.

Stellpflug et al. (2014) reported a clinical case of a nonfatal overdose with 25I-NBOMe. They identified a major metabolite of the compound in the urine that had a concentration 80-fold higher than the parent drug. The subject's urine was treated with β-glucuronidase and then analyzed using ultraperformance liquid chromatography electrospray ionization plus tandem mass spectrometry to identify a major metabolite with a mass that was one methyl group lower than the parent compound. Comparison of the full fragmentation pattern was then assessed and concluded to be an *O*-demethylated metabolite at the 2- or 5-position of the trisubstituted ring, but the investigators were not able to determine which position had been metabolized. They detected two other very minor metabolites that also appeared to be *O*-demethylated. The concentration of the unmetabolized parent 25I-NBOMe in the urine was 7.5 ng/ml, whereas the desmethyl metabolite was 600 ng/ml. This metabolite and the two other minor metabolites were not present in the urine in the absence of prior enzymatic treatment, indicating that they were all glucuronidated.

Most recently, Leth-Petersen et al. (2015) identified the basis for the high first-pass effect of NBOMe compounds and the likely basis for their inactivity after oral administration. Using in vivo studies in pigs, they determined that the 5-methoxy of the trisubstituted phenyl ring is rapidly *O*-demethylated. After intravenous administration of 25B-NBOMe [*N*-(2-[^{11}C]methoxybenzyl)-2, 5-dimethoxy-4-bromophenethylamine (Cimbi-36)] to a pig, analysis revealed that plasma levels of the parent drug rapidly declined, with a new metabolite rapidly appearing and accumulating in plasma. At the 30-minute mark, there was more than twice as much of this metabolite present in plasma as there was of the parent compound. This metabolite was definitively identified as the 5-*O*-glucuronide using liquid chromatography/mass spectrometry and chemical synthesis. Evidently, the highly hydrophobic nature of the *N*-benzyl phenethylamines readily targets them to the mixed function oxidases in the endoplasmic reticulum, where they are efficiently 5-*O*-demethylated and then very quickly glucuronidated.

III. Mechanism of Action

Stanislov Grof characterized LSD as a powerful "nonspecific amplifier of the unconscious" (Grof, 1975). This empirical observation was based on his personal supervision of more than a thousand clinical administrations of LSD. Barr et al. (1972) also stated that "…the phenomena induced by LSD… cannot be predicted or understood in purely pharmacological terms; the personality of the drug taker plays an enormous and critical role in determining how much effect there will be and of what particular type."

However, until we understand the fundamental nature of consciousness and its underlying neuronal substrates, as well as the unconscious, it will not be possible to scientifically test Grof's hypothesis. What can be discussed are the findings that point to involvement of specific receptors in certain brain areas that lead to the overt effects of psychedelics. In addition, recent brain scanning technologies, including PET, fMRI, EEG, magnetoencephalography (MEG), and pharmacological magnetic resonance imaging (phMRI), have also allowed the identification of key brain areas that must be involved in the actions of psychedelics.

One should keep in mind that the effects of psychedelics are highly variable and are not necessarily dose dependent. At low doses of LSD (e.g., <100 μg), sensory and cognitive processes may be distorted and altered but the user generally remains aware that the effects are attributable to having ingested the drug. For the purposes of clinical investigations, such doses allow the use of various questionnaires, instruments, and interviews to determine the intensity and qualitative aspects of the drug effect. Even lower doses of LSD are popular for recreational use or group events in which the user wishes to remain in contact with their surroundings.

By contrast, high doses have a greater propensity to transport the user to an alternate reality, where they lose contact with their everyday environment. These

occasions are often described as "peak experiences," "transcendent," or "mystical" and are profoundly altered states of consciousness. Users may feel that they have transcended time and space or encountered their concept of "God," or they may feel that they have encountered otherworldly beings, feelings of being at one with the universe, reliving past memories, and so forth. With respect to medical value, this state of consciousness is most closely associated with dramatic therapeutic improvement. Although this phenomenon is more likely to occur after high doses of psychedelics, it can occur at nearly any dose if the set and setting have been optimized to promote such an ASC. These experiences are often characterized as among the most meaningful of the subject's life (e.g., see Griffiths et al., 2006) and can lead to persisting positive effects on attitudes, mood, and behavior.

A. Evidence for Agonist or Partial Agonist Action at Serotonin 5-Hydroxytryptamine 2A Receptors

It was only a decade after the discovery of the remarkable psychopharmacology of LSD that the presence of serotonin was demonstrated in the mammalian brain (Twarog and Page, 1953). A comparison of the chemical structures of LSD and serotonin (shown earlier) led to early hypotheses that the action of LSD was due to an interaction with serotonin systems in the brain. Ten years after the discovery of LSD, Gaddum (1953) reported that LSD antagonized the action of serotonin in peripheral tissues. Only 1 year later, Gaddum and Hameed (1954) and Woolley and Shaw (1954) independently proposed that the effects of LSD might result from serotonin receptor blockade in the CNS. Shaw and Woolley (1956) later modified their hypothesis to include the possibility that LSD might mimic the actions of serotonin. Numerous studies in the subsequent decade examined the possibility that LSD blocked the actions of serotonin, but it was a concept that proved untenable. It was clear, however, that LSD did have a potent effect on brain serotonin systems, elevating whole brain serotonin content (Freedman, 1961) and reducing brain levels of the major metabolite of serotonin, 5-hydroxyindole acetic acid (Rosecrans et al., 1967).

Ultimately, Andén et al. (1968) suggested that LSD might have direct agonist actions at serotonin receptors in the brain. Subsequently, studies from numerous laboratories provided support for that idea, with an initial focus on serotonin 5-HT_{1A} receptors (see discussion in Nichols, 2004). When serotonin receptor–selective antagonists became available, it was Glennon et al. (1983, 1984) who demonstrated in a rat drug discrimination model that the 5-HT_2 antagonists ketanserin and pirenperone blocked the discriminative cue of a psychedelic. Further studies in numerous laboratories over the next 2 decades, primarily with rodents, then focused attention on the 5-HT_{2A} receptor as the primary target for psychedelics. Agonist or partial agonist activity at the serotonin 5-HT_{2A} receptor was ultimately concluded to be a necessary pharmacology for psychedelic effects, but it may not be sufficient to explain all of the qualitative differences between different drugs. As Ray (2010) pointed out, different molecules may also have significant affinity for other types of brain receptors.

The first definitive experiment pointing to the central role of the 5-HT_{2A} receptor for the action of psychedelics in humans came from a clinical study by Vollenweider et al. (1998), who showed that the effects of psilocybin were blocked by the 5-HT_{2A} receptor–selective antagonist ketanserin or the atypical antipsychotic risperidone but were enhanced by the dopamine antagonist and typical antipsychotic haloperidol. These data provided the first evidence that psilocybin-induced effects in humans were due to 5-HT_{2A} receptor activation. Subsequently, Vollenweider and colleagues have carried out several additional clinical studies, discussed later, of various aspects of the action of psilocybin and have shown that ketanserin can block most of those effects.

Kometer et al. (2012) carried out a randomized, double-blind study in 17 healthy human subjects. On 4 separate days, subjects received placebo, psilocybin (215 μg/kg), the 5-HT_{2A} antagonist ketanserin (50 mg, p.o.), or psilocybin plus ketanserin. Mood states were assessed, and behavioral and event-related potential measurements were used to quantify facial emotional recognition and goal-directed behavior toward emotional cues. Psilocybin was found to enhance positive mood and attenuate negative facial expression recognition. Furthermore, psilocybin increased goal-directed behavior toward positive compared with negative cues, facilitated positive but inhibited negative sequential emotional effects, and valence-dependently attenuated the P300 component. Ketanserin given alone had no effect but blocked the psilocybin-induced mood enhancement and decreased recognition of negative facial expression. This study demonstrated that psilocybin shifts the emotional bias across various psychologic domains and that activation of 5-HT_{2A} receptors is central in mood regulation and emotional face recognition in healthy subjects. The authors suggest that their findings have implications not only for the pathophysiology of dysfunctional emotional biases, but they may also provide a framework to delineate the mechanisms underlying psilocybin's putative antidepressant effects.

Quednow et al. (2012) investigated the role of 5-HT_{2A} receptors in automatic (sensorimotor gating) and controlled (Stroop interference) inhibition processes in a model psychosis approach using psilocybin (260 μg/kg) in 16 healthy humans pretreated either with the 5-HT_{2A}–selective receptor antagonist ketanserin (40 mg) or placebo, using a placebo-controlled, crossover, counterbalanced, and double-blind design. They found that psilocybin-induced deficits in automatic and

controlled inhibition were significantly attenuated by ketanserin. They also replicated their previous findings that most of the subjective hallucinogenic effects of psilocybin were abolished by ketanserin.

Kometer et al. (2013) assessed the effects of psilocybin (215 μg/kg) on both α oscillations that regulate cortical excitability and early visual evoked P1 and N170 potentials in 16 healthy human subjects. They employed a double-blind, placebo-controlled, within-subject, randomized design. Psilocybin generally significantly increased 5D-ASC scores after placebo pretreatment, but not after ketanserin pretreatment. Psilocybin strongly decreased both prestimulus parieto-occipatal α power and decreased N170 potentials associated with the appearance of visual perceptual alterations, including visual hallucinations. Preadministration of the 5-HT_{2A} antagonist ketanserin (50 mg) blocked all of these effects. The authors conclude that 5-HT_{2A} receptor activation by psilocybin profoundly modulates the neurophysiological and phenomenological indices of visual processing. They further propose that 5-HT_{2A} receptor activation may induce a processing mode in which stimulus-driven cortical excitation is overwhelmed by spontaneous neuronal excitation through modulation of α oscillations.

Indirect evidence for a role of 5-HT_{2A} receptors in mediating psychedelic-induced hallucinations comes from a study by Huot et al. (2010). In that study, [^{3}H]ketanserin binding was used to compare 5-HT_{2A} receptor density in postmortem brains of patients with Parkinson's disease (PD) who experienced visual hallucinations with the brains of PD patients who did not experience hallucinations. Six brains from patients with idiopathic PD who experienced visual hallucinations were compared with six PD patients without visual hallucinations and five healthy, age-matched controls. In PD patients with visual hallucinations, [^{3}H]ketanserin binding was increased 45.6% in inferolateral temporal cortex compared with PD patients who did not have visual hallucinations. The authors suggest that increased 5-HT_{2A} density in the inferolateral temporal cortex may be the basis for visual hallucinations in PD patients and that 5HT_{2A} antagonists may alleviate this symptom.

Similarly, Ballanger et al. (2010) measured 5-HT_{2A} binding in vivo using [^{18}F]setoperone PET in brains of seven PD patients with visual hallucinations and seven age-matched PD patients without visual hallucinations. Patients with visual hallucinations had significantly increased 5-HT_{2A} receptor binding in several cortical regions and one subcortical region. These increased levels of 5-HT_{2A} receptor expression were clustered mainly in the ventral visual pathway. With the evidence of increased 5-HT_{2A} receptor expression as a possible basis for visual hallucinations in PD patients, a serotonin 5-HT_{2A} inverse agonist, perhaps not surprisingly, demonstrated phase 3 clinical efficacy in treating several symptoms of PD psychosis, including visual hallucinations (Hacksell et al., 2014).

Halberstadt et al. (2011a) examined the effects of several psychedelics on the mouse head twitch response (HTR) in wild-type (WT) male C57BL/6J or 5-HT_{2A} knockout (KO) mice. They also assessed investigatory and locomotor activity in the mouse behavioral pattern monitor (BPM). Psilocin and 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT) produced the HTR in WT mice but not in KO mice. Psilocin and 5-MeO-DMT reduced locomotor activity, investigatory behavior, and center duration in the BPM, and these effects were blocked by the selective 5-HT_{1A} antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide), indicating that psilocin and 5-MeO-DMT act as mixed 5-HT_{1A}/5-HT_{2A} agonists. Halberstadt and Geyer (2011) reviewed the extensive literature covering various indoleamines as well as LSD and concluded that although the phenethylamines primarily exert their effects through activation of 5-HT_{2A} receptors, indoleamines can have a significant behavioral component mediated by activation of 5-HT_{1A} receptors.

Quednow et al. (2010), using [^{18}F]altanserin PET, found that the intensity of psilocybin-induced subjective effects measured using the 5D-ASC was directly correlated with the level of 5-HT_{2A} receptor occupation by psilocin in the ACC and medial prefrontal cortex (mPFC).

B. Production of Tolerance

Repeated administration of psychedelics leads to a very rapid development of tolerance known as tachyphylaxis, a phenomenon believed to result from 5-HT_{2A} receptor downregulation. Daily administration of LSD leads essentially to complete loss of sensitivity to the effects of the drug by day 4 (Cholden et al., 1955; Belleville et al., 1956). Likewise, in humans, daily administration of the hallucinogenic amphetamine 2,5-dimethoxy-4-methylamphetamine (DOM) also led to significant tolerance to the drug effect by day 3 (Angrist et al., 1974). In humans, cross-tolerance occurs between mescaline and LSD (Balestrieri and Fontanari, 1959) and between psilocybin and LSD (Isbell et al., 1961). Tolerance and cross-tolerance to hallucinogens also develops in animal models (Freedman et al., 1958; Smythies et al., 1966; Appel and Freedman, 1968; Winter, 1971; Freedman and Boggan, 1974; Wallach et al., 1974; Trulson et al., 1977; Commissaris et al., 1980).

Several studies have shown that rapid tolerance to psychedelics correlates with downregulation of 5-HT_{2A} receptors. For example, daily LSD administration selectively decreased 5-HT_2 receptor density in the rat brain (Buckholtz et al., 1985, 1990). Not only LSD, but the hallucinogenic amphetamines DOB and 2,5-dimethoxy-4-iodoamphetamine (DOI) also produced 5-HT_2 receptor

downregulation after repeated dosing in rats (Buckholtz et al., 1988). McKenna et al. (1989) also reported that chronic treatment of rats with DOI led to downregulation of brain 5-HT_2 receptors. The structures of DOM, DOB, and DOI are shown in Fig. 4.

Repeated treatment of rats with DOM also led to rapid desensitization and downregulation of central 5-HT_2 receptors (Leysen et al., 1989), with a significant reduction in the total number of cortical 5-HT_2 sites after only two drug treatments (2.5 mg/kg s.c. every 8 hours). Smith et al. (1999) similarly found that tolerance to the discriminative stimulus effect of DOI in rats correlated with downregulation of 5-HT_{2A} receptors, but they observed no change in density of 5-HT_{2C} receptors. Short agonist treatment also led to desensitization of 5-HT_{2A} receptor–mediated phosphoinositide (PI) hydrolysis in transfected cell lines (Ivins and Molinoff, 1991; Roth et al., 1995; Gray and Roth, 2001).

In contrast with most other G protein–coupled receptors (GPCRs), the 5-HT_{2A} receptor undergoes downregulation in response either to agonist or antagonist treatment (Gray and Roth, 2001). Two nonconserved residues in the 5-HT_{2A} receptor, S421 in the C terminus, and S188 in intracellular loop 2, have been reported to be essential for agonist-induced desensitization in cloned receptors expressed in human embryonic kidney 293 (HEK-293) cells (Gray et al., 2003). When either residue was mutated to alanine, 5-HT–induced desensitization was markedly attenuated.

Damjanoska et al. (2004) examined levels of $G_{\alpha q}$, $G_{\alpha 11}$, RGS4, and RGS7 proteins after chronic *R*-(−)-DOI treatment of rats. They used comparison of 5-HT versus guanosine 5′-3-*O*-(thio)triphosphate (GTPγS)–stimulated phospholipase C (PLC) activity (inositol-1,4,5-triphosphate production) and DOI-induced increases in plasma levels of adrenocorticotrophic hormone, corticosterone, and oxytocin. Rats were injected with *R*-(−)-DOI (1 mg/kg, i.p.) for 1, 4, and 7 days, or saline. 5-HT–stimulated PLC activity decreased 24% after 4 days and 30% after 7 days of DOI treatment. DOI treatment did not affect GTPγS-stimulated PLC activity in the frontal cortex. DOI treatment for 1 or 54 days did not alter Gαq; however, treatment for 7 days decreased Gαq by 47%, although levels of Gα11 were not changed. RGS4 and RGS7 levels did not change. Basal plasma adrenocorticotrophic hormone, corticosterone, and oxytocin levels were not significantly altered after any time of DOI treatment, but daily DOI for 4 days significantly reduced the response to a DOI challenge. Data suggest that desensitization of 5-HT_{2A} receptor signaling is not due to reduced ability of $G_{\alpha q/11}$ proteins to stimulate PLC but rather is due to a change in 5-HT_{2A} receptors or their coupling to G proteins. The desensitization of 5-HT_{2A} receptors is most likely attributable to post-translational modifications of the receptor (e.g., phosphorylation), $G_{\alpha q}$ or $G_{\alpha 11}$ proteins, altering the 5-HT_{2A} receptor to G protein interface.

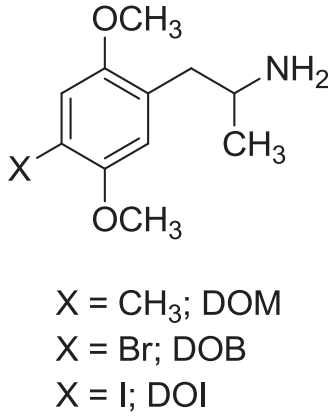


Fig. 4. Chemical structures of DOM, DOB, and DOI.

Shi et al. (2008) examined agonist-induced desensitization of 5-HT_{2A} receptors in the rat hypothalamic paraventricular nucleus (PVN) with 1 mg/kg *R*-(−)-DOI. Treatment with *R*-(−)-DOI for either 4 or 7 days caused a significant approximately 50% decrease in high-affinity 5-HT_{2A} binding, compared with saline-treated controls. Oxytocin and acetylcholine (ACh) release induced by 5-HT_{2A} receptor activation were also suppressed in a dose-dependent manner after treatment with DOI. By contrast, Western blot analysis revealed a significant increase in receptor protein. Taken together, the decreased high-affinity binding and decreased hormone release, but increased receptor protein, suggested a functional uncoupling of the 5-HT_{2A} receptors after chronic treatment with DOI.

LSD and DOB induce a ketanserin-sensitive increase in shaking behavior in mice and rats, which includes both head twitches and wet dog shakes. Substantial evidence suggests that shaking behavior primarily results from metabotropic glutamate mGlu2/3-sensitive glutamate release downstream of frontocortical 5-HT_{2A} activation (this review). Buchborn et al. (2015) investigated whether behavioral tolerance to LSD and DOB resulted from adaptations of 5-HT_{2A} and mGlu2/3 signaling, or of 5-HT_{2A} and/or overall-glutamate binding sites in the frontal cortex. Male Sprague-Dawley rats were administered LSD tartrate (0.025 mg/kg, i.p.) or DOB HCl (0.25 mg/kg, i.p.) seven times over 4 consecutive days (a low dose every morning and a high dose on the evening of days 1–3). Immediately after agonist administration, the occurrence of body shaking behavior was monitored for 30 minutes. Twenty-four hours after the last treatment, rats were decapitated and the frontal cortices were dissected and homogenized. For binding studies, aliquots containing 175–200 μg protein were incubated at 37°C with either [^{3}H]spiroperidol (0.25 nM, 30 minutes) or [^{3}H]glutamate (50 nM, 40 minutes). For measurement of 5-HT_{2A} and mGlu2/3 coupling to G proteins, crude synaptic membrane pellets were resuspended and aliquots containing 15–20 μg protein were incubated with 3 μM GDP and 0.05 nM [^{35}S]GTPγS.

Both LSD (0.025 mg/kg, i.p.) and DOB (0.25 and 0.5 mg/kg, i.p.) induced significant shaking behavior,

with LSD being about 10-fold more potent than DOB, although the maximal effect of LSD was much lower. LSD induced more wet dog shakes than head twitches, but the pattern was reversed for DOB. Ketanserin, a 5-HT_{2A}–selective antagonist, (0.5 or 1.0 mg/kg, i.p., 30 minutes before agonist), blocked the overall shaking behavior of both psychedelics. Both LSD and DOB shaking behavior significantly decreased over the time course of the experiment (Buchborn et al., 2015).

Repeated DOB treatment significantly reduced DOB-sensitive [^{3}H]spiroperidol binding to membranes of the frontal cortex, but glutamate-sensitive [^{3}H]glutamate binding remained unaffected. By contrast, repeated LSD treatment significantly reduced frontocortical [^{3}H]glutamate binding, but there was only a nonsignificant trend for reduction of [^{3}H]spiroperidol binding. After repeated DOB, but not LSD, treatment, there was a significant decrease in DOB-induced [^{35}S]GTPγS binding to frontocortical membranes. LY354740 [(1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid], a mGlu2/3 agonist, induced [^{35}S]GTPγS binding that was significantly reduced after repeated treatment with either DOB or LSD. For DOB-tolerant animals, both 5-HT_{2A} and [^{3}H]glutamate binding were highly correlated with the number of shaking behaviors observed on the last day of repeated DOB treatment, whereas only [^{3}H]glutamate binding correlated with shaking behavior for LSD-tolerant animals.

Buchborn et al. (2015) concluded that the differential receptor adaptations observed for DOB and LSD, respectively, indicate that tolerance to serotonergic hallucinogens can arise at two levels. That is, if a psychedelic (e.g., LSD) for some reason fails to downregulate 5-HT_{2A} receptors, glutamate receptors might adapt instead and thus prevent cortical overstimulation.

Moreno et al. (2013) investigated the effects of chronic treatment with the mGlu2/3 receptor inverse antagonist LY341495 [2-[(1*S*,2*S*)-2-carboxycyclopropyl]-3-(9*H*-xanthen-9-yl)-D-alanine] on the 5-HT_{2A} receptor–dependent cellular and behavioral responses induced in mice by LSD. Male 129S6/SvEv mice or mGlu2-KO mice were injected daily for 21 days with the mGlu2/3 inverse agonist LY341495 (1.5 mg/kg, i.p.) or vehicle. Mice were euthanized by cervical dislocation 1 day after the last injection of LY341495 and changes in [^{3}H]ketanserin and [^{3}H]LY341495 binding were assessed in the bilateral somatosensory cortex. The head-twitch behavioral response was also measured 1 day after the last injection with chronic LY341495. Induction of *c-fos*, *egr-1*, and *egr-2* by LSD (0.24 mg/kg) was measured 1 day after the last injection with chronic LY341495.

Analysis of the maximum number of binding sites (B_{max}) by Moreno et al. (2013) revealed a lower density of mGlu2/3 receptors in the somatosensory cortex from mice chronically treated with LY341495. The number of [^{3}H]ketanserin binding sites was also decreased in WT mice, but not in mGlu2-KO mice; there was no effect on K_D. The head-twitch behavior induced by LSD was also decreased in mice chronically treated with LY341495. A single dose of LSD induced expression of *c-fos* in mice chronically treated with saline, but not in mice chronically treated with LY341495. A single dose of LSD induced expression of *egr-1* in the somatosensory cortex of mice chronically treated with saline, but not in mice chronically treated with LY341495. A single dose of LSD also induced *egr-2* expression in both saline-injected mice and in mice chronically treated with LY341495, but *egr-2* expression induced by LSD was lower in mice chronically treated with LY341495 compared with that observed in mice chronically treated with vehicle.

This study by Moreno et al. (2013) demonstrates that chronic treatment with the mGlu2/3 receptor inverse agonist LY341495 downregulates 5-HT_{2A} receptor binding in the mouse somatosensory cortex through a mechanism that requires expression of the mGlu2 receptor, because this effect was not observed in mGlu2 KO mice. The lower density of 5-HT_{2A} receptors correlates with changes in hallucinogen-like behavioral and cellular responses that require expression of the 5-HT_{2A} receptor in the mouse brain cortex. Thus, chronic treatment with LY341495 decreases LSD-dependent head-twitch behavior as well as LSD-dependent induction of expression of *c-fos*, *egr-1*, and *egr-2* in the mouse somatosensory cortex. In conclusion, data from Moreno et al. (2013) support the hypothesis that chronic blockade of mGlu2 receptor–dependent signaling downregulates 5-HT_{2A} receptor binding in the mouse somatosensory cortex and its hallucinogen-like cellular signaling and behavioral effects. Given that mGlu2 receptors are located presynaptically, their blockade would lead to excessive glutamate release, potentially resulting in feedback downregulation of 5-HT_{2A} receptors expressed on the pyramidal apical dendrites.

C. Functional Selectivity at the Serotonin 5-Hydroxytryptamine 2A Receptor

Although there is very strong evidence that psychedelics act by an agonist or partial agonist action at 5-HT_{2A} receptor, the past 15 years has seen increasing awareness of the fact that GPCRs can and do couple to more than one intracellular signaling pathway. That is, although the canonical signaling pathway for the 5-HT_{2A} receptor is $G_{\alpha q}$ coupling and activation of PLC, it is now known that other signaling pathways can be activated. The relative activation of these different pathways is ligand dependent, has most often been referred to as "functional selectivity" (and also as ligand bias) (Urban et al., 2007), and has recently been reviewed (see Seifert, 2013; Zhou and Bohn, 2014).

One can readily envision that when an endogenous neurotransmitter (e.g., serotonin) binds within the orthosteric site of one of its receptors, the receptor protein will collapse around the ligand to generate a

transient and distinct ligand-receptor ensemble. That ensemble will result in conformational changes on the intracellular face of the receptor that lead to complementary association with a subset of available cellular signaling molecules. For example, the ethylamine side chain of serotonin is relatively flexible, and the receptor and the ligand will "adapt" to each other through complementary steric, electronic, and conformational changes in both the ligand and the receptor to generate a transient and specific ligand-receptor ensemble. By contrast, LSD is a synthetic conformationally rigid tetracyclic agonist molecule. When it binds to the same receptor, LSD and the receptor will again "adapt" to each other through complementary steric, electronic, and conformational changes. With LSD, however, because of the differences in the overall molecular structures of serotonin and LSD, and the flexibility of serotonin versus the conformational rigidity of LSD, the LSD-receptor ensemble will differ from the one formed when serotonin binds to the receptor. One can easily imagine that each and every structural change made in a series of agonist molecules might lead to distinct ligand-receptor complexes (i.e., a ligand-dependent state) and that these different complexes may lead to activation of different subsets of intracellular signaling molecules. Thus, although functional selectivity has already been demonstrated for the 5-HT_{2A} receptor, it presently remains unknown which particular signaling pathway(s) may be most relevant for the actions of psychedelics. Therefore, when a molecule is classified as a 5-HT_{2A} agonist, what exactly does that mean in terms of cellular responses? Furthermore, how will different proportions of intracellular signaling events affect the qualitative aspects of a "psychedelic" intoxication? It will take a great deal more research before these questions can be answered.

The most well understood and recognized signaling pathway mediated by the 5-HT_{2A} receptor is coupling to $G_{\alpha q}$, resulting in stimulation of PI-specific PLC (Conn and Sanders-Bush, 1984; Roth et al., 1984). This enzyme hydrolyzes phosphatidylinositol membrane lipids at the *sn*-3 position, generating inositol-1,4,5-triphosphate and diacylglycerol (Conn and Sanders-Bush, 1986; Williams, 1999). The inositol phosphates lead to release of Ca^{+2} from intracellular stores and diacylglycerol remains bound to the membrane and activates protein kinase C (PKC).

For a long time, it was assumed that PI hydrolysis signaling was most relevant for the action of psychedelics, but this hypothesis has certain problems. To begin with, LSD has very low efficacy in activating PI turnover (Sanders-Bush et al., 1988; Egan et al., 1998). In addition, Rabin et al. (2002) noted a lack of correlation between the behavioral potency in drug substitution in rats trained to discriminate LSD or DOM from saline, and efficacy in stimulating PI hydrolysis. They concluded that 5-HT_{2A}–mediated stimulation of PI hydrolysis did not appear to be the critical signaling mechanism involved in the discriminative stimulus effects of hallucinogens. Similarly, Roth et al. (1997a) found no significant relationship between high-affinity agonist binding and ability to stimulate PI turnover, and they proposed that additional transition states of the receptor-ligand complex must be essential for agonist efficacy.

Activation of 5-HT_{2A} receptors also leads to stimulation of phospholipase A_2 (PLA_2), which preferentially hydrolyzes arachidonic acid (AA)–containing phospholipids at the *sn*-2 position to produce free AA and lysophospholipid. This PLA_2 pathway is independent of PLC-mediated signaling (Berg et al., 1998; Kurrasch-Orbaugh et al., 2003b) and has been demonstrated in hippocampal slices (Felder et al., 1990) and cellular systems (Berg et al., 1994; Tournois et al., 1998). The PLA_2 signaling pathway is more complex than the PI turnover cascade, apparently involving multiple G proteins and the extracellular signal-regulated kinase (ERK) 1/2 and p38 mitogen-activated protein kinases (MAPKs), at least in NIH3T3 cells (Kurrasch-Orbaugh et al., 2003a).

Although the significance of this pathway has not been investigated in detail, Qu et al. (2003) found that administration of 2.5 mg/kg DOI to rats led to significantly increased incorporation of [^{3}H]AA in brain membranes. Quantitative autoradiography revealed large increases in [^{3}H]-labeled AA incorporation, particularly in the neocortex. The largest increases were seen in brain regions having high densities of 5-HT_{2A} receptors, compared with 5-HT_{2C} sites.

It is clear that specific ligands interact with the 5-HT_{2A} receptor to activate the PLC and PLA_2 pathways to different extents. At the rat 5-HT_{2A} receptor expressed in NIH3T3 cells, for example, LSD, DOB, psilocin, and 5-MeO-DMT have different EC_{50} values and intrinsic activities in activating the AA and PI turnover pathways (Kurrasch-Orbaugh et al., 2003b). In that study, nearly all of the compounds studied had greater potency in inducing AA release than in stimulating PI turnover. Some of these differences are noteworthy; for example, in psilocin, the potency for activating AA release was nearly 30-fold greater than for stimulating PI turnover.

Moya et al. (2007) compared two homologous series of substituted psychedelic phenethylamines and phenylisopropylamines for signaling at the 5-HT_{2A} receptor through PLC and PLA_2 responses. They employed Chinese hamster ovary (CHO)-FA4 cells stably expressing the human 5-HT_{2A} receptor that had similar maximal responses for inositol phosphate (IP) accumulation and AA release in response to serotonin. Relative efficacies for AA release and IP accumulation varied within the series, and the substituted amphetamines had higher efficacy in both pathways. The amphetamines also produced a more robust in vivo rat HTR, mediated through the 5-HT_{2A} receptor.

The kinases participating in 5-HT_{2A} receptor phosphorylation had proven elusive, but Sheffler et al. (2006) first identified RSK2, one of the p90 ribosomal S6 kinases (RSKs), which is a member of the ERK/MAPK cascade. They reported that RSK2 has a regulatory function on GPCR signaling by exerting a "tonic brake" on second messenger production. RSK2 was coimmunoprecipitated with the FLAG–5-HT_{2A} receptor transiently expressed in HEK-293 cells, indicating an in vitro association. Sheffler et al. (2006) showed that RSK2 interacts with intracellular loop 3 (i3) of the 5-HT_{2A} receptor, within a conserved region containing an RSK2-like consensus phosphorylation motif (^{275}RAKLSD^{280}). A number of comparison studies were carried out with fibroblasts derived from $RSK2^{+/+}$ and $RSK2^{-/-}$ mice. In both types of fibroblasts, 5-HT induced increased PI accumulation that was abolished by the specific 5-HT_{2A} antagonist M100907. In $RSK2^{-/-}$ fibroblasts, however, PI hydrolysis was enhanced relative to $RSK2^{+/+}$ fibroblasts. Controls were run to determine that there were no global changes in gene expression in the absence of RSK2 and that the augmented signaling in $RSK^{-/-}$ fibroblasts was not due to changes in expression of genes that regulate GPCR second messenger production. The authors also demonstrated that basal and 5-HT–stimulated ERK1/2 phosphorylation was increased in $RSK2^{-/-}$ compared with $RSK2^{+/+}$ fibroblasts. Their results showed that RSK2 exerts a tonic brake on 5-HT_{2A} receptor signaling. The absence of RSK2 led to an increase in agonist efficacy, measured as PI hydrolysis, without a change in agonist potency. Their findings implied that RSK2 acts proximal to activation of the receptor, at the level of receptor–G protein coupling, perhaps by phosphorylation of the receptor.

In a follow-up study, Strachan et al. (2009) carried out a more detailed examination of RSK2 and its relationship to 5-HT_{2A} receptor signaling, finding that RSK2 phosphorylates a conserved Ser314 within i3. In this study, they reported that RSK2 negatively regulates signaling in fibroblasts not only during the initial 0- to 90-second phase of signaling, but during the 0- to 60-minute extended periods of signaling. Again, using mouse $RSK2^{-/-}$ fibroblasts, they ectopically expressed WT RSK2, N-terminal kinase-dead RSK2 (K100A), or C-terminal kinase-dead RSK2 (K451A) constructs. They determined that $RSK2^{-/-}$ fibroblasts transfected with WT RSK2 and RSK2-K451A, but not with the RSK2-K100A mutant, expressed amounts of RSK2 similar to $RSK2^{+/+}$ fibroblasts. $RSK2^{-/-}$ fibroblasts expressing WT RSK, but not kinase-dead RSK2 (K451A) had attenuated agonist-mediated 5-HT_{2A} signaling levels comparable to $RSK2^{+/+}$ fibroblasts, indicating that RSK2 kinase activity was required to regulate 5-HT_{2A} receptor signaling. Incubation of purified 5-HT_{2A} receptors with activated RSK2 and [γ^{32}P] ATP led to robust [^{32}P] incorporation into the receptor, and the selective RSK inhibitor SL0101 (3-[(3,4-di-*O*-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]-5,7-dihydro-2-(4-hydroxyphenyl)-4*H*-1benzopyran-4-one) blocked this phosphorylation. These data supported the conclusion that activated and purified RSK2 phosphorylates the 5-HT_{2A} receptor in vitro.

Using a peptide composed of the i3 loop amino acids 252–328, purified and activated RSK2, and [γ^{32}P]ATP, Strachan et al. (2009) were able to demonstrate robust [^{32}P] incorporation into the purified i3 loop protein. The residue within i3 that was phosphorylated was then identified from among the 18 potential Ser/Thr kinase phosphorylation sites using trypsin digestion and tandem mass spectrometry. A major phosphorylation site was identified as Ser314, with a minor site at Ser280. The investigators then created S280A and S314A mutants, rendering these sites phosphorylation deficient. Activated RSK2 incorporated [^{32}P] into both WT and the S280A mutants to a similar extent, but the S314A mutation completely abolished RSK2 phosphorylation compared with the WT peptide. These results thus confirmed S314 as the phosphorylation site within i3 of the 5-HT_{2A} receptor. This assignment was verified using a phosphospecific antibody against phospho-Ser314. These studies were all carried out with the synthetic i3 peptide; the next experiments focused on the intact full-length 5-HT_{2A} receptor, where it was confirmed that Ser314 was indeed phosphorylated. Strachan et al. (2009) then immunopurified WT and 5-HT_{2A}–S314A receptors from $^{32}P_i$-labeled fibroblasts after RSK2 activation by epidermal growth factor (EGF). EGF time-dependently activated RSK2 in both types of fibroblasts. However, although phosphorylation was detected in the WT 5-HT_{2A} fibroblasts, no phosphorylation could be detected in the 5-HT_{2A}–S314A fibroblasts, showing that activated RSK2 phosphorylates Ser314 not only in vitro but also in intact cells. Finally, the authors compared the IP accumulation and intracellular Ca^{2+} release signaling pathways in both WT and 5-HT_{2A}–S314A expressed in intact cells. Three 5-HT_{2A} receptor agonists [5-HT, DOI, and α-methylserotonin (AMS)] all showed increased efficacy and potency in phosphorylation-deficient 5-HT_{2A}–S314A fibroblasts, compared with WT receptors, both in mobilizing significantly more Ca^{2+} and in IP accumulation. Taken together, these studies show that removing the phosphorylation site at Ser314 in the 5-HT_{2A} receptor i3 loop renders it resistant to negative regulation by RSK2, indicating that Ser314 phosphorylation possibly uncouples the 5-HT_{2A} receptor from its cognate G protein. Strachan et al. (2009) also showed that RSK2 was necessary for EGF-mediated heterologous desensitization of the 5-HT_{2A} receptor, showing for the first time that a growth factor can heterologously desensitize 5-HT_{2A} receptor signaling.

In a third study, Strachan et al. (2010) carried out a focused screen to evaluate the effect of RSK2 expression

on the signaling of a set of chemically diverse 5-HT_{2A} receptor agonists using IP accumulation, Ca^{2+} release, and ERK1/2 phosphorylation as readouts. They found that genetic deletion of RSK2 significantly increased relative efficacies of agonists at multiple effector pathways. 5-HT, AMS, and DOI elicited significantly greater maximal increases in IP accumulation, Ca^{2+} release, and ERK1/2 phosphorylation in RSK2 KO mouse embryonic fibroblasts (MEFs). The relative efficacies of quipazine, 5-MeO-DMT, lisuride, and meta-chlorophenylpiperazine (m-CPP) were significantly increased at all three effector readouts in RSK2 KO MEFs. In contrast with effects on maximal signaling, relative agonist potencies were not potentiated, with few exceptions. The ratios of the E_{max} responses for RSK2 KO MEFs and WT MEFs differed for each agonist and response, with the greatest changes seen for IP accumulation and ERK1/2 phosphorylation. Strachan et al. (2010) compared responses in WT and KO MEFs for a variety of agonists and partial agonists, with a detailed discussion of the effects; overall, their data suggested that 5-HT_{2A} agonists were differentially responsive to RSK2 deletion. Their study provides the first evidence that a relatively minor change in the cellular kinome is sufficient to elicit profound alterations in relative agonist efficacy, and they demonstrate that patterns of functional selectivity can vary depending on the cellular milieu of the receptor studied.

In addition to RSK2, other kinases can affect 5-HT_{2A} signaling. For example, PKC phosphorylation of serine residue S291 in the 5-HT_{2A} receptor is also thought to play a role in modulation of receptor signaling. Using five different ligands that activate the 5-HT_{2A} receptor, Raote et al. (2013) showed that different biochemical pathways are involved in receptor trafficking. These investigators used the fluorescence of rat 5-HT_{2A} enhanced green fluorescent protein (EGFP) to follow receptor internalization. This transcript was stably transfected into HEK-293 cells to generate what the authors designated as an SB1 cell population. Serotonin, dopamine, DOI, and clozapine all produced robust internalization, whereas the 5-HT_{2A} antagonist ketanserin did not. PKC activation by a phorbol ester (PMA) was sufficient to cause endocytosis in the absence of any agonist. In an earlier study, the authors had shown that PKC activation was required for internalization by 5-HT, but not by dopamine. DOI, but not clozapine, also required PKC to cause internalization. Sphingosine, an inhibitor of PKC activation, almost completely prevented 5-HT– or DOI–mediated receptor internalization but did not affect dopamine-mediated internalization. The inverse agonist clozapine caused receptor internalization independent of PKC activation. Serine residue 291 in the 5-HT_{2A} receptor was identified as a possible PKC phosphorylation site, which when mutated to an alanine (5-HT_{2A}–S291A–EGFP) completely blocked internalization in response to 5-HT and DOI, but internalization by dopamine and clozapine was not affected, with behavior like the WT receptor. Because 5-HT_{2A} receptor internalization can be induced by PKC activation in the absence of ligand, the investigators hypothesized that the PKC-phosphorylation–deficient 5-HT_{2A}–S291A mutant would be insensitive to PMA-induced internalization. Consistent with their hypothesis, no internalization could be detected after PKC activation by 4 nM PMA. The recycling time for the 5-HT_{2A} receptor after treatment with the different agonists was also shown to be ligand dependent, in which 5-HT or dopamine receptors internalized and were completely recycled after 2.5 hours, whereas recycling of DOI-internalized receptors was only complete in 7.5 hours. In addition, receptors that required PKC for internalization also required protein phosphatase 2A, a pH-sensitive, endosome-localized enzyme that may play a role in receptor recycling internalized in a phosphorylation-dependent manner.

Cussac et al. (2008) reported differential agonist action for a series of serotonergic ligands, including LSD and DOI, and using CHO cells stably expressing the human 5-HT_{2A} receptor. [They also used cells transfected with the human 5-HT_{2B} and 5-HT_{2C} (VSV isoform) receptors, obtaining generally similar results, but the discussion here will focus on their work with the 5-HT_{2A} receptor.] They measured specific activation of $G_{q/11}$ proteins using a scintillation proximity assay and used a fluorescent imaging plate reader assay to measure intracellular Ca^{2+} responses. Serotonin and 5-carboxytryptamine gave a 20- to 50-fold greater potency for Ca^{2+} release than measured for $G_{q/11}$ activation, whereas DOI showed only a modest 2-to 3-fold preference for Ca^{2+} release. As anticipated, M100907 potently blocked serotonin-stimulated $G_{q/11}$ proteins. LSD showed a 20-fold higher potency to stimulate $G_{q/11}$ than to induce Ca^{2+} release. The most striking separation between activities was for the nonhallucinogenic 5-HT_{2A} agonist lisuride, which was as potent as DOI in stimulating $G_{q/11}$, more than 1000-fold more potent than at Ca^{2+} release, and was a partial agonist for the two pathways. Interestingly, Ca^{2+} mobilization is classically considered to be a downstream consequence of $G_{q/11}$ activation and subsequent PLC stimulation. Yet the results presented here suggest that $G_{q/11}$ signaling may not be the only determinant of Ca^{2+} signaling. The main result of this study, however, was the ability of different agonists to differentially activate two signaling pathways in the same cell type.

β-Arrestins are intracellular scaffolding proteins that can attenuate or facilitate GPCR signaling, and represent another potential signaling path that may depend for their activation on specific ligands. Schmid et al. (2008) tested whether 5-HT_{2A} receptor regulation by β-arrestins contributes to serotonergic responsiveness in vivo by comparing WT mice with mice that lack β-arrestin-2. L-5-Hydroxytryptophan (5-HTP) produced

the HTR in WT mice but gave a greatly attenuated response in β-arrestin-2 KO mice. DOI, however, produced an HTR of equal magnitude in both genotypes, indicating that β-arrestin-2 mediates the 5-HTP–induced HTR, whereas the HTR produced by DOI is β-arrestin-2 independent. In addition, the investigators used transfected MEFs derived from β-arrestin-1 and β-arrestin-2-KO embryos. Culturing WT MEFs transfected with a yellow fluorescent protein (YFP) C-terminally tagged 5-HT_{2A} receptor in complete media (containing 10% fetal bovine serum) revealed that the majority of the 5-HT_{2A}–YFP was internalized in WT MEFs. This expression could be reversed by removing the serum from the media for a 2-hour incubation, indicating that serotonin in the media was probably causing receptor internalization. Consistent with this reasoning, adding serotonin to serum-free media for the culturing resulted in 5-HT_{2A}–YFP internalization in WT MEFs. The β-arrestin-1 KO and β-arrestin-2 KO MEFs both retained 5-HT_{2A}–YFP surface expression, even in media containing serum; adding serotonin to the media did not lead to internalization. Yet DOI produced 5-HT_{2A}–YFP internalization not only in WT MEFs but also in β-arrestin-1 KO and β-arrestin-2 KO MEFs. These findings demonstrate that DOI-induced 5-HT_{2A} receptor internalization is β-arrestin independent, whereas serotonin-mediated internalization requires β-arrestins.

Examining ERK1/2 signaling, Schmid et al. (2008) reported that serotonin induced robust ERK1/2 phosphorylation in β-arrestin KO cells, which was significantly greater than that produced by DOI. Both serotonin and DOI induced ERK1/2 phosphorylation in KO cells, but the degree of stimulation was much lower than in WT cells. Inhibition of PLC by U73122 (1-[6-[[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino] hexyl]-1*H*-pyrrole-2,5-dione) blocked approximately one-third of serotonin-mediated activation of ERK1/2 in WT cells in the canonical pathway but completely blocked DOI-induced activation. In β-arrestin KO MEFs, U73122 completely prevented ERK1/2 phosphorylation induced by either serotonin or DOI. Thus, when the 5-HT_{2A} receptor is expressed in MEF cells, DOI stimulates ERK1/2 primarily through a PLC-dependent pathway, whereas serotonin activates ERK1/2 predominantly through a β-arrestin–dependent pathway.

To examine in vivo signaling, Schmid et al. (2008) treated mice with either 5-HTP or DOI, and the frontal cortices were dissected 15 minutes after drug treatment, when behavioral responses were maximal. Serotonin induced ERK1/2 phosphorylation in WT mice but not in β-arrestin-2 KO mice, whereas DOI led to significant ERK1/2 activation in both genotypes. These results demonstrate that β-arrestin-2 is necessary for serotonin-induced ERK1/2 phosphorylation in the frontal cortex, but that DOI can activate ERK1/2 independent of β-arrestin. These data emphasize that the nature of the ligand determines the receptor signaling pathway activated, which will determine the nature of the ultimate physiologic response produced by that ligand.

Schmid and Bohn (2010) demonstrated in vivo functional selectivity at the 5-HT_{2A} receptor by serotonin and *N*-methyltryptamines, as well as in the mouse frontal cortex and in primary cortical neurons, where they showed that the actions of these neurotransmitters were functionally distinct. In their earlier study, Schmid et al. (2008) showed that in mice lacking β-arrestin-2, an intraperitoneal dose of 100 mg/kg 5-HTP failed to induce the HTR in mice. When the dose of 5-HTP was doubled, however, the HTR in β-arrestin-2 KO mice approached that observed in WT mice. After intracerebroventricular injection, low doses of serotonin produced a robust HTR in mice that markedly exceeded those observed in β-arrestin-2 KO mice. In fact, with higher doses of serotonin, the HTR in β-arrestin-2 KO mice even exceeded the response in their WT littermates. The HTR induced by either serotonin or 5-HTP was blocked by M100907, which demonstrated its mediation through the 5-HT_{2A} receptor. Although serotonin is principally metabolized by MAO-A to afford the inactive metabolite 5-hydroxyindole acetic acid, at high concentrations of serotonin, metabolism can occur by *N*-methyltransferases to give *N*-methylserotonin and *N,N*-dimethylserotonin (bufotenin). To determine whether these metabolites might be formed and relevant, mice were pretreated with the MAO-A–elective inhibitor clorgyline. After clorgyline administration, the HTR dose-response curve was left-shifted and occurred in β-arrestin-2 KO mice at 5-HTP doses that were ineffective if given alone. Again, M100907 blocked these responses. When methimazole, an *N*-methyltransferase inhibitor was preadministered, the number of HTR responses after 5-HTP treatment was decreased in both WT and β-arrestin-2 KO mice, although the inhibitory effect was more marked in the β-arrestin-2 KO mice. The effects of clorgyline and methimazole pretreatment suggest that the HTR after 5-HTP treatment may be due to *N*-methyltryptamines instead of serotonin itself. Direct intracerebroventricular injection of *N*-methylserotonin gave a greater HTR in β-arrestin-2 KO mice than in their WT littermates. Systemic injection of 5-MeO-DMT gave a greater HTR in β-arrestin-2 KO mice than in their WT littermates. These results were interpreted to suggest that *N*-methyltryptamines do not require β-arrestin-2 for the HTR, and that β-arrestin-2 may be a negative regulator in this cascade because KO mice consistently display greater responses to *N*-methyltryptamines. Thus, the behavioral response to serotonin in β-arrestin-2 KO mice may fundamentally differ from that in WT mice.

Schmid and Bohn (2010) then assessed activation of the serine threonine kinase Akt in the frontal cortex of WT and β-arrestin-2 KO mice after treatment with 5-HTP (100 mg/kg) or 5-MeO-DMT (10 mg/kg). This

dose of 5-HTP produced the HTR only in WT mice, and the dose of 5-MeO-DMT induced the greatest HTR in both genotypes. Treating WT mice with 5-HTP led to phosphorylation of Akt at threonine 308, but no Akt phosphorylation was observed in KO mice. 5-MeO-DMT did not lead to Akt phosphorylation in either genotype, but there was no difference in Akt phosphorylation after vehicle treatment in either genotype. These findings suggest that serotonin and 5-MeO-DMT have different abilities to activate Akt, and that serotonin requires β-arrestin-2, whereas 5-MeO-DMT does not. The 5-HT_{2A} receptor was then immunoprecipitated from the frontal cortex of both WT and β-arrestin-2 KO mice after drug treatment. A dose of 5-HTP in WT that stimulates Akt phosphorylation in the cortex revealed a depletion of protein PSD-95 from the complex and recruitment of β-arrestin-2, Src, and Akt. In the cortex of β-arrestin-2 KO mice, however, no depletion of PSD-95 or recruitment of Src or Akt was observed in response to 5-HTP. After 5-MeO-DMT treatment, no recruitment of β-arrestin-2, Src, or Akt to the 5-HT_{2A} receptor occurred in either genotype. These results demonstrate that β-arrestin-2 is essential for mediating serotonin-induced assembly of the 5-HT_{2A}/Src/Akt complex, and that 5-MeO-DMT differs from serotonin by not recruiting this complex.

Schmid and Bohn (2010) followed up on this in vivo finding by studying Akt phosphorylation in primary neuronal cultures from the frontal cortex of WT and β-arrestin-2 KO neonates. Serotonin (1 μM) gave a robust phosphorylation of Akt in WT cortical neurons, but neither *N*-methylserotonin nor 5-MeO-DMT activated Akt. Pretreatment with M100907 blocked Akt phosphorylation in WT neurons after serotonin treatment, showing that the effects of serotonin were mediated through the 5-HT_{2A} receptor. None of the tested agonists led to Akt phosphorylation in neurons from β-arrestin-2 KO neonates. Inhibiting individual components of the signaling cascade in the neurons also prevented Akt phosphorylation. That is, pretreatment with the phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (2-morpholin-4-yl-8-phenylchromen-4-one) prevented serotonin-induced phosphorylation of Akt in WT neurons, as did treatment with the Src inhibitor PP2 [4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo[3,4-*d*]pyrimidine].

To disrupt the function of the signaling complex in vivo, the HTR in normal C57BL/6J mice was assessed after intracerebroventricular administration of inhibitors of PI3K, Src, and Akt prior to treatment with 5-HTP. Each of the three different inhibitors reduced the HTR by approximately 50%. The Akt inhibitor had no effect on the number of HTRs after 5-MeO-DMT treatment, however. By contrast, pretreatment of β-arrestin-2 KO mice with PI3K, Src, or Akt inhibitors prior to 5-HTP did not reduce the HTR, suggesting that the HTR in β-arrestin-2 mice is independent of these signaling components. Schmid and Bohn (2010) suggested that the HTR induced by high doses of 5-HTP or serotonin in WT mice could reflect activity of both serotonin and its *N*-methylated derivatives. Thus, when WT mice were pretreated with the Akt inhibitor together with an *N*-methyltransferase inhibitor prior to 5-HTP, the HTR was nearly abolished.

These results demonstrate functional selectivity at the 5-HT_{2A} receptor, in which serotonin and its *N*-methylated derivatives promote differential signaling in the mouse frontal cortex and in primary cortical neurons and thus have different mechanisms underlying manifestation of the HTR. That is, serotonin activation leads to a signaling complex that involves β-arrestin-2, Src, and Akt, whereas *N*-methylated derivatives produce the HTR through a signaling mechanism that is independent of β-arrestin-2 and does not require activation of Akt. Bohn and Schmid (2010) reviewed functional selectivity of the 5-HT_{2A} receptor and the role of arrestin as one of the signaling pathways.

Different agonist ligands acting at the 5-HT_{2A} receptor also can lead to different downstream gene expression patterns. González-Maeso et al. (2003) quantified concentration-dependent gene changes in response to various agonists in HEK-293 cells expressing the human 5-HT_{2A} receptor, followed by examination of the in vivo gene expression responses in the mouse somatosensory cortex. They identified 23 transcripts in these cells for which expression levels were regulated after application of 10 μM 5-HT. Concentration-response curves for gene induction by four distinct agonists in these cells showed that the agonists differed in their ability to activate different genes; the different cellular signaling patterns translated into unique transcriptome fingerprints. The psychedelics LSD and DOI induced the mouse HTR, whereas the nonhallucinogenic ergoline lisuride failed to induce the HTR. In 5-$HT_{2A}^{-/-}$ mice, neither LSD nor DOI produced the HTR, demonstrating that the HTR was mediated by the 5-HT_{2A} receptor.

González-Maeso et al. (2003) then compared effects on in vivo gene expression of three agonists evaluated in the HTR. Transcript levels in the mouse somatosensory cortex 1 hour after agonist injection were compared by quantitative reverse-transcription (RT) polymerase chain reaction (PCR) to those from vehicle-injected controls. Thirteen transcripts showed significant changes after at least one agonist, with each agonist showing a unique and reproducible transcriptome fingerprints. Of the genes identified, only early growth response protein 1 (*egr-1*), early growth response protein 2 (*egr-2*), and *period-1* were similarly activated by DOI and LSD but were unaffected by lisuride. Their data support the hypothesis that agonists acting at the same receptor in vivo can induce unique patterns of signaling, thereby eliciting different behavioral responses. Unique behavioral and signaling responses elicited by each agonist studied in vivo appear to result

from agonist-specific differences in the activation of multiple signaling pathways coupled to the 5-HT_{2A} receptor. Only the psychedelics DOI and LSD induced increased expression of *egr-1*, *egr-2*, and *period-1* transcripts and were activated by changes in cortical signaling that appear to be specific effects of the two psychedelics, which correlate with the generation of the mouse HTR.

Nichols and Sanders-Bush (2002) carried out the first unbiased microarray screen on the action of LSD in the rat brain by assessing the effects of intraperitoneal administration of 1.0 mg/kg LSD in the prefrontal cortex (PFC) 90 minutes after drug administration. Their first screen yielded a collection of five genes that were upregulated by LSD identified as serum glucocorticoid kinase (*sgk*), inhibitor of nuclear factor κB (*Iκβ-α*), neuron-derived orphan receptor 1 (*nor1*; *nr4a3*), *ania3*, and *krox-20*. RNase protection was used to validate these genes as differentially expressed, in the PFC, along with *Arc* and *c-fos*. *Krox-20* is also known as early growth response protein, or *egr-2*, and is the same gene identified by González-Maeso et al. (2003) whose expression is specifically increased in response to treatment with hallucinogenic 5-HT_{2A} agonists, as contrasted with a nonhallucinogenic 5-HT_{2A} agonist (i.e., lisuride). *Krox-20* (*egr-2*) has been shown to be necessary for normal brain development and may be involved in the maintenance of long-term potentiation (LTP) (see references in Nichols and Sanders-Bush, 2002).

Nichols et al. (2003) subsequently examined the time course, dose response, and sensitivity of the LSD response to 5-HT_{2A} and 5-HT_{1A} receptor antagonists. Most gene expression peaked at 90 minutes after drug administration and returned to baseline 3–5 hours after LSD treatment. The *nor1* gene, however, remained maximally elevated through the final 5-hour time point. At the 0.20-mg/kg dose of LSD, two genes (*krox-20*, *Iκβ-α*) were significantly upregulated, and most expression levels increased with successively higher (0.5–1.0 mg/kg) doses of drug. The transcriptional effects of LSD were unaffected by pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 but were significantly attenuated by the 5-HT_{2A} receptor–selective antagonist M100907 [(*R*)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperinemethanol], with the exception of *sgk* and *Iκβ-α*, which were unaffected by MDL100907. Thus, the majority of LSD-related gene expression alterations were induced through 5-HT_{2A} receptor activation, but other receptors contribute to its effects (Nichols et al., 2003).

Extending this work further, Nichols and Sanders-Bush (2004) performed a second microarray screen using a different Affymetrix gene chip version, identifying and validating three additional transcripts increased by 1.0 mg/kg LSD in the rat PFC: MAP kinase phosphatase 1 (*mkp1*), core/enhancer binding protein β (*C/EBP-β),* and the novel gene, induced by lysergic acid diethylamide 1 (*ilad1*; subsequently renamed arrestin domain containing 2 or *arrdc2*). As with the other LSD-induced differentially expressed genes, these also followed a dose- and time-dependent expression pattern. At the highest 1.0-mg/kg dose of LSD, expression of *mkp1*, *C/EBP-β*, and *ilad1* was only partially blocked by MDL100907, indicating that activation of multiple receptors probably contributes to the effects of LSD on gene expression at this dose. Indeed, LSD is a relatively nonselective serotonin and dopamine receptor ligand, with high to moderate affinity for a number of receptors that may contribute to its effects (Nichols, 2004).

The general functions of the genes induced by LSD are varied, and little is known for some genes mentioned above. A common theme linking the transcriptional changes, however, appears to be an effect on synaptic plasticity. For example, *Ania3* is a splice variant within the Homer1 gene family that encodes synaptic proteins, and *Ania3* has been implicated in metabotropic glutamate receptor (mGluR)–mediated plasticity. *C/EBP-β* is known to affect memory consolidation and synaptic strength; *Iκβ-α* inhibits nuclear factor κB, which is important in synapse regulation; *nor1* is a member of the *Nr4a* family of activity-dependent transcription factors, demonstrated to be important for transcription-dependent LTP in the hippocampus; and *sgk* plays a role in long-term memory and the expression of LTP in hippocampal neurons (see references in Nichols and Sanders-Bush, 2004). The way in which these genes contribute to downstream transcriptional, structural, and functional sequelae of neuronal activation, however, remains poorly understood.

Martí-Solano et al. (2015) compared relative signaling bias of several putative 5-HT_{2A} agonists in both the PI hydrolysis and AA release pathways. They relied on extensive molecular dynamics to generate binding poses for the ligands in the receptor and then considered how the ligand poses could potentially engage different residues within the receptor to explain observed signaling bias. Although in principle one could employ such an approach to identify structural determinants for functional selectivity, the molecular dynamics should be guided by published mutagenesis studies. Although some of the compounds studied by Martí-Solano et al. (2015) did have a high bias for PI signaling over AA release, the basis for their structural conclusions must be considered suspect because of significant flaws in their molecular modeling. For example, Braden and Nichols (2007) showed that TM5 serine $239^{(5.43)}$ in the 5-HT_{2A} receptor was critical for high affinity and potency of 4- or 5-oxygenated tryptamines, potentially serving as a hydrogen bond donor to the ligand, but Martí-Solano et al. (2015) essentially ignore its role in their molecular dynamics simulations. In addition, they (incorrectly) state that "S5.43 is able to establish indirect interactions with different serotonergic agonists," citing

Braden and Nichols (2007). What Braden and Nichols actually report was that the 5-HT_{2A}–S5.43A mutant receptor had markedly reduced affinity for 5-HT, 5-methoxytryptamine, and 5-MeO-DMT, consistent with the loss of a hydrogen bond (0.5–1.5 kcal/mol). Furthermore, the potency of 5-oxygenated tryptamines to activate the PI hydrolysis pathway was also significantly attenuated in the S5.43A mutant. Martí-Solano et al. (2015) point out that in their molecular dynamics–derived ligand binding mode S5.43 does not show direct contacts with serotonin, but rather indirect ones via N6.55. Other aspects of the binding modes they present also are unexpected, because F6.52 (known to engage the aromatic rings of 5-HT_{2A} agonists) does not engage the indole ring of serotonin or any of the other ligands they explored. Previous site-directed mutagenesis studies have identified both F6.51 and 6.52 to be within the 5-HT_{2A} agonist binding pocket (Choudhary et al., 1993, 1995; Roth et al., 1997b; Braden et al., 2006). It also is well known that the 2-methoxy of phenethylamines (e.g., 2,5-dimethoxy-4-nitrophenethylamine, one of the ligands studied) is crucial for activity, yet their molecular dynamics simulations and docking poses assign no evident role to a complementary residue in the receptor. Although Martí-Solano et al. (2015) argue that interaction with N6.55 in helix 6 favors receptor conformations with a preference to signal through the AA pathway, with S5.46 responsible for facilitating signaling through the IP pathway, significant problems with their docking poses call these conclusions into question. Nonetheless, if properly done, the type of approach employed by Martí-Solano et al. (2015) likely could be used to help in the identification of the structural basis for functional selectivity.

D. Role of Glutamate

Research over the past 2 decades has clearly shown that psychedelics enhance glutamatergic transmission in the cortex at the neuronal level and also in behavioral responses. At the neuronal level, using whole-cell recording in rat brain slides, Aghajanian and Marek (1997) first reported that 5-HT induced a calcium-dependent rapid and dramatic increase in frequency and amplitude of spontaneous (nonelectrically evoked), glutamatergic excitatory postsynaptic potentials (EPSPs)/excitatory postsynaptic currents (EPSCs) in cortical pyramidal cells of layer V. The effect was most robust in the mPFC and other frontal areas with a high expression of 5-HT_{2A} receptors in pyramidal apical dendrites. The effect was completely blocked by M100907 and also by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate antagonist LY293558 [(3*S*,4a*R*,6*R*,8a*R*)-6-[2-(2*H*-tetrazol-5-yl)ethyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxylic acid].

In a subsequent study, it was found that Sr^{2+} fully substituted for Ca^{+2} in supporting the 5-HT–induced increase in spontaneous EPSC frequency, but only partially with respect to amplitude (Aghajanian and Marek, 1999b). In the presence of Sr^{2+}, however, late asynchronous evoked EPSPs followed each electrical stimulus, with an absence of synchronous EPSCs. Serotonin reduced the amplitude of synchronous evoked EPSCs, with an increase in frequency of spontaneous excitatory postsynaptic currents (sEPSCs) both before and after electrical stimulation. Although late evoked EPSCs were not evident, when 5-HT was washed out and synchronous evoked EPSCs and sEPSCs were returning to normal, late evoked EPSCs began to appear that resembled the asynchronous evoked EPSCs observed after Sr^{2+} substitution. It was speculated that 5-HT produced a hyperpolarizing effect, mediated through 5-HT_{1A} receptors, which might be masking the 5-HT_{2A}–mediated excitatory late component. As the 5-HT was washed out of the preparation, the 5-HT_{2A} receptor–mediated late component would be unmasked. After application of DOI, a mixed 5-$HT_{2A/2C}$ agonist that lacks 5-HT_{1A} effects, the late component was evoked by every stimulus in all tested cells. Application of M100907 reversed the DOI effect. The authors note that their data with Ca^{2+} and Sr^{2+} point to a presynaptic 5-HT_{2A} action, although 5-HT_{2A} receptors are known to be expressed primarily postsynaptically on pyramidal cell apical dendrites.

Delving more deeply into these mechanistic findings, Marek et al. (2000) studied the effect of the selective mGlu2/3 agonist LY354740 and the selective mGlu2/3 antagonist LY341495 on the 5-HT–induced EPSPs and electrically evoked EPSPs in pyramidal cells from layer V in the rat mPFC. They also examined the effect of these two ligands on the effects of DOI, reporting that the mGlu2/3 antagonist LY341495 enhanced the frequency and amplitude of 5-HT–induced EPSCs by 30%–65% and 12%–21%, respectively. The mGlu2/3 agonist LY354740 was equipotent in suppressing both 5-HT–induced EPSCs and DOI-enhanced electrically evoked late EPSPs. Their findings were consistent with the hypothesis that mGlu2/3 receptors function as inhibitory autoreceptors in cortical glutamatergic terminals that are positively regulated by activation of 5-HT_{2A} receptors. Using autoradiography, these authors further demonstrated that the highest density of mGlu2/3 receptor binding in the mPFC is expressed in cortical layers I and Va, in a laminar distribution similar to 5-HT_{2A} receptor expression.

Administration of LSD to rats (0.5 mg/kg, i.p.) led to a significant increase in *fos*-like immunoreactivity in the rat PFC and ACC that was completely blocked by systemic pretreatment with the specific 5-HT_{2A} antagonist MDL100907 (Gresch et al., 2002). Double staining for both *fos* immunoreactivity and the 5-HT_{2A} receptor revealed that LSD did not induce *fos* in pyramidal cells expressing 5-HT_{2A} receptors in either the PFC or parietal cortex. Increased *fos* expression was induced in cortical cells in layers III and IV, with only rare

occurrence of a doubly labeled pyramidal cell, suggesting *fos* induction by some indirect mechanism. Based on the work from Aghajanian's laboratory (Aghajanian and Marek, 1997; Marek and Aghajanian, 1998a), the authors speculated that this activation could result from glutamatergic thalamocortical inputs.

In vivo microdialysis after systemic administration of DOI revealed significantly increased extracellular glutamate in the rat somatosensory cortex (Scruggs et al., 2003). Intracortical reverse dialysis of DOI also increased extracellular glutamate. The increase in glutamate after intracortical DOI was blocked by the selective 5-HT_{2A} antagonist M100907. Similarly, using microdialysis, extracellular glutamate also was significantly increased in the rat PFC 30 minutes after 0.1 mg/kg intraperitoneal LSD administration and continued for 30 minutes. This glutamate release was blocked by preadministration of 0.05 mg/kg M100907 15 minutes prior to LSD administration (Muschamp et al., 2004). Reverse dialysis of LSD for 30 minutes into the rat PFC, followed by perfusion of drug-free solution for 45 minutes, led to a significant increase of glutamate that remained elevated for at least 45 minutes after the LSD perfusion ended. DOM (0.6 mg/kg, i.p.) similarly increased extracellular glutamate measured in the rat PFC (Muschamp et al., 2004).

Studies by Lambe and Aghajanian (2006) further examined the basis for the enhanced late wave of extrasynaptic transmission of glutamate in PFC brain slices of the rat. They retarded the diffusion of glutamate beyond the synapse by increasing the viscosity of the extracellular space using a solution of dextran, a procedure known to suppress glutamate spillover. They also assessed the subtypes of *N*-methyl-D-aspartate (NMDA) receptors involved in the late wave of increased glutamate produced by LSD and DOI. They found that the wash-in of a 5% dextran solution dramatically attenuated the late EPSCs induced by LSD or DOI without changing the fast EPSC. The suppression of the late EPSCs (UP states) by dextran suggested that the late wave of glutamate release induced by psychedelics stimulates extrasynaptic, rather than synaptic, glutamate receptors. Thus, the NMDA receptor subtype 2B antagonists Ro25-6981 [(αR,βS)-α-(4-hydroxyphenyl)-β-methyl-4-(phenylmethyl)-1-piperidinepropanol] and ifenprodil markedly suppressed the DOI-induced late EPSCs but had no effect on the fast EPSC. The effect of psychedelics on UP states also could be partially mimicked by a glutamate uptake inhibitor after blocking mGluR2/3. Their results indicate that psychedelics increase glutamate spillover in a phasic manner.

Two explanations had been offered for the increase in cortical glutamate after application of a 5-HT_{2A} agonist. The first was that glutamate was released from terminals arising from the mediodorsal thalamus (Aghajanian and Marek, 1997; Marek and Aghajanian, 1998a; Scruggs et al., 2000). Some evidence for that mechanism was provided by Marek et al. (2001), who found that lesions of the medial thalamus in rats led to a significant decrease in the frequency of 5-HT–induced EPSCs recorded from layer V pyramidal cells. Autoradiographic experiments showed that medial thalamic lesions led to a significant decrease in the density of group II mGlu2/3 receptors in the mPFC, consistent with the loss of presynaptic glutamate terminals. Unexpectedly, however, these lesions led to *increased* density of cortical 5-HT_{2A} receptors, a finding that would not be entirely consistent with presynaptic 5-HT_{2A} receptor localization.

A major problem with the presynaptic glutamate release hypothesis was the fact that immunocytochemistry studies had revealed that the majority (73%) of 5-HT_{2A} immunopositive profiles were postsynaptic processes, mostly proximal and distal dendritic shafts, with only 24% of identifiable immunoreactive profiles on presynaptic structures (Miner et al., 2003). These latter structures only rarely formed synaptic contacts in single sections, and 5-HT_{2A} receptor labeling was not typically observed in presumed glutamate axon terminals, a finding consistent with other studies that had failed to identify substantial numbers of 5-HT_{2A} immunoreactive axon terminals with features characteristic of glutamate profiles. Interestingly, the remaining labeled profiles (4%) were glial processes, suggesting that perhaps activation of 5-HT_{2A} receptors on glial processes might also induce glutamate release. These results were not compatible with the hypothesis that 5-HT_{2A} receptors might serve as presynaptic heteroreceptors on mediodorsal thalamic glutamate terminals in the middle layers of the PFC.

Evidence against the hypothesis of presynaptic glutamate release from thalamic glutamate terminals was provided by Puig et al. (2003), who examined the effect of intravenous administration of cumulative doses of DOI on pyramidal cell firing in vivo in the mPFC of control animals and compared the responses with those observed in animals with electrolytic lesions of the mediodorsal and centromedial nuclei of the thalamus, which project densely to the mPFC, as well as several other nuclei that also project to the mPFC. They found that DOI evoked a dramatic increase in the firing rate of a subpopulation of mPFC pyramidal neurons that was unaffected by prior lesioning of thalamic nuclei that project to the mPFC, and the authors concluded that DOI does not affect pyramidal cell firing by acting on presynaptic 5-HT_{2A} receptors putatively located on thalamocortical afferents to the mPFC.

In a subsequent study, Celada et al. (2008) reported that systemic administration of DOI markedly reduced the amplitude of low frequency oscillations in the mPFC, an effect that was completely blocked by preadministration of the selective 5-HT_{2A} antagonist M100907. They also compared responses to DOI in control rats with rats that had been given electrolytic lesions of several thalamic nuclei that project to the

mPFC. DOI was found to be equally effective in both control rats and in rats with thalamic lesions, again refuting the earlier hypothesis of the role of putative thalamocortical 5-HT_{2A} receptors as mediators of hallucinogen action.

A second explanation for the increase in cortical glutamate after hallucinogens was offered by Lambe and Aghajanian (2001), who suggested that 5-HT_{2A} receptor activation on postsynaptic cells might lead to release of a retrograde messenger. This substance would then diffuse out from the postsynaptic membrane and block K^+ channels on presynaptic glutamate terminals, leading to glutamate release. Neither of these hypotheses survived further scientific scrutiny, however, because the ultimate source of glutamate was later identified by Béïque et al. (2007), as discussed later.

Molinaro et al. (2009) studied the in vivo interaction between 5-HT_{2A} and mGlu2/3 receptors in the mouse frontal cortex. Male CD1 mice first were injected intracerebroventricularly with [*myo*-^{3}H]inositol. Twenty-four hours later, mice were treated with LiCl subcutaneously, followed 1 hour later by drug administration. The mGlu1/5 agonist dihydroxyphenylglycine given intracerebroventricularly increased [^{3}H]IP formation by approximately 50% in the frontal cortex. DOI (2 mg/kg, i.p.) was as effective as dihydroxyphenylglycine in stimulating [^{3}H]IP in the frontal cortex. The effect of DOI on [^{3}H]IP formation was abolished by pretreatment with 5 mg/kg ketanserin, indicating an action through 5-HT_{2A} receptors. Pretreatment of mice with the mGlu2/3 agonist LY379268 [(1*R*,4*R*,5*S*,6*R*)-4-amino-2-oxabicyclo[3.1.0]hexane-4,6-dicarboxylic acid] (10 mg/kg, i.p.) 5 minutes before DOI reduced stimulation of PI hydrolysis, indicating that mGlu2/3 receptor agonists selectively inhibit 5-HT_{2A} receptor–mediated PI hydrolysis; this effect was blocked by the mGlu2/3 antagonist LY341495. LY566332 [*N*-(4′-cyano-biphenyl-3-yl)-*N*-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride], an mGlu2 positive allosteric modulator also attenuated DOI-stimulated PI hydrolysis when combined with a subthreshold dose of LY379268. Coadministration of the mGlu2/3 agonist LY379268 with the mGlu2/3 antagonist LY341495 blocked the effect of LY379268. These investigators also used mGlu2($^{-/-}$), mGlu3($^{-/-}$), and double-KO mGlu2($^{-/-}$)/mGlu3($^{-/-}$) mice. None of the mGlu2/3 ligands had any effect on PI hydrolysis in the absence of DOI. In WT, mGlu2/3($^{-/-}$), and mGlu3($^{-/-}$) mice, LY379268 reduced DOI-stimulated levels of PI hydrolysis to a similar extent, but it did not reduce DOI-stimulated PI hydrolysis in the cortex of the double mGlu2($^{-/-}$)/mGlu3($^{-/-}$) mice. Local intracortical injection of LY379268 attenuated DOI-stimulated PI hydrolysis in the ipsilateral frontal cortex to an extent similar to that seen in mice after systemic injection of LY379268. DOI (2 mg/kg) or LY379268 (10 mg/kg) increased phosphorylated ERK1/2 by approximately 50% in the frontal cortex, but no increase in ERK1/2 phosphorylation was seen in the mouse cortex when mice were treated with the combination of DOI and LY379268. In cortical slices, however, the mGlu2/3 agonist LY379268 amplified DOI-stimulated PI hydrolysis, and this effect was blocked by the mGlu5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP). The authors propose that the slice preparation may unmask an mGlu2/3 interaction with mGlu5 receptors, which can confound data obtained with LY379268 and DOI, indicating that cortical slice preparations are not appropriate for the study of 5-HT_{2A} and mGlu2/3 receptor interactions. Their findings suggest that in frontal cortex mGlu2/3 receptors negatively regulate the G_q phospholipase C3 pathway that is activated by 5-HT_{2A} receptors.

Wischhof et al. (2011) examined interactions between 5-HT_{2A} and mGlu2/3 receptors in the orbitofrontal cortex (OFC) and mPFC with respect to impulsive choice and impulsive action in Lister Hooded rats. Impulsive choice was assessed in rats trained in a delay-discounting T-maze task, after bilateral infra-OFC infusions of DOI or the mGlu2/3 agonist LY379268. In a second group of rats trained in the five-choice serial reaction time task (5-CSRTT), impulsive action was assessed after bilateral intra-mPFC infusions of DOI and LY379268. Intra-OFC DOI increased impulsive choice, an effect that was blocked by LY379268. LY379268 had no effect on choice when given alone. Impulsive over-responding in the 5CSRTT was observed after intra-mPFC DOI, an effect that was attenuated by coinjection of both DOI and LY379268. Their results indicate that 5-HT_{2A} receptors in the OFC and mPFC are differentially involved in the regulation of impulsive choice and impulsive actions.

Wischhof and Koch (2012) followed up on their earlier study by further examination of the interactions between the 5-HT_{2A} and mGlu2/3 receptors involved in impulse control. Hooded Lister rats were trained in the 5-CSRTT and treated with DOI and/or the mGlu2/3 agonist LY379268. Drug-induced changes in neuronal activity were measured by *c-fos* immunoreactivity. Colocalization of *c-fos* and GABAergic markers was detected by double immunofluorescence labeling. Impulsive over-responding induced by DOI was reduced in animals pretreated with LY379268, whereas LY379268 had no effect by itself on 5-CSRTT performance. DOI treatment led to increased *c-fos* immunoreactivity in the frontocortical and limbic brain areas, an effect that was blocked by pretreatment with LY379268. Double immunofluorescence labeling revealed colocalization of DOI-elicited *c-fos* with glutamate decarboxylase GAD_{67}–positive GABAergic cells lacking parvalbumin, whereas LY379268 increased *c-fos* immunoreactivity in both GABAergic and non-GABAergic cells. They conclude that impulsivity may possibly be due to a primary increase in glutamatergic transmission that is mediated by 5-HT_{2A} receptor activation.

The selective mGlu2/3 agonist LY379268 also attenuated the DOI-induced increase in *c-fos* mRNA in rat

mPFC slices (Zhai et al., 2003). DOI enhanced the amplitude of the complex EPSP evoked in pyramidal neurons by 30%, an effect that was blocked by LY379268, demonstrating that excitatory glutamatergic responses of prefrontal cortical pyramidal neurons are positively and negatively modulated by agonists at 5-HT_{2A} and mGlu2/3 receptors, respectively.

Applying these approaches to the mouse, the selective mGlu2/3 agonists LY379268 and LY354740 suppressed the increased frequency of spontaneous EPSPs induced by bath-applied DOI in layer V pyramidal cells, recorded in slices of mouse medial frontal cortex (Kłodzinska et al., 2002). These two mGlu2/3 agonists also inhibited mouse head twitches induced by DOI.

Using a variety of different techniques, Béïque et al. (2007) challenged the hypothesis that activation of 5-HT_{2A} receptors led to the production of a retrograde transmitter, which resulted in release of glutamate from thalamocortical afferents. In one experiment, they transfected cortical pyramidal cells with a construct coding for the C-terminal portion of PLCβ1, which acts as a dominant negative to suppress $G\alpha_{q/11}$ signaling. The 5-HT_{2A} agonist AMS induced a small inward current in nontransfected cells, but not in neighboring neurons transfected with PLCβ-ct. The investigators reasoned that if postsynaptic 5-HT_{2A} receptor signaling induces glutamate release from presynaptic terminals, then inhibition of postsynaptic 5-HT_{2A} signaling should also inhibit the ability of the cells to increase sEPSCs. They found, however, that the ability of AMS to increase frequency of sEPSCs was no different between control cells and those transfected with PLCβ-ct.

Béïque et al. (2007) then examined prefrontal cortical neurons from 5-HT_{2A} KO mice. Reasoning that if 5-HT_{2A} signaling led to an increase in sEPSC by release of a retrograde transmitter, transfection of a cell with the 5-HT_{2A} receptor should rescue the ability of AMS to increase sEPSCs only in cells where 5-HT_{2A} receptors were expressed. AMS did not induce an inward current in control (nontransfected cells) but elicited an inward current in neighboring neurons transfected with the 5-HT_{2A} receptor. The authors found that expression of 5-HT_{2A} receptors in pyramidal cells from 5-HT_{2A} KO mice rescued the ability of 5-HT_{2A} receptors to signal an inward current but not the ability to increase sEPSC frequency. Thus, these results failed to support the retrograde message hypothesis. In another experiment, they used GTPγS, a nonhydrolyzable GTP analog, to render G protein–mediated responses irreversible. AMS induced a small inward current that recovered on agonist removal from control cells, as well as an irreversible inward current in cells loaded with GTPγS. Yet AMS induced comparable increases in sEPSCs in both control and GTPγS-loaded neurons. Importantly, however, the sEPSC increases for both types of cells recovered after agonist removal, something that would not be expected in cells with irreversible signal production by GTPγS. These experiments were very strong evidence that 5-HT_{2A} receptor signaling was not generating a retrograde messenger.

However, in current-clamp recordings in acute rat PFC slices, Béïque et al. (2007) noticed a subpopulation of large neurons in deep layers that was highly sensitive to 5-HT and that responded with strong membrane depolarizations capable of initiating spiking activity. Approximately one-third of the largest cells were excited by 10–30 μM 5-HT. Thus, their results indicated that within the cortex, there is a subpopulation of 5-HT_{2A}–expressing cells that when excited by a 5-HT_{2A} agonist leads to increases in the frequency of sEPSCs in layer V pyramidal neurons. Finally, they note earlier work by Marek and Aghajanian (1998a), who showed that μ-opioid agonists abolished the ability of 5-HT to increase sEPSC activity and who also suggested that there was possibly a subcortical source for afferents on which 5-HT_2 receptors could induce glutamate release. Thus, Béïque et al. (2007) carried out current-clamp recordings from the deep large cells they had identified and found that the selective μ-opioid agonist D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin, (DAMGO) DAMGO completely blocked the ability of AMS to depolarize and excite these neurons. Thus, this subpopulation of cells in deep layers of the cortex that are very sensitive to 5-HT_{2A} agonists also expresses μ-opioid receptors. Taken together, their results indicate that the increased sEPSC activity seen in PFC layer V pyramidal neurons in response to 5-HT_{2A} receptor stimulation results from excitation of a subpopulation of pyramidal cells in the deep layers of the PFC, and not through activation of thalamocortical axon terminals by a retrograde messenger. Therefore, 5-HT_{2A} receptors in the PFC enhance overall excitability of the PFC network by regulating the properties of a key subpopulation of pyramidal neurons.

Much of the work on neuronal effects of psychedelics has been obtained from single cell recordings from brain slices, which do not represent an intact and functioning cortex. The neocortex is constantly active in vivo, as cortical and subcortical networks generate rhythmic patterns of activity at a variety of frequencies (Steriade et al., 1993). The propagation and synchronization of slow cortical oscillations depends at least in part on corticocortical connections and is proposed to be generated by recurrent excitation among large networks of cortical neurons. Unfortunately, no one has yet examined the effect of hallucinogens on spontaneous rhythmic activity in cortical circuits, experiments that would likely provide important new data. For example, Shu et al. (2003) demonstrated that operation of local cortical circuits could generate activity that exhibited a proportional increase in feedback excitation and inhibition, keeping the network in relative balance. It would be most interesting to carry out a similar experiment, but with the addition of DOI or LSD to observe the effect on cortical circuit activity.

All of the in vitro electrophysiological studies of cortical slices discussed in this review appear to have employed a "standard" and essentially identical slice bath composition. Sanchez-Vives and McCormick (2000) noted that when ferret prefrontal cortical slices are maintained in vitro in the traditional bathing medium, no spontaneous rhythmic activity is observed. By contrast, when these investigators used a bath solution with an ionic composition that closely mimicked brain interstitial fluid, spontaneous rhythmic oscillations appeared that could be continuously maintained and were nearly identical to those observed in vivo. Slow oscillation was most robust and occurred first in or near cortical layer V, after a short delay by activity in deeper layers. The activity maximum in layer V was always larger and persisted longer than in any other layers and seemed to be initiated in layer V as an excitatory interaction between pyramidal neurons that propagated through the neocortex. These workers suggest that the basic operation of cortical networks is the generation of self-maintained depolarized states that are tightly regulated through interaction with local GABA/GABAergic neurons and intrinsic membrane conductances. They suggest that the ability of cortical networks to generate persistent and recurring activities even in the absence of ongoing subcortical inputs may be a process that underlies perceptual influences on sensory information processing.

Most recently, Nichols and Martin (2015) discovered a subpopulation of cortical cells that are activated by the 5-HT_{2A} receptor agonist DOI, and this subpopulation may represent the cells described by Béïque et al. (2007). Nichols and Martin (2015) found that only about 3%–5% of total cortical cells were activated by DOI but the neurons within this subpopulation had a 10-fold higher expression of 5-HT_{2A} receptor mRNA than the nonactivated neuronal population. This activated cellular subpopulation included pyramidal neurons, interneurons, glia, and astrocytes. High levels of both 5-HT_{2A} receptor protein expression and cellular activation were observed in the claustrum, where nearly one-half of the neurons were activated by DOI. Furthermore, Nichols and Martin (2015) reported that DOI activated only subsets of inhibitory GABA interneurons, which included somatostatin and parvalbumin GABA interneurons. The reader is directed to the section in this review on the claustrum, which demonstrates that the claustrum would be a logical target for activation by psychedelics, particularly because it is involved in corticocortical interactions.

There is also extensive evidence for the interaction of glutamate systems in serotonin 5-HT_{2A} receptor–mediated behaviors. For example, both competitive and noncompetitive NMDA antagonists given intracerebroventricularly were able to potentiate the HTR in mice produced by a subsequent intracerebroventricular injection of 5-HT, providing the first evidence of glutamatergic modulation of serotonergic function in intact mice (Kim et al., 1998). Competitive and noncompetitive NMDA receptor antagonists also markedly enhanced the 5-HT–induced HTR in mice that had been treated with *p*-chlorophenylalanine to deplete endogenous serotonin (Kim et al., 1999).

In particular, data indicate that group II mGluR agonists can counteract the effects of psychedelic 5-HT_{2A} agonists. For example, DOI-induced rat head shakes mediated by 5-HT_{2A} receptor activation were enhanced by pretreatment with either competitive or noncompetitive NMDA antagonists (Dall'Olio et al., 1999). Preadministration of the mGlu2/3 receptor agonist LY354740 attenuated the frequency of DOI-induced head shakes in rats, whereas administration of the selective mGlu2/3 antagonist LY341495 potentiated DOI-induced head shakes in rats (Gewirtz and Marek, 2000). As noted above, DOI-induced head twitches in mice were inhibited in a dose-dependent manner by the selective mGlu2/3 agonists LY354740 and LY379268 (Kłodzinska et al., 2002). This action is presumably due to a presynaptic effect on glutamate neurons, in which mGlu2/3 agonists suppress glutamate release and antagonists block the presynaptic autoreceptor agonist effect of endogenously released glutamate (Conn and Pin, 1997).

In a study by Winter et al. (2000a), rats were trained in a two-lever fixed ratio (FR) 10 schedule of reinforcement to discriminate saline from a training dose of 0.6 mg/kg DOM. Pretreatment of the trained rats with PCP, an NMDA antagonist, dramatically shifted the dose-response curve leftward. When a dose of 0.1 mg/kg DOM was administered, rats emitted only 32% DOM-appropriate responding. Yet when combined with a range of doses of the noncompetitive NMDA antagonists PCP, dizocilpine, or ketamine, DOM-appropriate lever selection was increased to 73%, 84%, and 79%, respectively. Consistent with other studies, the data show that the stimulus effects of phenethylamine hallucinogens are potentiated by pretreatment with noncompetitive NMDA antagonists.

Using a two-lever FR10 food-reinforced drug discrimination task, the stimulus effect of LSD was enhanced by pretreatment with both the mGlu2/3 antagonist LY341495 and the NMDA antagonist PCP. Stimulus control by LSD was significantly but incompletely blocked by the mGlu2/3 agonist LY379268, whereas the 5-HT_{2A} antagonists pirenperone and M100907 completely antagonized stimulus control by LSD (Winter et al., 2004). By contrast, the mGlu2/3 antagonist LY341495 had no effect on stimulus control by PCP, and the training dose of PCP was significantly, but incompletely, antagonized by the mGlu2/3 agonist LY379268.

Although the in vivo functional interaction between 5-HT_{2A} receptors and mGlu2 receptors is now widely accepted, González-Maeso et al. (2008) proposed that the 5-HT_{2A} receptor formed a heterodimer with the

mGlu2 receptor and identified this complex as a possible site of action for hallucinogenic drugs. They reported that the 5-HT_{2A} and mGlu2 receptors directly interact in recombinant cell lines and are present in the same neuronal cells in culture. Their results indicated that this heterodimeric complex enhanced $G_{\alpha i}$ activation by psychedelic 5-HT_{2A} agonists, a signaling event proposed to be involved in hallucinogen-specific signaling (González-Maeso et al., 2007). Fribourg et al. (2011) further extended the original findings presented by González-Maeso et al. (2008).

Delille et al. (2012) subsequently coexpressed mGlu2 receptors in an inducible manner in a constitutive 5-HT_{2A} background in HEK-293 cells. They determined the reciprocal influence of the two receptors on receptor expression and measured intracellular Ca^{+2} as well as cAMP levels after stimulation with agonists, antagonists, psychedelics, and positive allosteric modulators. The formation of the 5-HT_{2A}–mGlu2 heterocomplex did not result in functional cross-talk. In addition, psychedelic 5-HT_{2A} agonists had no effect on $G_{\alpha i}$-mediated signaling. The results of Delille et al. (2012) indicate that the functional interaction of mGlu2 and 5-HT_{2A} receptors may not be mediated by heterodimer interaction.

In a subsequent report, Delille et al. (2013) reviewed the evidence for a heterodimer presented by González-Maeso et al. (2008), including their report of coimmunoprecipitation of 5-HT_{2A} and mGlu2 receptors, data from bioluminescence resonance energy transfer assays, and many other detailed data. Delille et al. (2013) note that formation of a heterodimer in a recombinant system is not proof for its occurrence in vivo. They further point out that the preponderance of evidence suggests that mGlu2 receptors are expressed presynaptically, whereas the 5-HT_{2A} receptor is predominantly expressed postsynaptically. Delille et al. (2013) also point out that most anatomic data indicate that the predominant fraction of 5-HT_{2A} and mGlu2 receptors is expressed in different compartments, somatodendritic (postsynaptic) or axons (presynaptic), respectively. Furthermore, an allosteric interaction of 5-HT_{2A} and mGlu2 receptors has not been independently replicated in any other laboratory. They conclude that until ultrastructural studies can be completed, the physiologic relevance of a 5-HT_{2A}–mGlu2 heterodimer "remains questionable."

E. A Role for γ-Aminobutyric Acid

Although the focus of most research on amino acid neurotransmitters in the frontal cortex has been on glutamate, GABA interneurons play an important role. Using in vivo microdialysis in the rat mPFC, administration of DOI through the perfusion probe led to a significant dose-dependent increase in extracellular GABA (Abi-Saab et al., 1999). Double-labeling immunohistochemical examination of cortical cells after systemic administration of DOI showed a significant increase in the number of interneurons expressing both GAD and *fos*-like immunoreactivity. These authors concluded that 5-HT regulates cortical GABA interneurons, an effect similar to that seen in piriform cortex interneurons (Marek and Aghajanian, 1994). In the rat frontal cortex, 5-HT–enhanced spontaneous inhibitory postsynaptic potentials in pyramidal cells can be produced through activation of 5-HT_{2A} receptors located on GABAergic interneurons (Zhou and Hablitz, 1999). Thus, activation of 5-HT_{2A} receptors in the cortex can produce both excitation and a feed-forward inhibition of cortical pyramidal cells.

F. Possible Role of Other Receptors

Ray (2010) reported on receptor screening of 25 hallucinogens and analogs by the National Institute of Mental Health Psychoactive Drug Screening Program, with affinities of 10 additional drugs taken from the literature. The 35 drugs of the study had very diverse patterns of interaction, which may underlie some of the qualitative psychopharmacological differences between the drugs. Functional effects of the various compounds were not studied, however, which would have strengthened the conclusions and given more detailed insight into the possible relevance of receptors where some of the tested drugs had relatively high affinity.

The most consistent finding for involvement of other receptors in the actions of psychedelics is the 5-HT_{1A} receptor. That is particularly true for tryptamines and LSD, which generally have significant affinity and functional potency at this receptor. It is known that 5-HT_{1A} receptors are colocalized with 5-HT_{2A} receptors on cortical pyramidal cells (Martín-Ruiz et al., 2001), where the two receptor types have opposing functional effects (Araneda and Andrade, 1991). In addition to functioning as somatodendritic autoreceptors in the raphe, postsynaptic 5-HT_{1A} receptors are also localized in a number of other important brain regions. Their highest density is found in limbic regions of the brain such as the hippocampus (Hamon et al., 1990), areas where emotion and affect would be modified by agonist and antagonist drug interactions. Autoradiographic studies demonstrated the presence of 5-HT_{1A} receptors in layer V of the rat PFC (Glaser et al., 1985; Pazos and Palacios, 1985), and neurons in the human neocortex contain mRNA for the 5-HT_{1A} receptor, with pyramidal cells in layer III more heavily labeled than those in layer V (Burnet et al., 1995).

LSD and potent tryptamine hallucinogens such as 5-MeO-DMT and psilocin all have high affinity for 5-HT_{1A} receptors (McKenna et al., 1990; Blair et al., 2000). LSD is a full agonist at central 5-HT_{1A} receptors linked to inhibition of adenylyl cyclase (De Vivo and Maayani, 1986). Deliganis et al. (1991) reported that DMT affinity for 5-HT_{1A} receptors (K_i = 130 nM), was reduced (K_i = 464 nM) by addition of guanyl nucleotides, suggesting that DMT was an agonist. This hypothesis

was confirmed by their finding that DMT was equally efficacious to the 5-HT_{1A} agonist 8-OH-DPAT [7-(dipropylamino)-5,6,7,8-tetrahydronaphthalen-1-ol] in inhibiting forskolin-stimulated cAMP formation in rat hippocampal homogenate. DMT also enhances the acoustic startle response (ASR) in rats (Davis and Sheard, 1974), an effect now attributed to 5-HT_{1A} receptor activation (Nanry and Tilson, 1989). The closely related *N,N*-diethyltryptamine is also a full agonist in inhibiting forskolin-stimulated cAMP production in cloned human 5-HT_{1A} receptors expressed in CHO cells (Blair et al., 2000). Similarly, 5-MeO-DMT is a full agonist at 5-HT_{1A} receptors (Dumuis et al., 1988; Blair et al., 2000). No clinical research has yet identified a 5-HT_{1A} receptor–mediated component of action for any psychedelic, but it would seem surprising if the psychopharmacology of psychedelics was not affected to some extent when the molecule had a potent 5-HT_{1A} agonist effect, given the brain areas that express high levels of this receptor.

The evidence for involvement of 5-HT_{1A} receptors in the behavioral actions of psychedelics has been gleaned primarily from animal studies. Halberstadt and Geyer (2011) reviewed the evidence and concluded that the 5-HT_{1A} receptor can play an important role in the behavioral effects of tryptamine-type psychedelics. Several examples illustrating the importance of 5-HT_{1A} receptor activation in the action of tryptamine hallucinogens are provided in the later sections of this review on animal models, but two examples are provided now to illustrate how these conclusions were developed.

As one illustration, Fantegrossi et al. (2008b) examined the effects of *N,N*-dipropyltryptamine (DPT) in the mouse HTR and in rat drug discrimination assays. Rats were trained to discriminate LSD, psilocybin, or MDMA from saline; in addition, one group of rats was trained to discriminate DPT from saline. The paradigm was a two-choice FR10, with diluted sweetened condensed milk as the reinforcer. In the mouse, DPT elicited the HTR in a dose-dependent fashion, producing a maximum of 15 twitches at 3 mg/kg. Pretreatment with 1 mg/kg of the 5-HT_{1A} antagonist WAY-100635 gave a 3-fold rightward shift in the dose-response curve, indicating competitive antagonism at the 5-HT_{1A} receptor. A 10-mg/kg dose of DPT produced a greater HTR, but a 3-mg/kg dose of WAY-100635 gave no greater antagonism than the 1-mg/kg dose. Pretreatment with 0.1 mg/kg of the 5-HT_{2A}–selective antagonist M100907, however, nearly abolished the HTR. In drug discrimination studies in LSD-trained rats, the training stimulus generalized to DPT with approximately 60% LSD-appropriate responding at the 3-mg/kg dose of DPT. Pretreatment with 0.3 mg/kg WAY-100635 produced a parallel rightward shift in the DPT dose-effect curve, but 0.1 mg/kg M100907 completely blocked the LSD-like discriminative stimulus effects of 1.5 or 3.0 mg/kg DPT. In psilocybin-trained rats, DPT gave an intermediate degree of generalization (55%) at a dose of 3 mg/kg. Neither 0.3 mg/kg WAY-100635 nor 0.1 mg/kg M100907 affected the psilocybin-like discriminative effects of DPT, but surprisingly, a combination of the two antagonists completed abolished the stimulus effects of DPT. DPT dose-dependently and fully substituted in MDMA-trained rats, and pretreatment with 0.3 mg/kg WAY-100635 had no effect on the discrimination. By contrast, 0.1 mg/kg M100907 completely blocked the MDMA-like stimulus properties of DPT.

Fantegrossi et al. (2008b) also trained rats to discriminate 1.5 mg/kg DPT from saline. The discriminative stimulus effects of DPT were not affected by prior treatment with WAY-100635, but they were significantly attenuated by pretreatment with 0.1 mg/kg M100907. A combination of the two antagonists again essentially abolished the stimulus effects of DPT. Both the mouse HTR and drug discrimination data from this study indicate that DPT has potent effects as a 5-HT_{2A} agonist, but that some component of the mouse HTR is mediated by the 5-HT_{1A} receptor. With respect to the drug discrimination assays, the authors suggest that the 5-HT_{1A}–mediated components of the stimulus properties of DPT may be more salient at lower doses, whereas the stimulus properties resulting from activation of the 5-HT_{2A} receptor are more prominent at higher doses of DPT.

A second example is work by Halberstadt et al. (2011a), who compared the effects of psilocin with those of 1-methypsilocin and 5-MeO-DMT in mice using the BPM and the mouse HTR. 1-Methylpsilocin is a derivative of psilocin that is reported to act as a selective 5-HT_{2C} receptor agonist (Sard et al., 2005). Psilocin at doses of 0.6–2.4 mg/kg produced an inverted U-shaped dose-response curve for the HTR. In 5-HT_{2A} KO mice, however, psilocin did not produce the HTR. The HTR also was produced by 1-methylpsilocin, which was only about one-fourth the potency of psilocin, and again, the HTR was absent for 1-methylpsilocin in 5-HT_{2A} KO mice. The HTR was also produced by 5-MeO-DMT at 10 and 20 mg/kg. As with psilocin and 1-methylpsilocin, 5-MeO-DMT did not induce the HTR in 5-HT_{2A} KO mice. In the BPM, 4.8 mg/kg psilocin was maximally effective in significantly reducing locomotor activity, yet hole poking, rearing, and center duration were significantly reduced at doses $\leq$ 1.2 mg/kg, a dose that had no effect on locomotor activity. Pretreatment with the 5-HT_{1A} antagonist WAY-100635 (0.5 mg/kg) completely blocked the ability of psilocin to reduce locomotor activity. WAY-100635 partially blocked the ability of psilocin to reduce hole-poking behavior but failed to reverse the reduced rearing behavior induced by psilocin.

In the study by Halberstadt et al. (2011a), the response to psilocin was not affected by deletion of the 5-HT_{2A} receptor gene. The 5-HT_{2C} antagonist SB-242084 (6-chloro-5-methyl-*N*-{6-[(2-methylpyridin-3-yl)oxy]pyridin-3-yl}indoline-1-carboxamide) slightly potentiated the

ability of psilocin to reduce motor activity but had no effect on rearing, hole pokes, or center duration. Doses of 10 or 20 mg/kg 5-MeO-DMT also reduced distance traveled in the BPM, with the larger dose having a more prolonged effect. Both doses of 5-MeO-DMT also significantly reduced hole pokes throughout the test. Pretreatment with 0.5 mg/kg WAY-100635 significantly blocked the distance traveled by mice given 5-MeO-DMT, similar to psilocin. Also like psilocin, pretreatment with SB-242084 potentiated the effects of 5-MeO-DMT in reducing distance traveled. Halberstadt et al. (2011a) concluded that the reduction in locomotor activity, investigatory behavior, and center duration produced by psilocin were all mediated by the 5-HT_{1A} receptor because of the blockade of these effects by WAY-100635 and the fact that neither the 5-HT_{2C} antagonist SB-242084 nor 5-HT_{2A} receptor gene deletion had any effect on these behaviors. The effects of 5-MeO-DMT on locomotor activity were also blocked by WAY-100635. By contrast, 1-methylpsilocin had no effect on locomotor activity in the BPM, despite the fact that it produced the HTR. These findings are further evidence that the 5-HT_{1A} receptor can play a role in the behavioral effects of indoleamine-type psychedelics.

Is the serotonin 5-HT_{2C} receptor involved in the actions of psychedelics? All known psychedelics are agonists at both the 5-HT_{2A} and 5-HT_{2C} receptors. The most salient behaviors induced in rodents by psychedelics generally have been shown due to activation of the 5-HT_{2A} receptor. Nevertheless, higher doses of particular psychedelics may lead to activation of the 5-HT_{2C} receptor, which often functionally opposes the effects of 5-HT_{2A} receptor activation. For example, low doses of DOI increase locomotor activity in mice, whereas higher doses attenuate it, leading to an inverted U type of dose-response curve. The same phenomenon can be observed in the mouse HTR. This effect has been attributed to activation of 5-HT_{2A} receptors at low doses, but 5-HT_{2C} receptor agonist activity at higher doses (Halberstadt et al., 2009). This interaction is explored more fully in the section in this review on the use of animal models.

Although it is believed that dopaminergic systems are not directly involved in the mechanism of action of classic serotonergic hallucinogens, LSD is a unique agent with known high affinity and agonist activity at dopamine receptors (e.g., see Watts et al., 1995). Marona-Lewicka et al. (2005) first demonstrated that the effect of LSD as a training stimulus occurs in two temporal phases. When rats are trained to discriminate 0.08 mg/kg LSD, given 30 minutes prior to training (LSD30 rats), the stimulus generalizes to classic psychedelics and is blocked by 5-HT_{2A} receptor antagonists. If, however, LSD (0.16 mg/kg) is administered 90 minutes prior to training (LSD90 rats), the stimulus is no longer blocked by 5-HT_{2A} antagonists, does not generalize to classic psychedelics, and is blocked by dopamine D_2-like antagonists. In rats trained to discriminate LSD administered 90 minutes prior to training, the cue generalized to the dopamine D_2-like agonists apomorphine, *N*-propyldihydrexidine, and quinelorane. Although a slightly larger dose of LSD is required to maintain the salience of the LSD cue out to 90 minutes, if the higher dose of LSD is administered 30 minutes prior to training, the cue is still found to be mediated by 5-HT_{2A} receptor activation. Therefore, the time between administration of LSD and training determines the nature of the discriminative cue.

In a subsequent study, Marona-Lewicka and Nichols (2007) compared the stimulus effects of LSD administered either 30 or 90 minutes prior to training and employed a number of different agonist and antagonist ligands to elucidate the nature of the cues. Mescaline, DMT, *N,N*-diethyltryptamine, and psilocin fully substituted in LSD30 rats but produced only saline-appropriate responding in LSD90 rats. The highly selective 5-HT_{2A} antagonist MDL11939 [α-phenyl-1-(2-phenylethyl)-4-piperidine methanol] blocked the LSD30 cue, but not the LSD90 cue, confirming that activation of the 5-HT_{2A} receptor is not essential for the delayed effect of LSD. The 5-HT_{1A} antagonist/D_4 agonist WAY-100635 did not block the LSD-90 cue, but surprisingly fully substituted in LSD90 rats. Nevertheless, earlier studies had shown that WAY 100635 had potent dopamine D_4 agonist properties, and that rats could be trained to discriminate the dopamine D_4 agonist effect of WAY-100635 from saline (Chemel et al., 2006; Marona-Lewicka and Nichols, 2009).

Thus, in a third study, Marona-Lewicka et al. (2009) examined whether the LSD90 cue resulted from dopamine D_4 receptor activation. Again, LSD30 and LSD90 rats were tested and compared with DOI-trained rats. Combinations were tested of training drugs with the 5-HT_{1A} antagonist/D_4 agonist WAY-100635, the selective D_4 antagonists L-745,870 [3-[[4-(4-chlorophenyl) piperazin-1-yl]methyl]-1-1*H*-pyrollo[2,3-*b*]pyridine] and A-381393 [2-[4-(3,4-dimethylphenlyl)piperazin-1-ylmethyl]-1*H* benzoimidazole], the selective D_4 agonist ABT-724 [2-[(4-pyridin-2-ylpiperazin-1-yl)methyl]-1H-benzimidazole)], and the atypical antipsychotic drugs clozapine and olanzapine. WAY-100635 produced full substitution in LSD90 rats, only partial substitution in LSD30 rats, and saline-appropriate responding in DOI-trained rats. ABT-724 partially mimicked the LSD30 and LSD90 cues but produced no substitution in DOI-trained rats. In combination tests, ABT-724 potently enhanced the LSD90 cue; the D_4 antagonists significantly attenuated the LSD cues but had no effect on the DOI cue. It was concluded that dopamine D_4 receptor activation plays a key role in the discriminative stimulus properties of LSD when administered to rats 90 minutes prior to training but is not involved in the cue produced by DOI. Furthermore, functional tests in human embryonic kidney cells stably expressing the human $D_{4.4}$ receptor demonstrated that

LSD was a full agonist with an EC_{50} virtually identical to the standard D_2-like agonist quinpirole (11 versus 9.6 nM, respectively) for inhibiting forskolin-stimulated cAMP accumulation.

The significance of this delayed dopamine D_4-mediated pharmacology of LSD in rats remains unclear, but Daniel X. Freedman first described the clinical effects of LSD as occurring in two temporal phases: an early "psychedelic" phase, followed by a later phase 4–6 hours after LSD administration, and at times out to 10 hours, where subjects reported "they had been at the least self-centered, and usually suspicious, with ideas of reference or even paranoid convictions" (Freedman, 1984). The only other report to indicate that LSD had two phases of action was the finding that LSD-induced spontaneous behavioral effects in rats occurred in two phases, an initial suppression of behavioral responding, followed by a subsequent increase in locomotor activity that was not observed with other serotonergic agonists (Mittman and Geyer, 1991). Because of the limited number of pretreatments employed in that study, one could not clearly deduce a mechanistic basis for these different effects. Nonetheless, the delayed increase in locomotor activity that is peculiar to LSD would be consistent with a dopaminergic action. Although the dopaminergic properties of LSD have been recognized for many years (Nichols, 1976); also see references in Watts et al., 1995), the significance of this dopaminergic action has perhaps not been widely appreciated.

With Freedman's (1984) comments in mind, it was noted that the rats trained with 0.16 mg/kg LSD for several months began to show increased hyperactivity, irritability, and hyper-reactivity to external stimuli, behavioral effects that had not been previously reported by others who had used this training dose of LSD for drug discrimination studies. Based on the dopaminergic nature of the LSD90 cue, it was speculated that these animals might manifest behaviors that resembled core symptoms of schizophrenia. Thus, these animals were further investigated by Marona-Lewicka et al. (2011) to determine whether chronic treatment of rats with LSD might represent a new animal model of schizophrenia. Drug discrimination followed the methods described in their earlier studies and used a two-lever FR10 food-reinforced paradigm. A variety of different drugs were used in combination tests and antagonism tests to examine in more detail the differences between animals trained with the lower 0.08 mg/kg LSD dose 30 minutes prior to training (LSD30 rats), which did not lead to long-term behavioral changes, and the animals trained 90 minutes after 0.16 mg/kg (LSD90 rats).

In separate experiments, groups of rats were given either 0.08 or 0.16 mg/kg LSD every other day for 3 months. These rats did not receive drug discrimination training but were used for behavioral tests and for analysis of mRNA isolated from the mPFC 1 month after cessation of LSD treatment. In the drug discrimination experiments, M100907, olanzapine, and clozapine completely blocked the LSD30 cue, but haloperidol produced only a slight 20% nonsignificant inhibition of the cue. In LSD90 rats M100907 produced a maximum of only 32% inhibition of the cue, but ziprasidone, olanzapine, clozapine, and haloperidol reduced drug-appropriate responding up to 80%. The nonselective NMDA antagonists PCP, ketamine, and MK-801 [(5*R*,10*S*)-(−)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cylcohepten-5,10-imine] were also tested in both groups of rats, in both young and old (>12 months) rats. MK-801 and PCP produced partial substitution in both young and old LSD30 rats, with more than 50% disruption at higher doses. Ketamine was recognized as saline by some rats but produced behavioral disruption at higher doses. In young LSD90 rats, all three NMDA antagonists produced partial substitution (60%–71%); however, in older rats (>12 months), all three NMDA antagonists surprisingly produced full substitution with little disruption at high doses.

Two weeks after ending the 3-month 0.16 mg/kg LSD treatment, rats had significantly elevated locomotor activity compared with saline-injected controls, which was not blocked by M100907 but was blocked by haloperidol and olanzapine. Rats also were hyper-reflexive to external stimuli. One month after cessation of 0.16 mg/kg LSD treatment, rats also had significantly altered social behavior, with reduced sniffing, grooming, and following and markedly enhanced aggressive (boxing, kicking, wrestling) and exploratory (sniffing, rearing, hole poking) behaviors. The preference for sucrose solution in control rats was also lost in rats chronically treated with 0.16 mg/kg LSD, indicating a state of anhedonia in these rats. On the basis of these results and their earlier studies, the authors suggest that rats chronically treated with LSD may represent a new animal model of psychosis, with the advantage that the animals can be used long after the LSD treatment has been ended.

Altered behaviors that persisted at full strength long after LSD was discontinued (Marona-Lewicka et al., 2011) suggested that long-term LSD administration in rats permanently shifted brain neurochemistry and gene expression from a normal to a pathologic state resembling that observed in rodent models of schizophrenia. To investigate how long-term LSD administration affected gene expression in the brain, Martin et al. (2014) performed RNA sequencing on RNA isolated from the mPFC of rats 4 weeks after cessation of a 90-day treatment protocol with LSD or saline. They identified several hundred relatively low-magnitude (<2-fold) but significant transcriptional changes in the mPFC of LSD-treated animals long after drug administration ended. Functional clustering analysis indicated that the altered genes were significantly concentrated in pathways related to neurotransmission, synaptic plasticity, and metabolism (Martin et al.,

2014). Several unanticipated clusters of genes were identified that included those involved in RNA processing and endocrine function, with a significant enrichment of altered transcripts that have been implicated in schizophrenia by others, including genes for the dopamine D_1 and D_2 receptors, brain-derived neurotrophic factor (BDNF), receptor tyrosine-protein kinase erbB-4 (ERBB4), and various NMDA and GABA receptor subunits (see references in Martin et al., 2014).

Minuzzi et al. (2005) used [^{11}C]raclopride PET in living pig brain to examine the effects of LSD on dopamine $D_{2/3}$ receptor binding. They observed an unusual progressive displacement of the PET ligand that only reached a maximum 240 minutes after LSD administration. The authors speculate that "This time course seems consistent with the prolonged psychoactive action of LSD in humans." Optimal receptor occupancy might be expected fairly quickly after intravenous LSD administration, however, not 4 hours later. The authors also suggest that "…LSD might evoke a serotonin 5-HT_2 receptor–mediated sensitization of dopamine $D_{2/3}$ receptors," a mechanism that also has been offered to explain an enhanced behavioral response to amphetamine after a 2- or 3-hour pretreatment with DOI or LSD (Marona-Lewicka and Nichols, 1997). One might speculate that the initial phase of pharmacology for LSD involves activation of serotonin 5-HT_{2A} receptors, and that this action leads to a sensitization of dopaminergic systems in the CNS. Then, the intrinsic dopaminergic effects of LSD would become potentiated, leading to a delayed dopamine-mediated action in the effect of LSD. It also seems possible that one of the metabolites of LSD (e.g., 13-hydroxyLSD; see Parli et al., 1978) might be a highly potent dopaminergic agonist; thus far, no one has studied the pharmacology of hydroxylated metabolites of LSD.

Interestingly, Fontanilla et al. (2009) reported that DMT bound to σ-1 receptors and inhibited voltage-gated sodium channels in both native cardiac myocytes and heterologous cells expressing the σ-1 receptor. DMT reduced locomotor activity in WT mice, but not in σ-1 KO mice. Patch-clamp recordings in HEK-293 cells stably expressing the human cardiac Na^+ channel hNav1.5 showed that application of 100 μM DMT inhibited the I_{Na} by 62%. The K_D for DMT at σ-1 receptors, however, was 14.75 μM. The authors conclude on the basis of this and other data in their report that DMT may serve as an endogenous sigma receptor ligand. Although that conclusion may be valid, the low K_D for DMT at the σ-1 receptor, and the fact that only trace amounts of DMT are apparently produced within the body and are rapidly broken down by MAOs would seem to argue that the relevance of σ receptor activation to the psychedelic effects of DMT is questionable, at best.

In addition to direct receptor effects, Cozzi et al. (2009) have demonstrated that DMT, DPT, and *N,N*-diisopropyltryptamine also inhibit the serotonin transporter (SERT) in human platelets, with K_I values of 4 μM, 8.88 μM, 0.59 μM, and 2.3 μM, respectively. They also were inhibitors of rat vesicle monoamine transporter 2 expressed in Sf9 cells, where they were somewhat less potent. However, they were poor inhibitors of [^{3}H]paroxetine binding to the SERT and of [^{3}H] dihydrotetrabenazine binding to vesicle monoamine transporter 2. These high binding to uptake ratios were taken to support the hypothesis that the studied tryptamines were transporter substrates, rather than uptake blockers. The authors suggest that as substrates they might be accumulated within neurons and perhaps function as releasable transmitters. Another possibility, not considered by the investigators, is that these tryptamines might be taken up into serotonin neuron terminals and might displace stored intraneuronal serotonin, in a mechanism similar to a 5-HT releasing agent such as MDMA. The release of stored serotonin would enhance synaptic levels of 5-HT, potentially adding an additional component to the pharmacology of certain tryptamines.

Nagai et al. (2007) studied the ability of a variety of psychoactive drugs to block reuptake of dopamine, 5-HT, and NE into rat brain synaptosomes. Several known psychedelic phenethylamines were studied, as were selected tryptamines. They included DPT, one of the drugs examined by Cozzi et al. (2009). The six phenethylamines had very weak uptake inhibition properties, with IC_{50} values ranging from 30 to 80 μM. DPT had IC_{50} values for reuptake inhibition of dopamine, 5-HT, and NE of 23 μM, 2.9 μM, and 9.1 μM, respectively, somewhat less potent than those found by Cozzi et al. (2009) using human platelets. The ability of the compounds to induce release of stored monoamines from rat brain synaptosomes also was studied. The six phenethylamines were virtually inactive, as was DPT, having no effect at 10^{-4} M of the drug. These results seem to suggest that, in general, effects of psychedelics on monoamine reuptake and release may not be relevant and that direct receptor actions remain most important.

IV. Where Is the Serotonin 5-Hydroxytryptamine 2A Receptor Expressed?

To invoke a mechanism of action for psychedelics that involves activation of serotonin 5-HT_{2A} receptors, it is instructive to examine brain areas where that receptor is highly expressed. One would expect to find high receptor levels in key brain regions that are responsible for sensory processing and cognition. The 5-HT_{2A} receptor is in fact expressed in those very brain areas that include, among others, the neocortex, thalamus, locus coeruleus (LC), and ventral tegmental area (VTA), and recognizing the functional importance of these brain regions will help to understand the complex pharmacology of psychedelics.

A. Serotonin 5-Hydroxytryptamine 2A Receptor Expression in the Cortex

The PFC is the executive area of the brain where incoming sensory information and affective output from the limbic areas converges, creating our sense of awareness of our environment and being involved in higher cognitive functions. The PFC in primates coordinates actions such as complex cognitive and affective functions, with many reciprocal fiber connections from other neocortical areas, as well as with numerous subcortical and limbic structures. The concept that psychedelics would have their major site of action in the cortex is certainly consistent with the powerful psychoactive effects of these substances.

Early receptor mapping studies in rat brain using autoradiography with tritiated antagonist ligands identified brain areas with high 5-HT_2 receptor density (Pazos et al., 1985). Highest binding was seen in the claustrum, but with high expression also in all laminae of the neocortex. The highest receptor density within the cortex was localized to a continuous band that included lamina IV and extended into lamina III, depending on the area studied. In humans, PET with *N*1-[^{11}C]-methyl)-2-bromo-LSD revealed highest binding in the frontal and temporal cortices, lower expression in parietal cortex and motor regions, with intermediate levels in basal ganglia, and only low levels in thalamus (Wong et al., 1987).

Pazos et al. (1987) examined 5-HT_2 receptor distribution in the human brain using light microscopic autoradiography with the 5-HT_{2A} antagonist ligand [^{3}H]ketanserin. A heterogeneous distribution of 5-HT_2 receptor densities was observed, with high expression localized over layers III and V of several cortical areas, including the frontal, parietal, temporal, and occipital lobes, the anterogenual cortex, and the entorhinal area. Their findings were consistent with the observation of a dense band of 5-HT_2 receptors in upper cortical layer V in register with a dense plexus of fine 5-HT axons (Blue et al., 1988). A mRNA in situ hybridization study of human cortex demonstrated the 5-HT_{2A} receptor on both pyramidal and nonpyramidal cells (Burnet et al., 1995).

Autoradiography in the rat brain using *R*-(−)-[^{125}I] DOI revealed highest binding in the claustrum and the frontal cortex (McKenna and Saavedra, 1987). Lower expression was observed in the caudate, nucleus accumbens, and olfactory tubercle. Other autoradiographic and in situ hybridization studies have observed high densities of 5-HT_{2A} receptors and transcripts in the cortex (Blue et al., 1988; Mengod et al., 1990; Wright et al., 1995).

Séguéla et al. (1989) found that cortical synaptic 5-HT terminals always made asymmetric junctions exclusively located on dendritic spines and shafts, expressed more frequently on spines in the deep frontal and the upper occipital cortex. They reported that cortical 5-HT innervation in the adult rat was predominantly (approximately 60%) nonjunctional throughout the neocortex.

Willins et al. (1997), using 5-HT_{2A} receptor antibodies, reported dense 5-HT_{2A} receptor expression in apical dendrites of pyramidal cells in the rat cortex, with most of the 5-HT_{2A}–like immunoreactivity associated with the plasma membrane. A small amount of labeling was also seen on cortical interneurons. Localization of 5-HT_{2A} receptors on cortical pyramidal cells is consistent with electrophysiological data suggesting that psychedelics have excitatory effects on projection neurons in the neocortex (Araneda and Andrade, 1991; Ashby et al., 1994). Furthermore, microiontophoresis of serotonin in "hot spots" near the border of layers I/II and IV/Va induced EPSC frequency increases in layer V pyramidal cells (Aghajanian and Marek, 1997), an area with dense 5-HT_{2A} receptor expression. This finding is consistent with the hypothesis that the most potent 5-HT_{2A} receptor–mediated cortical actions of psychedelics occur at hot spots on proximal apical dendritic shafts.

A later study of 5-HT_{2A} receptor localization in the rat cortex by Miner et al. (2003) employed immunoperoxidase labeling to determine the localization of 5-HT_{2A} receptors in the middle layers of the rat PFC. Using a polyclonal antibody, they found most 5-HT_{2A} receptors to be expressed on postsynaptic structures, predominantly on proximal and distal dendritic shafts, apparently on both pyramidal and local circuit neurons. Most often, Miner et al. (2003) observed that 5-HT_{2A} receptors were restricted to a particular area of the dendrite, usually extrasynaptic regions apposed to unlabeled dendrites. They reported that 73% of the immunopositive sites were postsynaptic, and 58% of those were on dendritic shafts, with 42% expressed in dendritic spines. This study provided the first evidence of extensive localization of 5-HT_{2A} receptors to the heads and necks of dendritic spines, and the findings were consistent with those of Séguéla et al. (1989), who found that synaptic serotonin terminals always made asymmetric junctions exclusively found on dendritic spines and shafts, appearing more frequently on spines than shafts in the deep frontal and the upper occipital cortex. A postsynaptic localization is also consistent with the reports by Xia et al. (2003a,b), who demonstrated that 5-HT_{2A} receptors interact with PSD-95, the major protein of postsynaptic densities in asymmetric synapses.

The finding by Miner et al. (2003) that a significant fraction of 5-HT_{2A} receptors were localized to extrasynaptic portions of dendritic shafts suggests that serotonin within the PFC may exhibit at least some of its actions through volume transmission mechanisms (e.g., see Agnati et al., 1995; Zoli et al., 1998). This hypothesis is consistent with immunocytochemical studies by Jansson et al. (2001), in which 5-HT_{2A} receptor labeling was located distal from 5-HT terminals in other regions of rat cortex. Miner et al. (2003)

also found little evidence for significant presynaptic 5-HT_{2A} receptor expression. Séguéla et al. (1989) estimated that only about 38% of serotonin axons in the cortex engaged in synaptic contact. Furthermore, serotonin reuptake transporters are frequently located at extrasynaptic sites in the PFC (Miner et al., 2000). On the basis of their results as well as other data, Miner et al. (2003) proposed that cortical 5-HT innervation is primarily nonjunctional and that the entire cortical volume may be reached by serotonin. They suggest that some of the cortical actions of 5-HT may be constantly exerted, with more or less efficacy at the various 5-HT receptors, thus providing widespread global and/or sustained influence in the neocortex.

Nevertheless, Miner et al. (2003) did identify a significant 24% fraction of 5-HT_{2A} receptor immunoreactive profiles that were presynaptic. The structures were thin, were unmyelinated, rarely formed synaptic contacts in single sections, and sometimes contained dense-core vesicles, suggesting that they might be monoaminergic axons. Miner et al. (2003) suggested that these might include dopaminergic fibers, consistent with a report by Pehek et al. (2001) that 5-HT_{2A} receptors may modulate cortical dopaminergic function.

Using double in situ hybridization to quantify mRNA colocalization of the 5-HT_{2A} receptor with the vesicular glutamate transporter 1, and with the GABAergic marker GAD65/67, and in parvalbumin and calbindin GABAergic cell populations, de Almeida and Mengod (2007) reported that 86%–100% of glutamatergic cells in cortical layers II–V in both monkey and human brain expressed 5-HT_{2A} receptor mRNA. Layer VI had only 13%–31% expression. In GABAergic interneurons, 5-HT_{2A} mRNA was expressed in 45%–69% of parvalbumin and 61%–87% of calbindin-positive cells. Parvalbumin cells are fast-spiking interneurons with the morphology of chandelier and large basket cells, whereas calbindin cells are nonfast-spiking interneurons with a double-bouquet cell morphology (see Mengod et al., 2015, and references therein). Thus, the authors concluded that the majority of glutamatergic neurons express 5-HT_{2A} receptors, whereas this expression occurs only in a limited population of GABAergic neurons.

To determine the specific cell types expressing 5-HT_{2A} receptors in the cortex, Weber and Andrade (2010) used BAC transgenic mice engineered to express EGFP under the control of the 5-HT_{2A} receptor promoter. EGFP expression was used to identify 5-HT_{2A} gene-expressing neurons both in vivo and in vitro. They also used 5-HT_{2A} KO mice, as well as a third strain of mice that expressed YFP under control of the Thy-1 promoter in pyramidal cells of layer Vb. Data were compared using these three transgenic mice to demonstrate that 5-HT_{2A} receptors in the cortex are expressed predominantly by three discrete populations of cells: layer V pyramidal cells of the anterior cerebral cortex, a subpopulation of GABAergic interneurons restricted to the middle layers of the cortex, and nonpyramidal cells of the subplate/layer VIb. A large percentage of the GABAergic interneurons were parvalbumin-expressing fast-spiking interneurons, consistent with work from other laboratories (de Almeida and Mengod, 2007; Puig et al., 2010). Taken together with results from others, the authors indicate that "5-HT_{2A} receptors are expressed at loci critical for controlling information processing in cerebral cortex" (Weber and Andrade, 2010).

Most recently, Mengod et al. (2015) reviewed the cartography of 5-HT_{1A} and 5-HT_{2A} receptor subtypes in the PFC and its projections. They conclude that in the rat PFC, 5-HT_{2A} receptors are expressed in pyramidal tract neurons that project to the dorsal raphe nucleus, VTA, and nucleus accumbens.

Nordstrom et al. (2008) used [^{11}C]*N*-methylspiperone (NMS) PET analysis in four human subjects with the 5-HT_{2A} receptor inverse agonist ACP-103 [*N*-(4-fluorophenylmethyl)-*N*-(1-methylpiperidin-4-yl)-*N*′-(4-(2-methylpropyloxy)phenylmethyl)carbamide]. Cortical [^{11}C]NMS binding was measured by PET after escalating single oral doses of ACP-103. Displacement of [^{11}C]NMS was about half-maximal after a 5-mg dose and near maximal displacement after 10- to 20-mg doses.

Ettrup et al. (2010) evaluated [^{11}C]CIMBI-5 [*N*-(2-methoxybenzyl)-2,5-dimethoxy-4-iodophenethylamine] (25I-NBOMe) as an agonist radioligand for PET imaging of 5-HT_{2A} receptors. [^{11}C]CIMBI-5 showed high cortical uptake in vivo in pig brain. CIMBI-5 was the first 5-HT_{2A} agonist PET ligand developed. [^{11}C]CIMBI-5 and the 5-HT_{2A} antagonist PET ligand [^{18}F]altanserin showed similar cortex-to-cerebellum uptake and had similar target-to-background ratios. In a subsequent publication, Ettrup et al. (2011) examined a series of nine structural congeners of CIMBI-5 to identify one with improved target-to-background binding. Their most promising candidate proved to be [^{11}C]Cimbi-36, which was further characterized by Finnema et al. (2014) as an improved agonist PET radioligand for in vivo imaging of 5-HT_{2A} and 5-HT_{2C} receptors in the nonhuman primate brain.

B. Serotonin 5-Hydroxytryptamine 2A Receptor Expression in the Thalamus and Reticular Nucleus

In addition to the cortex, the thalamus and reticular nucleus of the thalamus may also be important sites of action for psychedelics. The 5-HT_{2A} receptor is expressed in the thalamus, primarily in sensory and "nonspecific" nuclei (Cornea-Hébert et al., 1999). In the rat brain, significant levels of 5-HT_{2A} receptor mRNA are expressed in the reticular nucleus, lateral geniculate nucleus, zona incerta, and the anterodorsal and ventromedial nucleus of the thalamus (Cyr et al., 2000). Pompeiano et al. (1994) also found 5-HT_{2A} mRNA in the reticular nucleus, lateral geniculate, and zona incerta, but none in the midline or intralaminar nuclei. The

thalamus, along with the amygdala, represents the major source of glutamate afferents innervating the neocortex. The thalamus not only processes somatosensory inputs, but it also receives afferents from both the raphe nuclei and the LC (Asanuma, 1992). It is interesting that very small bilateral lesions in the intralaminar nuclei, which project axons widely to all cortical areas, can lead to permanent loss of consciousness (Bogen, 1997).

The reticular nucleus is of particular interest here because it is thought to serve as a sort of gate for processing signals to the cortex. It is a thin sheet of GABAergic neurons that has functionally distinct afferent and efferent connections with thalamic nuclei, the neocortex, the basal forebrain, and the brainstem. Rodríguez et al. (2011) found that the two major postsynaptic serotonin receptors in the reticular nucleus were of the 5-HT_{1A} and 5-HT_{2A} subtypes. Serotonergic projections from the dorsal raphe and supralemniscal nucleus (B9) were demonstrated to be the principal sources of raphe projections to the reticular nucleus.

The reticular nucleus is known to regulate the flow of information between the thalamus and the cortex and sends inhibitory projections into the thalamus, apparently serving a negative-feedback regulatory role in thalamic function. It has been proposed to serve as a sort of "searchlight" of attention (Crick, 1984; Sherman and Guillery, 1996) and to control elements of signal to noise or the quality of information being sent to the cortex (see Vollenweider and Geyer, 2001, and references therein).

C. Serotonin 5-Hydroxytryptamine 2A Receptor Expression in Primary Visual Cortex V1

One of the prominent clinical features of psychedelic drugs is their effect on visual perception, even at relatively low doses. Subjects report visual phenomena such as "walls breathing," "curtains waving," or undulating patterns in carpets, visual arabesques, complex textures, and so forth. Certainly these illusory effects could arise through a variety of mechanisms, because 5-HT_{2A} receptors are expressed in many areas of the brain responsible for cognition and sensory processing. It has not been widely appreciated, however, that mammalian primary visual cortex area V1, the largest known visual cortical area, expresses a high density of 5-HT_{2A} receptors (Watakabe et al., 2009; Moreau et al., 2010). Microiontophoresis of DOI into macaque V1 gave a bidirectional modulatory effect on the neuron's firing rate. Analysis of recordings from 44 neurons showed that DOI facilitates visual responses of neurons with a low firing rate but suppressed those of neurons with a high firing rate. This effect of DOI was blocked by ketanserin. The authors suggest that neurons in the input layers of V1, which abundantly express the 5-HT_{2A} receptor, may act as gain controllers by enhancing weak signal response and suppressing excessive response.

D. Effects of Psychedelics on Raphe Cell Firing

Midbrain raphe cells send serotonergic projections throughout the forebrain and are the source of serotonin afferents in the PFC (Moore et al., 1978). Raphe cells fire at a characteristic regular rate that is generally correlated with a mammalian organism's level of vigilance or attention. When the organism is awake, the cells fire at a characteristic slow regular rate; as the organism grows drowsy, the rate of firing slows. During the rapid eye movement (REM) sleep stage, raphe cell firing essentially stops completely. As a consequence of slowed raphe firing, serotonin release in projection areas declines or ceases.

Early experiments found that LSD potently suppressed cell firing in the dorsal raphe nucleus if it was given systemically (Aghajanian et al., 1968, 1970) or applied by microiontophoresis directly to the raphe cell bodies (Aghajanian et al., 1972). Tryptamine psychedelics all inhibited dorsal raphe cell firing (Aghajanian et al., 1970; Aghajanian and Hailgler, 1975; deMontigny and Aghajanian, 1977), leading Aghajanian and Hailgler (1975) to hypothesize that this suppressant effect on raphe cell firing might be the underlying basis for the action of psychedelics.

In subsequent studies, phenethylamine psychedelics failed to have a direct suppressant effect on raphe cell firing. After additional experiments, it was ultimately discovered that suppression of raphe cell firing by psychedelic tryptamines resulted from stimulation of 5-HT_{1A} somatodendritic autoreceptors, and nonhallucinogenic 5-HT_{1A} agonists were identified that also suppressed raphe firing (Sprouse and Aghajanian, 1987, 1988). Phenethylamine-type psychedelics such as mescaline lack 5-HT_{1A} agonist activity, so this hypothesis for the mechanism of action of psychedelics was, therefore, not tenable. Nevertheless, phenethylamine-type psychedelics do suppress firing of a subset of raphe cells when given systemically but not when administered directly into the raphe (Aghajanian et al., 1970; Haigler and Aghajanian, 1973). This suppressant effect by phenethylamine psychedelics is thought to occur through an indirect GABA-mediated mechanism (Liu et al., 2000; Martín-Ruiz et al., 2001). Thus, an increased GABA release onto raphe cells may explain the previous observation of an indirect suppression of 5-HT cells in the dorsal raphe induced by phenethylamine psychedelics in vivo.

E. Serotonin 5-Hydroxytryptamine 2A Receptor Expression in the Ventral Tegmental Area

Anatomic and physiologic data suggest that a likely site for serotonergic modulation of dopaminergic transmission is through direct actions on mesolimbic and mesocortical dopaminergic transmission in the VTA (see Doherty and Pickel, 2000, and references therein). A variety of reports provide evidence for direct and

indirect modulation of VTA dopaminergic neurons through activation of 5-HT_{2A} receptors in this region. Cornea-Hébert et al. (1999) identified somatodendritic localization of 5-HT_{2A} receptors in the VTA, and depolarization of dopamine cells in VTA slice preparations can be blocked by the 5-HT_{2A}–selective antagonist ketanserin (Pessia et al., 1994).

An electron microscopy immunocytochemical study showed localization of 5-HT_{2A} receptors in both the parabrachial and paranigral regions of the VTA, with label identified primarily as dendrites and unmyelinated axons (Doherty and Pickel, 2000). Dendrites commonly showed 5-HT_{2A} receptor immunoreactivity colocalized with tyrosine hydroxylase. Thus, 5-HT_{2A} receptor activation may directly affect local dendritic release of dopamine as well as release of dopamine in mesocortical and mesolimbic terminal fields. A substantial number of 5-HT_{2A}–labeled dendrites were also detected that did not contain tyrosine hydroxylase immunoreactivity, suggesting 5-HT_{2A} receptor modulation of other nondopaminergic, perhaps GABAergic, interneurons in the VTA.

In a study using fluorescence immunohistochemistry with confocal microscopy, Nocjar et al. (2002) found that 5-HT_{2A} receptors were colocalized, in part, to tyrosine hydroxylase–containing cells throughout all subnuclei of the VTA, most prominently in the anterior region, principally within the rostral and midparanigral, parabrachial, and intrafascicular nuclei. Thus, activation of 5-HT_{2A} receptors by psychedelics would be expected to modulate dopaminergic activity of VTA cells either directly or indirectly through nondopaminergic neurons, and effect excitatory dopamine release from projections in cortical and limbic structures.

Pehek et al. (2001) used in vivo microdialysis in the rat mPFC to show that direct infusion of the selective 5-HT_{2A} antagonist M100907 through the dialysis probe produced a concentration-dependent block of K^+-stimulated dopamine release. Direct infusion of M100907 into the mPFC also blocked increased extracellular dopamine produced by systemically administered DOI. They concluded that activation of cortical 5-HT_{2A} receptors potentiated the phasic release of mesocortical dopamine.

Similarly, systemic administration of DOI to freely moving rats dose-dependently increased dialysate levels of dopamine and NE in the frontal cortex. This effect was abolished by the selective 5-HT_{2A} antagonist M100907, which by itself did not change dopamine or NE levels. By contrast, the selective 5-$HT_{2B/2C}$ antagonist SB-206553 (3,5-dihydro-5-methyl-*N*-3-pyridinylbenzo[1,2-*b*:4,5-*b*']dipyrrole-1(2*H*)-carboxamide hydrochloride) potentiated the effect of DOI. The authors concluded that 5-HT_{2A} receptors exert a phasic facilitatory influence on cortical levels of dopamine and NE, whereas 5-HT_{2C} receptors exert an inhibitory effect.

Finally, in healthy human volunteers, PET has been used to study a possible role for dopamine in the effects of psilocybin. An effective dose of psilocybin, which lacks significant affinity for dopamine receptors, significantly decreased binding of the dopamine D_2 antagonist [^{11}C]raclopride in both the caudate nucleus and putamen, a finding that would be consistent with an increase in extracellular dopamine (Vollenweider et al., 1999).

F. Effect of Psychedelics on the Locus Coeruleus

Although the majority of studies point to a major site of action for psychedelics in the frontal cortex, perhaps with important involvement of the thalamus, psychedelics also have a potent effect on the LC. This finding is very intriguing because the LC is a point of convergence for widely ranging somatosensory and visceral sensory inputs from all regions of the body. The LC has been likened to a "novelty detector" for salient external stimuli (Cedarbaum and Aghajanian, 1978; Aston-Jones and Bloom, 1981). The LC sends NE projections diffusely to all parts of the neuraxis, including the cerebral cortex (Aghajanian and Marek, 1999a). Within the cortex, 5-HT_{2A} and α_1-adrenergic receptors share a similar regional and laminar distribution, with heaviest concentrations in layer Va (see Marek and Aghajanian, 1999, and references therein). Furthermore, activation of either 5-HT_{2A} or α_1 adrenergic receptors modulates cortical pyramidal cells and interneurons in a parallel fashion (Marek and Aghajanian, 1994, 1996, 1999).

Using double in situ hybridization, Santana et al. (2013) performed a quantitative study of the expression of α_{1A}, α_{1B}, and α_{1D} adrenergic receptors in pyramidal vesicular glutamate transporter 1–positive and GABAergic (GAD65/67–positive) cells of rat PFC. Given the common signaling pathways shared by 5-HT_{2A} and α_1 adrenergic receptors, they examined the coexpression of both receptors in the rat PFC using double in situ hybridization histochemistry. They found that virtually all subdivisions of the PFC contained cells expressing one or more α_1 adrenergic receptors. The most abundant transcripts were those corresponding to α_{1A} and α_{1D} adrenergic receptors. The various α_1 adrenergic receptor transcripts showed an almost nonoverlapping regional distribution within the mPFC that was particularly evident for α_{1A} and α_{1D} adrenergic receptors. The former transcript was densely expressed in deep layers VIa and VIb of the medial, dorsal, and lateral (agranular insular) PFC and the claustrum, as well as in ventral areas such as the orbital and piriform cortices and the tenia tecta. By contrast, the α_{1D} adrenergic receptor transcript was especially abundant in layers II to III, and outer layer V in medial and dorsolateral aspects of the PFC. All three types of α_1 adrenergic receptors were observed to be present in pyramidal (vesicular glutamate transporter 1–positive) and GABAergic (GAD65/67–positive) neurons. Pyramidal neurons expressing α_{1A} adrenergic receptors were more abundantly localized in deep layers V to VI in the three mPFC subdivisions.

The regional distribution of α_1 adrenergic receptor and 5-HT_{2A} receptor mRNA was very similar in the

PFC, with the exception of layer VI, and the more ventral part of the infralimbic cortex, with low 5-HT_{2A} receptor mRNA expression, whereas virtually all other areas in the PFC showed an abundant expression of both receptor transcripts. Taking 5-HT_{2A}–expressing cells as 100%, the percentage of cells also expressing α_1 adrenergic receptors varied between 44% in the infralimbic area to 75% and 80%, respectively, in the prelimbic and cingulate areas. Each α_1 adrenergic receptor type was extensively coexpressed with 5-HT_{2A} receptors, indicating a convergence of excitatory noradrenergic and serotonergic signals in the same PFC neurons. The different α_1 adrenergic receptors also were expressed in a high proportion of GABAergic interneurons, similar to that seen in pyramidal neurons (69%–74% in superficial layers; 52%–73% in deep layers). Overall, the presence of α_1 adrenergic receptors in such a large proportion of pyramidal and GABAergic neurons suggested to the authors that NE can modulate the activity of most PFC output neurons via direct or indirect actions.

Systemic administration of LSD, mescaline, or other phenethylamine psychedelics to anesthetized rats decreased spontaneous activity of LC cells but surprisingly enhanced the activity of LC neurons evoked by sensory stimuli (Aghajanian, 1980; Rasmussen and Aghajanian, 1986; Rasmussen et al., 1986). Direct microiontophoretic application of the drugs onto LC cell bodies did not have the same effect, indicating some indirect effect of the drugs. The action did depend on 5-HT_{2A} receptor activation, however, because systemic administration of ritanserin, a 5-HT_{2A} antagonist, blocked the effect.

Similarly, systemic, but not local, administration of DOI suppressed spontaneous LC activity but enhanced responses to somatosensory stimulation, with both effects being blocked by systemic administration of ketanserin, a 5-HT_{2A}–selective antagonist (Chiang and Aston-Jones, 1993). DOI-induced suppression of LC firing was blocked by local infusion of GABA antagonists, and enhanced responses to external stimuli were blocked by an NMDA antagonist. These investigators proposed that systemic administration of 5-HT_2 agonists suppressed LC firing indirectly, through tonic activation of inhibitory GABAergic inputs to the LC. They proposed that the facilitating effect on sensory inputs was mediated through excitatory amino acid receptors in the LC.

As a "novelty detector," the LC has been viewed as enhancing the signal to noise ratio in modulating postsynaptic activity throughout the brain; suppression of basal activity concomitantly with enhanced responding to external sensory stimuli would amplify this effect (see Marek and Aghajanian, 1998b, and references therein). Thus, after application of a psychedelic, one might speculate that sensory events that would be perceived as ordinary might instead be perceived as having increased novelty. Indeed, it is a well known anecdote that under the influence of psychedelics, one may perceive very ordinary objects as new or novel.

The LC sends noradrenergic projections to the cortex, where α_1 adrenergic and serotonin 5-HT_{2A} receptors both share a similar laminar distribution and similar signaling pathways in pyramidal cells. Thus, changes in LC firing would also affect pyramidal cell excitability. It would be very surprising, therefore, if changes in LC firing induced by psychedelics also did not modulate the direct effects of 5-HT_{2A} agonists on cortical cells.

G. Serotonin 5-Hydroxytryptamine 2A Receptor Expression in the Amygdala

The amygdala is a structure located in the medial portion of the temporal lobe and is involved in numerous tasks that include the generation of emotional behavior, the formation of emotional memories related to fear and anxiety, and modulation of the consolidation of explicit memories for emotionally arousing events. Fear conditioning is known to be a model of emotional learning in which amygdala circuits play an important role (see references in Bombardi, 2014). The amygdala receives substantial serotoninergic innervation originating mainly from the dorsal raphe nucleus and, to a lesser extent, from the median raphe nucleus (Pralong et al., 2002; Hensler, 2006; Asan et al., 2013). Autoradiography with [^{3}H]ketanserin has shown light to moderate expression of 5-HT_{2A} receptors in most nuclei of the amygdala, with higher expression in the cortical nucleus and dorsolateral subdivision of the lateral nucleus (Pazos et al., 1985). Similarly, in situ hybridization has demonstrated that most nuclei of the amygdala have moderate levels of 5-HT_{2A} receptor mRNA, with higher levels in the cortical nucleus and dorsolateral subdivision of the lateral nucleus (Wright et al., 1995).

McDonald and Mascagni (2007) used single- and double-labeling immunohistochemical techniques to examine the expression of 5HT_{2A} (and other serotonin receptors) in rat basolateral amygdala. They obtained different patterns of immunostaining, depending on which of three different antibodies they employed. Two of the antibodies (BD and Ab51) gave immunostaining, suggesting that virtually all pyramidal cells in the basolateral nuclear complex of the amygdala express 5-HT_{2A} receptors that are primarily associated with dendrites.

Serotonin regulates amygdalar activity through activation of the 5-HT_2 receptor family, which includes the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. In the deep nuclei of the amygdala, the 5-HT_{2A} receptor is expressed on both excitatory (glutamatergic) pyramidal and inhibitory (GABAergic) nonpyramidal neurons, potentially playing a crucial role in the formation of emotional memories. In the rat, 100% of pyramidal cells in the deep nuclei express the 5-HT_{2A} receptor, where it is strongly expressed in the apical dendrites, similarly

to cortical pyramidal cells, and may induce excitatory synaptic currents. In the rat deep nuclei, 5-HT_{2A} immunoreactivity is seen in both GABAergic interneurons and projection neurons. 5-HT_{2A} receptor immunoreactivity is also observed in every superficial nucleus of the rat amygdala.

The 5-HT_2 receptor family plays a crucial role in regulating the activity of amygdalar microcircuits and projections, modulating both excitatory and inhibitory neurons, in parallel to what is seen in the cerebral cortex. Although the exact role of the 5-HT_{2A} receptor, and other 5-HT_2 receptor family members, is not well understood with respect to the amygdala, it is clear that the 5-HT_{2A} receptor plays an important role in emotional responses and is an important target to be considered in the actions of 5-HT_{2A} agonist psychedelics.

H. A Role for the Claustrum?

Crick and Koch (2005) speculated on the possible relationship of the claustrum to the processes that give rise to integrated conscious percepts. That is, they proposed that the claustrum may play a key role in information processing in the brain by correlating activity in different sensory cortices into one coherent activity that binds separate sensations into the unitary objects that we experience in consciousness. They suggested that the claustrum may be analogous to a conductor, coordinating the musicians in an orchestra. Autoradiography studies in the rat brain using tritiated antagonist ligands have identified brain areas that expressed 5-HT_2 receptors (Pazos et al., 1985). Although high receptor density was seen in all laminae of the neocortex, the highest binding was observed in the claustrum. Similarly, rat brain autoradiography using the 5-$HT_{2A/2C}$ agonist *R*-(–)-[^{125}I]DOI confirmed the highest binding in the claustrum as well as the frontal cortex (McKenna and Saavedra, 1987). The very high density of 5-HT_{2A} receptors in the claustrum indicated that further inquiry into the structure and function of the claustrum was warranted for this review. The claustrum lies at the confluence of a large number of simple loops with the cortex, so it is natural to ask whether the claustrum might be a previously unrecognized target for psychedelics.

The claustrum has remained one of the most enigmatic structures in the brain for several centuries. It is a thin, irregular sheet of gray matter, with one located on each side of the brain. It lies under the inner surface of the neocortex, below the general region of the insula, and above the outer surface of the putamen, with fiber tracts on each side: the extreme and external capsules.

Because of the claustrum's location, small size, and shape, however, it has been difficult to study its connections and functions. Nevertheless, it is generally accepted today that the claustrum exhibits widely distributed reciprocal anatomic projections to virtually all regions of the cortex as well as to many subcortical structures, including the hippocampus, amygdala, and caudate nucleus. Substantial evidence now exists that the major target of claustral projections is the cortex, and that the major input to the claustrum comes from the cortex (see reviews by Smythies et al., 2012, 2014; Baizer et al., 2014; Mathur, 2014; Torgerson and Van Horn, 2014).

Claustral neurons are of two kinds: type I and type II. Golgi type I neurons comprise approximately 85% of all claustral neurons and are evenly distributed throughout the body of the claustrum. They have spiny dendrites with axons projecting out of the claustrum either medially or laterally and are excitatory neurons that send projections to, and receive projections from, the cortex. They express the vesicular glutamate transporter Vglut2 and are thus inferred to be glutamatergic. The remaining cells are Golgi type II neurons, lacking spines, with axons that do not project outside of the body of the claustrum. These are thought to be inhibitory-like interneurons that provide a substrate for local information processing (Mathur, 2014). Anterograde labeling studies in the cat have demonstrated the abundance of claustral terminals in the inner parts of layers III (IV in some PFC zones) and VI, as well as in layer I, but layer V is remarkably free of terminals (Clascá et al., 1992).

Immunohistochemical studies in rats and cats suggest that the claustrum receives a diffuse serotonergic innervation, most likely from the brainstem dorsal raphe nucleus (Baizer, 2001; Rahman and Baizer, 2007). Although knowledge of claustral function is seriously lacking, most evidence suggests that the claustrum may serve as a subcortical relay nucleus through which different sensory cortices can access each other to associate modalities. The claustrum theoretically synchronizes cortical areas to accomplish the feat of crossing modalities.

Milardi et al. (2015) used constrained spherical deconvolution tractography to elucidate the complex relationships between the fiber systems in the claustrum. They detected four groups of white matter fibers connecting the claustrum to the cortex (anterior, posterior, superior, and lateral) and provided a detailed representation of brain areas connected to the claustrum.

Although Crick and Koch (2005) proposed that the claustrum might mediate consciousness, or provide perceptual integration across sensory modalities, Baizer et al. (2014) argue against this hypothesis, stating that intraclaustral processing would require that the claustrum be a continuous structure, which it is not in every species. Furthermore, as the cortex has expanded through evolution in different species, the size of the claustrum has actually decreased in relative size (Kowianski et al., 1999).

Most recently, Torgerson et al. (2015) carried out the first population-level study in 100 healthy human subjects to quantitatively examine the structural

connectivity of the claustrum using in vivo brain mapping and graph analytic methods. Using diffusion tensor imaging tractography and graph theoretical analytics, they computationally verified that the human claustrum is widely connected, with the highest density of fiber connections per unit volume of all brain regions examined. The claustra had their densest diffusion tensor imaging fiber connections to frontal cortices, with more modest degrees of connectivity to parietal, temporal, and occipital lobes, respectively. Their results are consistent with the notion that the claustrum plays a central role in linking multiple disparate structural brain networks. Torgerson et al. (2015) conclude that the claustrum occupies a unique, and presumably critical, "location" in the overall architecture of network connectivity in the brain, and they suggest that the "claustrum may serve to filter signals about the relationships of all the thousands of sensorimotor inputs from the outside world to and from frontal and cingulate processing subnetworks. Its differential influence on brain region subnetworks typically associated with high cognitive activity, attention, and action suggest that the claustrum plays a central role in linking multiple sensory networks with those regions that can interpret and take action on such information." They contend that their results are in concurrence with the proposal of Crick and Koch (2005).

Stiefel et al. (2014) presented a further argument to support a key role of the claustrum in consciousness, by considering the very potent hallucinogenic effects of salvinorin A. Salvinorin A is a natural product derived from *Salvia divinorum*, which is a specific high-affinity κ-opioid agonist (Roth et al., 2002). Stiefel et al. (2014) argue that its psychotomimetic effects may result from stimulation of κ receptors in the claustrum. They base their hypothesis on work by Peckys and Landwehrmeyer (1999), who measured κ-opioid mRNA receptor expression in the human brain using in situ hybridization. The strongest signal was found in the claustrum, and nearly all neurons exhibited high κ opioid receptor signals. In addition, Sim-Selley et al. (1999) measured a very high level of κ receptor-stimulated [^{35}S]GTPγS in the claustrum of the cynomolgus monkey brain, with "an area of especially high stimulation in the ventral claustrum, adjacent to the amygdala."

Interestingly, Koubeissi et al. (2014) reported the case of a 54-year-old patient with intractable epilepsy. Fifteen intraparenchymal electrodes were implanted in her brain to assess the origin of her seizures. One of the depth electrodes included a contact in the extreme capsule in close proximity to the anterior insular cortex and closest to the claustrum. Stimulation of this electrode led to immediate impairment of consciousness, 10 of 10 times, with the patient returning to baseline when the stimulation was stopped. Stimulation of neighboring contacts that were within 2.7 mm did not elicit such phenomena. Koubeissi et al. (2014) speculate that the claustrum "could be a key component of the network supporting 'conscious awareness' during wakefulness." The authors suggest that the claustrum might constitute a common gate to the external and internal awareness networks, perhaps being a component of the neural correlates of consciousness mediating increased synchronization between various cortical regions.

In a recent review, Goll et al. (2015) proposed that the claustrum serves the function of segregating attention between modalities, implicating the claustrum as a hub for attention. They propose that "the claustrum serves the function of segregating attention between modalities, enabling us to isolate objects of current priority for attention" (Goll et al., 2015). They draw certain parallels between the claustrum and the thalamus, proposing that both the thalamus and the claustrum have the capacity to focus attention, but at different stages of sensory processing.

All of these results, taken together, along with the structural and functional knowledge that currently exists for the claustrum, strongly indicate that the claustrum must be seriously considered as a previously unrecognized target for the action of the psychedelics. There has been increasing interest in the functional role of the claustrum, particularly since the Crick and Koch (2005) proposal, and a more intense research focus on this area of the brain seems likely to lead to very important results.

As this review was being finalized, Nichols and Martin (2015) reported that serotonergic hallucinogens preferentially activate subsets of cortical neurons, interneurons, and glial cells in the rat claustrum and induce rapid redistribution of 5-HT$_{2A}$ receptor protein within neurons. Using a refined process of cortical cell dissociation and sorting by flow cytometry, Nichols and Martin were able to identify, isolate, and separate highly purified individual cellular populations that included pyramidal neurons, the three subtypes of GABA interneurons, glial cells, and astrocytes. Sorted populations were processed for RNA extraction and subjected to quantitative RT-PCR for gene expression analysis. Only about 3% of total mPFC cells were activated in response to DOI; after identification, it was found that these activated cells displayed a 10-fold higher expression of 5-HT$_{2A}$ receptors than the non-activated population. The highest 5-HT$_{2A}$ receptor expression and cellular activation was seen in these cells, which were identified as claustral cells, in which nearly one-half of the neurons were directly activated by DOI.

V. Effects on Visual Perception

Binocular rivalry is a phenomenon that occurs when different images are simultaneously presented to each eye. When continually viewing this stimulus, observers

experience repeated switches between visual awareness of the two images. Frecska et al. (2004) examined the effects of the hallucinogenic brew ayahuasca on binocular rivalry in 10 subjects. As noted earlier, ayahuasca is a decoction made from two Amazonian plants, containing DMT as the active psychedelic component, and β-carboline alkaloids that inhibit the liver MAO that normally breaks down orally ingested DMT. Hence, ayahuasca might essentially be considered to be an orally active form of DMT. In this study, a variant of stimulus presentation called dichoptic stimulus alternation (DSA) was employed. In DSA, stimuli are applied to the eyes in rapid alterations instead of keeping the stimulus presented to each eye constant. The stimuli consisted of white binocular fixation guides centered within which is a disk filled either with horizontal or vertical gratings. Standard binocular rivalry was presented for 6 minutes to obtain the endogenous perceptual alternation rate. Dichoptic reversal stimuli were presented at 3.75, 7.5, 15, and 30 reversals per second in four 1.5-minute epochs. At a low DSA rate of 3.75 or 7.5 Hz, normal subjects reported horizontal and vertical switching at a very high rate. At 15 and 30 Hz DSA, however, a blended percept (crosshatch) was consistently reported. By contrast, after subjects had ingested ayahuasca, they were able to maintain much longer horizontal or vertical dominance periods, even when the stimuli were alternating at rates almost two orders of magnitude faster than their endogenous rivalry rate. To explain their results, the authors favor a view that the persistence of binocular rivalry at high DSA rates "requires a neural context that involves the primary visual cortex and visual pathways below."

Carter et al. (2004) studied whether psilocybin could impair motion processing in humans. They found that a 215-μg/kg dose of psilocybin selectively impaired global motion, but not local motion processing in nine healthy human subjects. They argue that because local motion discrimination is thought to be a relatively low-level process resolvable at the level of primary visual cortex V1, the lack of effect of psilocybin on local motion thresholds indicates that visual disturbances associated with the drug do not likely reflect changes at the level of the retina or in transfer of information from the retina through the lateral geniculate nucleus to primary visual cortex.

Carter et al. (2005b) studied perceptual rivalry with low-dose (115 μg/kg) and high-dose (250 μg/kg) psilocybin in 12 healthy human volunteers. Stationary green vertical and horizontal gratings were presented to the subject's left eye and right eye, respectively. Vertical and horizontal gratings were presented alternately, in rapid succession at 120 Hz. Subjects reported a dominance of vertical gratings by pressing one computer key, and by pressing another when they experienced a dominance of horizontal gratings. They could also respond if they experienced a stable mixed percept (grid or patchwork). Psilocybin dose-dependently increased binocular rivalry phase duration in a manner reflecting subjective changes in state of consciousness, as assessed with the 5D-ASC rating scale developed by Dittrich (1998).

Carter et al. (2007) explored the proposed relationship between binocular rivalry switch rate and subjective changes in psychologic state associated with 5-HT_{2A} receptor activation. Ten healthy human subjects were tested after administration of psilocybin (215 μg/kg) and after pretreatment with the selective 5-HT_{2A} antagonist ketanserin (50 mg). Psilocybin significantly reduced the rate of binocular rivalry switching. Pretreatment with 50 mg ketanserin blocked most of psilocybin's positive psychosis-like hallucinogenic effects but had no effect on the suppression of binocular rivalry switching induced by psilocybin, indicating that the psilocybin-induced rate reduction likely was not mediated by 5-HT_{2A} receptor activation.

Carter et al. (2005a) investigated the effects of psilocybin (215 μg/kg, p.o.) on attentional function in eight healthy volunteers using a multiple-object tracking task. The task required subjects to track a subset (up to eight) of 20 visually indistinguishable randomly moving green dots. This task is believed to test an individual's capacity to maintain multiple foci of attention simultaneously. They also tested spatial working memory using an electronic version of Corsi's block tapping task, in which subjects were required to remember and reproduce a sequence of up to nine spatial locations. Because the effects of psilocybin are thought to be mediated primarily by activation of the 5-HT_{2A} receptor, the selective 5-HT_{2A} antagonist ketanserin (50 mg, p.o.) was used to determine whether it would attenuate any psilocybin-induced changes in attentional tracking or spatial working memory. The 5D-ASC rating scale (Dittrich, 1998) was also used to assess the subjective effects of the drug.

Carter et al. (2005a) found that psilocybin significantly increased four of the five factors in the 5D-ASC, but only the factor of reduced vigilance remained significantly elevated after ketanserin pretreatment. Performance on the tracking task varied inversely with the number of targets subjects had to track, and it dropped off markedly when the number of targets exceeded three. The number of dots successfully tracked was significantly reduced from placebo in both the psilocybin and psilocybin plus ketanserin pretreatment conditions; ketanserin alone had no effect. In the spatial working memory task, psilocybin had no significant effect on the number of boxes remembered correctly in sequence "span length," indicating that psilocybin had no effect on spatial working memory performance. Thus, psilocybin impaired multiple-object tracking through a non–5-HT_{2A} receptor–dependent mechanism but had no effect on spatial working memory. In view of

the fact that psilocybin has agonist actions at the 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} receptors, the authors suggest that the deficit in attentional tracking might be due to the 5-HT_{1A} receptor agonist action of psilocybin. In that event, activation of somatodendritic autoreceptors in the raphe would lead to a reduction of 5-HT release into forebrain regions, possibly disrupting multiple-tracking ability mediated by those regions and consistent with work from other laboratories suggesting involvement of 5-HT and the 5-HT_{1A} receptor in attention (see references in Carter et al., 2005a).

To further characterize the role of 5-$HT_{1A/2A}$ receptors in visual processing, Kometer et al. (2011) assessed the effect of psilocybin (125 and 250 μg/kg) versus placebo on spatiotemporal dynamics of modal object completion in 17 healthy volunteers. Modal object recognition refers to the illusory perception of object boundaries and their enclosing surface when there is no direct sensory information to depict those boundaries or surfaces. The authors employed visual evoked potential recordings in conjunction with topographic mapping and source analysis. EEG recordings were made while participants viewed Kanizsa and non-Kanizsa figures. Behavioral responses were recorded and high-density electrical mapping with source analysis was used, allowing for the measurement of the spatiotemporal brain dynamics of visual modal object completion and its association with the appearance of visual hallucinations. Imaging studies have provided evidence that the intermediate lateral occipital complex (LOC) as well as the early visual area V2 probably play a major role in modal completion. Electrophysiological studies have demonstrated that modal object completion of simple figures such as Kanizsa figures is predominantly indexed by modulation of the N170 component (see references in Kometer et al., 2011).

Kometer et al. (2011) found that psilocybin increased reaction time, which was generally faster for Kanizsa than for non-Kanizsa figures. After psilocybin treatment, the P1 amplitude (90–144 milliseconds) was increased and was locally restricted to occipital electrode sites. Psilocybin dose-dependently decreased the N170 amplitude (148–223 milliseconds). Source estimation revealed activity within LOC and V2 in both hemispheres, with current source density stronger within the right-lateralized LOC and V2 in the Kanizsa compared with the non-Kanizsa condition. Psilocybin dose-dependently decreased the differential activation of the two stimulus conditions and reduced the current source density within the LOC, V2, and fusiform gyrus in both stimulus conditions. Psilocybin-induced current source-density reduction over the right-lateralized LOC, V2, and posterior parietal areas correlated significantly with the increased intensity of visual hallucinations. The three main findings of this study were as follows. First, there was a strong dose-dependent effect of psilocybin to decrease the N170 component, but there was a slight increase of the earlier visual P1 component over occipital sites. Second, the N170 component reduction was stronger for the Kanizsa figure condition than for the non-Kanizsa condition. Third, during this time range, the decrease in activation over the right-lateralized extrastriate and posterior parietal cortex was correlated with the reported intensity of visual hallucinations. The preferential reduction of the N170 amplitude in the Kanizsa compared with the non-Kanizsa condition indicates a key role for 5-$HT_{1A/2A}$ receptor(s) in object completion. Reduced extrastriate visual cortex activation during the time range of the N170 also identifies it as a potential key component of 5-HT_{2A} agonist–induced visual hallucinations.

Kometer et al. (2013) used a similar experimental approach to assess the effects of psilocybin on both α oscillations that regulate cortical excitability and early visual P1 and N170 potentials in 17 healthy humans. They also tested whether these effects were related to the formation of visual hallucinations. Parieto-occipital α oscillations are crucial for modulation of visual network excitability and strongly influence visual perception (see references in Kometer et al., 2013). The authors hypothesized that activating 5-HT_{2A} receptors with psilocybin might modulate α oscillations, leading to an altered excitability that would promote visual hallucination formation. They used a double-blind, placebo-controlled randomized design, in which subjects received pretreatments of placebo or ketanserin (50 mg, p.o.) and treatments of placebo or psilocybin (215 μg/kg). Stimuli were Kanizsa figures that induce the perception of an illusory triangle, or non-Kanizsa figures in which the figure alignment no longer induces that perception. EEG data were recorded and the P1 and N170 amplitudes were quantified, with a time frame from 80–120 milliseconds for the P1 and 150–190 milliseconds for the N170 amplitude.

Kometer et al. (2013) report that psilocybin robustly induced visual perceptual alterations that included complex visual hallucinations of scenes and pictures, visual elementary hallucinations of regular patterns, colors, light, and light flashes, but no such effects occurred after ketanserin pretreatment. Psilocybin selectively increased P1 amplitudes over the medial but not the lateral parieto-occipital regions of interest, and the psilocybin-induced increase over the medial region was blocked by ketanserin. The N170 amplitudes were decreased by psilocybin, and this effect was blocked by ketanserin. Correlation analysis with the original five main factors of the 5D-ASC revealed that the N170 decrease was selectively associated with visual perceptual alterations. Psilocybin also strongly decreased prestimulus α power (i.e., 98–12 Hz; -600 to -200 milliseconds), most evident in the right parieto-occipital region, and this decrease also was prevented by ketanserin pretreatment. The psilocybin-induced decrease in α power was significantly correlated with

the psilocybin-induced increase in the medial P1 visual potential. The high level of prestimulus α power was strongly attenuated by psilocybin, and this effect was reversed by ketanserin pretreatment, suggesting that 5-HT_{2A} receptor activation increases excitability of the visual network in the absence of externally presented stimuli by decreasing ongoing α oscillations. Furthermore, the strongly decreased prestimulus α power by psilocybin precluded observation of any subsequent stimulus-induced decrease in α power. The authors conclude that 5-HT_{2A} receptor activation induces a "dysbalance" between the excitability observed in the absence of an external stimulus and the excitability induced by the presentation of a stimulus, which occurs primarily by an attenuation of ongoing α oscillations. The fact that both the N170 potentials and perceptual alterations were blocked by ketanserin pretreatment led the authors to suggest that the decrease in N170 potentials is a crucial mechanism underlying 5-HT_{2A} receptor–mediated alterations of visual perception.

de Araujo et al. (2012) investigated the neural basis of imagery induced by the psychedelic Amazonian brew ayahuasca. They studied 10 regular ayahuasca users who participated in two fMRI sessions. The first scan was performed on the subjects before taking ayahuasca, after which they immediately drank ayahuasca. Psychologic changes were assessed at various times, and became significant at 40 and 80 minutes after ingestion. The second fMRI session began 40 minutes after drug intake when effects were at their maximum. Subjects performed three different visual tasks. First, they passively viewed natural images of people, animals, or trees. Then they were asked to close their eyes and mentally generate the same image they had just seen. Finally, they viewed a scrambled version of the image. Statistically significant brain areas of blood oxygen level–dependent (BOLD) signal increase were observed after ayahuasca intake, compared with the predrug condition. The increase in the BOLD signal was most pronounced in the bilateral occipital cortex, extending into the inferior and medial temporal lobe, and to parts of the frontal lobe. The signal amplitude after ayahuasca intake increased markedly in occipital areas during imagery, but not when viewing natural images. The activity of several cortical areas known to be involved in episodic memory and contextual association processing was also potentiated by ayahuasca during the imagery task. A connectivity analysis revealed that ayahuasca strongly altered fronto-occipital relationships, leading to marked changes in the timing of events across several brain regions. de Araujo et al. (2012) speculate that the robust visions induced by ayahuasca may even be initiated in the primary visual cortex.

VI. Effects on Sleep

Systemic administration of DOM (Dugovic et al., 1989) and DOI (Monti et al., 1990) was previously shown to reduce REM and slow wave sleep in the rat, and to increase wakefulness through a 5-HT_2 receptor–mediated action. In addition, Amici et al. (2004) had microinjected DOI or ketanserin directly into the laterodorsal tegmental nucleus, which had been found to express 5-HT_2 receptors (Morilak and Ciaranello, 1993). DOI significantly decreased the number but not the duration of REM sleep episodes. The authors cite previous work by others showing that silencing raphe neurons removes inhibition from cholinergic cells in the laterodorsal tegmental and pedunculopontine tegmental nuclei that are involved in generating REM sleep (see references in Amici et al., 2004). To determine the receptors responsible for these effects of racemic DOI, Monti and Jantos (2006a) microinjected serotonergic ligands directly into the dorsal raphe nucleus of 35 male rats. They also employed the selective 5-HT_{2A} antagonist EMD 281014 (7-[[4-[2-(4-fluorophenyl)ethyl]-1-piperazinyl]carbonyl]-1*H*-indole-3-carbonitrile hydrochloride) and the 5-HT_{2C}–selective antagonist SB-243213 (2,3-dihydro-5-methyl-*N*-[6-[(2-methyl-3-pyridinyl)oxy]-3-pyridinyl]-6-(trifluoromethyl)-1*H*-indole-1-carboxamide), which were microinjected into the DRN. Rats were implanted with Nichrome electrodes to record EEG from the frontal and occipital cortex and electromyography activity from the neck musculature. Microinjection of 2.8- and 5.6-μmol doses (to note, the publication erroneously reports millimolar doses) of DOI into the DRN reduced REM sleep during the first and second 2 hours of recording. Microinjection of either the 5-HT_{2A}–selective antagonist EMD 281014 or the 5-HT_{2C} antagonist SB-243213 prevented the DOI-induced suppression of REM. The authors argue that DOI inhibits firing in the DRN by activating 5-HT_{2A} and 5-HT_{2C} receptors on GABAergic projection interneurons that synapse on cholinergic neurons in the laterodorsal tegmental and pedunculopontine tegmental nuclei that are involved in REM sleep.

In a similar study from the same laboratory, with rats implanted for chronic sleep recordings, subcutaneous DOI significantly increased waking and light sleep but reduced slow wave sleep, REM sleep, and the number of REM episodes (Monti and Jantos, 2006b). The 5-HT_{2A}–selective antagonist EMD 281014 blocked the DOI-induced increase in waking and light sleep and reduction of slow wave sleep, but REM sleep remained suppressed. The 5-HT_{2C} antagonist SB-243213 was without effect on sleep effects induced by DOI. The results suggest that 5-HT_{2A} receptor–mediated mechanisms are responsible for effects of DOI on waking and slow wave sleep.

VII. Effects on Time Perception

One of the common subjectively perceived effects of psychedelics is a strongly altered experience of time (reviewed in Heimann, 1994). Brief periods, sometimes

a few minutes of clock time, may be perceived as having been hours. Time may also be compressed. For example, one LSD user indicated that he had watched the big bang and the evolution of the universe all in the space of only a few minutes. Animal studies of effects of psychedelics on time perception have been reported, a few of which will be briefly discussed here. Two studies of time perception after psilocybin administration to humans have also been reported and are also reviewed here.

To study the effect of DOI on the ability of rats to discriminate time intervals, Asgari et al. (2006a) trained 20 female rats in a discrete-trials psychophysical procedure to assess temporal discrimination. It had been previously shown that stimulation of 5-HT_2 receptors disrupted temporal differentiation in the free-operant psychophysical procedure. In this report, 50-second trials were carried out, with a light presented for t seconds, after which two levers were presented. A response on lever "A" was reinforced with a 45-mg food pellet when trials were $t < 25$ seconds, and a response on lever "B" was reinforced if $t > 25$ seconds, with a series of different times t. DOI (various doses) and ketanserin (2 mg/kg) were given subcutaneously, and M100907 (0.5 and 1.0 mg/kg) was given by i.p. injection. DOI (0.25 mg/kg) produced a significant increase in %B 12.5 seconds after trial onset and significant decreases in %B at 32.5, 37.5, 42.5, and 47.5 seconds after trial onset. DOI thus disrupted the rats' ability to discriminate durations of exteroceptive stimuli (temporal discrimination). The 5-HT_{2A}–selective antagonist ketanserin had no effect by itself but completely antagonized the effect of DOI. Likewise, 0.5 mg/kg M100907 had no effect by itself but completely antagonized the effect of DOI, but only at the 47.5-second time point. At 1.0 mg/kg, M100907 blocked the effect of DOI at all time points > 22.5 seconds, except 37.5 seconds after trial onset. Thus, DOI produced a dose-dependent disruption of temporal discrimination that was most evident at longer stimulus durations. The authors point out that it is not clear whether the effect of DOI is specific to the temporal dimension or whether it may reflect a more general breakdown of stimulus control. They also suggest the possibility that stimulus control is relatively weak and may be more vulnerable to disruption at longer stimulus durations. These results are similar to those of Body et al. (2003), who found that DOI also altered temporal differentiation in the free-operant psychophysical procedure in rats, an effect that was similarly blocked by ketanserin.

Asgari et al. (2006b) examined the effect of (−)-DOI (0.25 mg/kg, s.c.) on the fixed-interval peak procedure in 18 female rats. In fixed-interval trials (16 per session), reinforcement (45-mg food pellets) was delivered after the first response emitted after 30 seconds since the start of the trial. In probe trials (16 per session), there was no reinforcement and the response lever remained in the chamber for 120 seconds. Fixed-interval and probe trials were scheduled in a pseudo-random sequence. In probe trials, the response rate in trained vehicle-treated rats reaches a peak time (t_{peak}, the peak of the Gaussian component of the function) close to the 30-second designated time of reinforcer availability and then subsequently declines. After vehicle treatment, t_{peak} (33.2 ± 1.3 seconds) was close to the scheduled reinforcement time of 30 seconds. DOI significantly decreased t_{peak} to 29.7 ± 1.1 seconds and also reduced peak response rate compared with controls, and the effect was significantly antagonized by ketanserin tartrate (2 mg/kg, s.c.).

Hampson et al. (2010) reported that in an ongoing series of experiments, DOI had qualitatively different effects on temporal differentiation and temporal discrimination. They note that previous investigators (Asgari et al., 2006a) suggested that the disruptive effect of DOI on temporal discrimination might reflect a general breakdown of stimulus control rather than a specific interaction with timing processes. That is, disruption of temporal discrimination by DOI might reflect a global impairment of animals' ability to perform discriminative tasks. Therefore, one might expect that DOI would also disrupt stimulus control exerted by exteroceptive stimuli lacking temporal dimensions. These investigators tested this prediction by comparing the effects of DOI on two dimensions of a light stimulus: duration and intensity. Twelve female Wistar rats were used for DOI experiments [the authors also compared the effects of (+)-amphetamine] maintained at 80% of their free-feeding body weight. They used a two-lever paradigm with apertures 5 cm above and 2.5 cm on either side of a recess where 45-mg food pellets were provided. A light-emitting diode was located 2.5 cm above the food pellet delivery device whose intensity (luminance in cd/m^2) was varied by computer control. Trials began with illumination of the light-emitting diode above the central reinforcer recess at an intensity of 22 cd/m^2. For the temporal discrimination procedure, after a predetermined time t, the two levers were inserted into the chamber. A single response on either lever resulted in withdrawal of both levers, extinguishing of the light, and the chamber remained dark until the start of the next trial. The duration of stimulus presentation, t, in each trial varied between 2.5 and 47.5 seconds. If t was shorter than 25 seconds (the mean duration), a response on lever A resulted in reinforcer delivery, whereas a response on lever B had no consequence. Conversely, if t was longer than 25 seconds, a response on lever B resulted in reinforcer delivery, whereas a response on lever A had no consequence. For the light-intensity discrimination procedure, stimulus duration t was kept at 25 seconds for each trial, but the intensity of the light i varied in 10 distinct values between 3.6 and 128.5 cd/m^2. If $i < 22$ cd/m^2 (the geometric mean of the intensity range), a response on lever A resulted in reinforcer delivery,

whereas a response on lever B had no consequence. Conversely, if i was greater than 22 cd/m^2, a response on lever B led to reinforcer delivery, whereas a response on lever A did not. Doses of DOI were 0.0625, 0.125, and 0.25 mg/kg and were given 15 minutes prior to test sessions.

In this temporal discrimination task, Hampson et al. (2010) found that selection of lever B (%B) increased progressively as a function of stimulus duration t. The value of the indifference point T_{50}, was close to the midpoint of the range of durations (25 seconds). In the light-intensity discrimination task, %B increased progressively as a function of light intensity i. The value of the indifference point I_{50} was close to the geometric mean of the range of the light intensities (22 cd/m^2).

DOI was found to flatten the psychometric function, tending to displace it rightward, and increased the Weber fraction, but only for temporal discrimination. DOI at 0.25 mg produced a significant reduction of %B on temporal discrimination but had no effect on intensity discrimination. The authors present a detailed statistical analysis that included slope ε and Weber fraction, but they conclude that that the impairment of the precision with which the rats discriminated the durations of the stimulus was not due to a general disruption of stimulus control. Their results strongly suggest that DOI has a greater effect on temporal discrimination than on light-intensity discrimination.

Wittmann et al. (2007) carried out a study to elucidate the role of the serotonin system in time perception and temporal behavior. Theirs was the first study to systematically assess the effect of psilocybin on timing performance on standardized measures of temporal processing. They used a placebo-controlled, double-blind design in 12 healthy volunteers, administering placebo, a medium psilocybin dose of 115 μg/kg, or a high psilocybin dose of 250 μg/kg. The standardized measures of temporal processing included the temporal reproduction, sensorimotor synchronization, and tapping speed (personal and maximal). They tested spatial working memory span using the spatial span test. Psychometric measures included the 5D-ASC scale and the AMRS.

Wittmann et al. (2007) reported that high-dose psilocybin led to significant under-reproduction of time intervals 4 seconds and longer. Psilocybin significantly impaired the subject's ability to reproduce interval durations longer than 2.5 seconds, and it impaired the ability to synchronize to interbeat interval durations longer than 2 seconds. High-dose psilocybin also slowed the personal preferred tapping rate to 949 milliseconds from a baseline of 692 milliseconds but had no effect on the maximum tapping rate. No effect at the medium dose of psilocybin was observed. At the time of peak of effects, high-dose psilocybin (but not the medium dose) impaired spatial span task performance as indexed by span length. The investigators indicate that the disturbed timing abilities for sensorimotor synchronization and duration reproduction observed could reflect impairments of short-term memory, attention, or decision-making mechanisms. Given the selective effect of psilocybin on longer-duration intervals in both the temporal reproduction and sensory synchronization tasks, it was suggested that the observed temporal disturbance is induced through interference with cognitive processes like attention and working memory.

In a subsequent study, Wackermann et al. (2008) reanalyzed the data from the Wittmann et al. (2007) study and added an additional group of nine subjects that was administered a very low dose of psilocybin (12 μg/kg). Their new analysis employed a "dual klepsydra" model of duration reproduction. They measured changes in a dual klepsydra parameter designated κ, which proved to be a sensitive measure of the effect of a psychoactive substance on the internal representation of time. The results were similar to those from Wittmann et al. (2007), and the estimate of κ was significantly increased by psilocybin 90 minutes after the drug was administered, which was interpreted to mean that psilocybin led to a higher loss rate of the internal duration representation.

VIII. Use of Animal Models

What can animal models tell us about the unique psychopharmacology of psychedelics? Unfortunately, they cannot tell us as much as one would wish. Obviously, it is impossible to model the complexity of the psychopharmacology induced by psychedelics in humans with any nonhuman animal model. As far as we know, animals administered a psychedelic do not "hallucinate" or have the same sorts of sensory and cognitive effects that occur in humans. As with all animal models, the underlying assumption is that what happens in the animal system parallels, at least to a certain extent, what happens in the human. Fortunately, the key brain target for psychedelics, the serotonin 5-HT$_{2A}$ receptor, is a prominent player in the brain physiology of all mammalian species and its activation can produce measurable behaviors. Other ancillary receptors that may be involved in the actions of psychedelics, such as the serotonin 5-HT$_{1A}$ and glutamate mGlu2 receptors, also appear to have similar roles in the brain physiology of lower mammalian species. Thus, the observation of a specific and consistent behavior in an animal model after administration of a known psychedelic, and the finding that the behavior is primarily mediated by activation of the serotonin 5-HT$_{2A}$ receptor, is usually taken as a strong indication that the substance may have some sort of psychedelic action in humans. In that respect, animal models have most often been used to confirm an effect that is already known in humans. For example, when a new "research chemical" appears on the illicit market

and becomes popular for recreational use, animal models can be used to understand how this chemical compares with other known psychedelics. A sufficiently large database of known compounds in mouse and rat models has developed over the years so that it may be possible in some cases to predict whether a new chemical substance will possess psychedelic activity based on a behavioral readout.

More important, however, has been the use of animal models to dissect the underlying neuropharmacology and physiology of psychedelics. During the past 5 decades, when human research was essentially nonexistent, numerous laboratories continued to study the effects of psychedelics in animal models. In vitro and ex vivo receptor binding studies, production of second messenger signals, use of receptor-specific antagonists, and even whole-animal imaging have given insight into the possible pharmacological and neurochemical effects of psychedelics. Indeed, many of those experiments would have been impossible to carry out in humans. The result has been a further and much more detailed understanding of the role that the 5-HT_{2A} receptor, and other receptors, plays in normal brain function.

Although studies have appeared that employed psilocybin or LSD or a select few other agents, probably the majority of animal experiments have used the "psychedelic" 5-HT_{2A} agonist DOI. That is another unfortunate consequence of the current drug laws. DOI has never been popular as a recreational drug, nor has any clinical study been carried out to compare its effects with classic drugs such as LSD, mescaline, or psilocybin, and only anecdotal reports of its human psychopharmacology exist (e.g., Shulgin and Shulgin, 1991). Although DOI is quite potent, it likely never became popular as a street drug because of its very prolonged duration of action, so it had never been placed into Schedule I of the Controlled Substances Act (although as of November 2015, there are congressional moves afoot to change that). Therefore, DOI has been commercially available to qualified investigators and did not require a U.S. Drug Enforcement Administration license to work with it. That situation is somewhat problematic because the pharmacology of other psychedelics is often more complex. For example, LSD has effects at a variety of GPCRs other than the 5-HT_{2A} receptor, the presumed principal target for psychedelics. Although DOI appears to be primarily an agonist at 5-HT_{2A} and 5-HT_{2C} receptors, it lacks effects at most other receptors. By contrast, psilocybin and many other tryptamines are also agonists at the 5-HT_{1A} receptor. Although it is certainly true that the salient pharmacology of all psychedelics in animal models seems to be an agonist action at 5-HT_{2A} receptors, the complex neuropharmacology of other types of psychedelics, especially LSD, is not as well studied in animal models.

Overall, at least in rodent models, psychedelics seem to exacerbate neophobia, increase responsiveness to sensory stimulation, and interfere with response habituation across multiple sensory modalities and behavioral responses (Geyer and Vollenweider, 2008). A review of the use of animal models of serotonergic psychedelics was recently published (Hanks and González-Maeso, 2013).

A. Rat Models

1. Drug Discrimination. The drug discrimination procedure in rats has proven to be a sensitive and powerful technique that has allowed an analysis of the neuropharmacology of many classes of drugs with an action in the CNS, including the psychedelics. It has been widely used in numerous laboratories, and the topic of hallucinogens as discriminative stimuli was recently reviewed (Winter, 2009). No attempt will be made here to provide a comprehensive review of the drug discrimination literature on psychedelics published over the past 3 decades. Rather, a brief background and a few key experimental studies will be highlighted. With respect to the popularity of rat drug discrimination as a model for studying psychedelics, about two to three times as many publications are listed for the terms "rat drug discrimination" plus "hallucinogen" in the National Library of Medicine compared with what is now probably the second most popular technique, the mouse head-twitch assay.

The drug discrimination assay is based on the fact that rats can learn to recognize a drug-induced interoceptive cue. The rat learns to discriminate the drug effect (its interoceptive cue, or stimulus property) from the nondrug effect by a process known as differential reinforcement. The most popular method utilizes a two-lever operant test chamber and either a FR or variable interval (VI) schedule of reinforcement, whereby the rat receives reinforcement only after responding on the lever associated with administration of the training drug (e.g., a psychedelic). Responding on the lever associated with the nondrug treatment (e.g., saline) usually has no consequence. Although negative foot-shock reinforcement has been used on rare occasions, positive reinforcement is the vastly more prevalent method, usually using food pellets, diluted sweetened condensed milk, or water as the reinforcer. Responding is most robust when rats have had restricted access to food or water in their home cages. Once the rat has learned the discrimination task, dose-response curves can be generated and an ED_{50} can be determined. Specific antagonists can be used to assess the nature of the discriminative cue by blocking responding to the training drug, various treatments can be used to alter levels and function of neurotransmitter systems, and substitution tests can be performed with drugs suspected of having pharmacology similar to the training drug. Thus, the underlying pharmacological basis of the interceptive cue produced by the training drug or drugs with similar or identical pharmacology generally can be

elucidated, and experiments can be carried out that most often could not be done in humans.

Historically, Hirschhorn and Winter (1971) published the first report of a psychedelic producing a discriminative stimulus in rats. Using a 1-minute VI schedule of reinforcement with diluted sweetened condensed milk as the reinforcer, intraperitoneal injections of both LSD and mescaline were shown to produce discriminative stimuli in female rats. Based on testing of varying doses Hirschhorn and Winter (1971) found that 40 μmol/kg mescaline and 0.06 μmol/kg LSD were equivalent in their ability to produce discriminated responding when paired with saline. This potency ratio, approximately 670, is only 3- to 4-fold off from the ratio in humans (approximately 2500). ED_{50} values in several rat drug discrimination studies have been contrasted with their dosages in humans and a general correlation is seen, as shown in Table 1.

One of the features of drug discrimination that makes it so powerful has been referred to as the "third state hypothesis," advanced by Frey and Winter (1978). This principle essentially states that if an animal has been trained to discriminate saline from some training drug and another drug is administered, the animal will respond as if given the training drug if, and only if, the new drug has similar discriminative stimulus properties. Even if the new drug has centrally mediated effects on behavior, unless it has discriminative stimulus properties qualitatively similar (or identical) to the training drug, the animal will respond on the saline-appropriate lever. Thus, when challenged with a new drug, if the animal responds on the lever associated with the training drug, it is essentially telling the investigator "I think you gave me the training drug."

The drug discrimination procedure does suffer from the possibility of "false positives," whereby the rat responds on the lever associated with the training drug after receiving a drug that is known not to have similar psychopharmacology in humans. Appel et al. (2004) described the series of detailed experiments used in their laboratory to elucidate the nature of the interoceptive cue induced by the nonhallucinogenic ergoline lisuride, which produced a false positive in rats trained to discriminate saline from LSD. Appel et al. (2004) described the use of a variety of antagonists, a three-lever drug discrimination procedure, as well as direct intracerebroventricular injections. Today, the consensus is that discriminative responding after psychedelic administration is primarily mediated by an agonist or partial agonist activity at serotonin 5-HT_{2A} receptors (Winter, 2009), and the most efficacious drug for antagonizing hallucinogen-induced stimulus control is the highly selective 5-HT_{2A} antagonist M100907, as first demonstrated by Schreiber et al. (1994). Fiorella et al. (1995) used a series of nonselective 5-HT_{2A}/5-HT_{2C} antagonists to demonstrate through antagonist correlation analysis that 5-HT_{2A} receptor activation is primarily responsible for the stimulus effects of indoleamine and phenethylamine psychedelics. A more detailed historical background for this conclusion is presented in Nichols (2004).

It had generally been assumed that the canonical PI hydrolysis signaling pathway was the most relevant for the behavioral actions of psychedelics, but there are certain problems with this hypothesis. First, it is well known that LSD has very low efficacy in activating PI turnover (Sanders-Bush et al., 1988; Egan et al., 1998). Subsequently, Rabin et al. (2002) pointed out the lack of correlation between potency in drug substitution in rats trained to discriminate LSD or DOM from saline and efficacy in stimulating PI hydrolysis. They concluded that 5-HT_{2A}–mediated stimulation of PI hydrolysis does not appear to be the sole critical signaling mechanism involved in the discriminative effects of hallucinogens. Similarly, Roth et al. (1997a) found no significant relationship between high-affinity agonist binding and ability to stimulate PI turnover, and they proposed that additional transition states of the receptor-ligand complex must be essential for agonist efficacy.

The site in the rat brain where the stimulus properties of LSD, and presumably other psychedelics, is mediated has been identified as the ACC. Gresch et al. (2007) combined drug discrimination in rats with intracerebral microinjections of LSD into various brain areas of rats. Rats were first trained to discriminate subcutaneous injections of LSD from saline. After training, rats were implanted with bilateral cannulae in the ACC. Gresch et al. chose the ACC based on prior studies in their laboratory that had shown increased *c-fos* expression in the ACC, medial PFC, and amygdala after an acute dose of LSD (Gresch et al., 2002). Furthermore, they also had observed that behavioral tolerance to repeated LSD administration was associated with decreased 5-HT_{2A} receptor density in the mPFC and ACC (Gresch et al., 2005). Reasoning that the ACC was known to be involved in modulation of complex behaviors, the authors therefore examined the effect of bilateral infusion of LSD into the ACC. They found that local infusion of LSD dose-dependently substituted for systemically administered LSD, and also that systemic administration of the highly selective 5-HT_{2A} antagonist M100907 completely blocked the stimulus effect of locally infused LSD.

That is not to say that other receptor systems have not been identified as involved to some extent with specific psychedelic agents. For example, Reissig et al. (2005) examined the interoceptive cue produced by LSD in a two-lever FR10 paradigm in rats. Once stimulus control with LSD was established, they conducted combination and substitution tests with 5-HT_{1A} agonists and antagonists. Among other results, they observed an intermediate level of LSD substitution with the 5-HT_{1A} agonist, 8-OH-DPAT. Yet the training dose of LSD was unaffected by the 5-HT_{1A}–selective

TABLE 1
A comparison of human doses of selected hallucinogens with their potency using drug discrimination tests in LSD-trained rats

Where available, K_i values for cloned human 5-HT_{2A} and 5-HT_{2C} receptors are also listed for comparison. Compounds are ranked by relative human potency. Reproduced by permission of Elsevier from Nichols (2004).

Drug[a]	K_i for 5-HT_{2A}[b]	K_i for 5-HT_{2C}[b]	DD ED_{50}[c]	Potency Relative to LSD (Rat DD)	Human Dose[d]	Potency Relative to LSD (Human)
	nM		*μM/kg*		*mg*	
EthLAD	—	—	0.02	185	0.04–0.15	140
AllyLAD	—	—	0.013	285	0.08–0.16	110
LSD	2–4	3–6	0.037	100	0.06–0.20	100
ProLAD	—	—	0.037	100	0.10–0.20	90
DOB	0.6	1.3	1.06	2.3	1–3	7
DOI	0.7	2.4	0.28	9.2	1.5–3	6
DOM	19	ND	0.89	3.3	3–10	2
Psilocin	15–25	10	1.0	2.6	10–15	1
DMCPA	ND	ND	0.66	4.5	15–20	0.7
MEM	73	124	12	0.2	20–50	0.4
MMDA-2	ND	ND	7	0.4	25–50	0.4
Mescaline	550	300	34	0.08	200–400	0.04

AllyLAD, *N*-allyl-*N*(6)-norLSD; DD, drug discrimination; DMCPA, (±)-*trans*-2,5-dimethoxy-4-methylphenylcyclopropylamine; EthLAD, *N*-ethyl-*N*(6)-norLSD; MEM, (±)-2,5-dimethoxy-4-ethoxyamphetamine; MMDA-2, (±)-2-methoxy-4,5-methylenedioxyamphetamine; ProLAD, *N*-*n*-propyl-*N*(6)-norLSD.
[a]Drug abbreviations are from Shulgin and Shulgin (1991, 1997).
[b]Affinity of phenethylamines in cloned receptors taken from Nelson et al. (1999); mescaline affinity is from Monte et al. (1997).
[c]Drug discrimination data from Blair et al. (2000), Hoffman and Nichols (1985), Monte et al. (1997), and Nichols et al. (1984a,b, 1986, 1991).
[d]Averaged dose range from Shulgin & Shulgin (1991, 1997) (LSD = 100).

antagonist WAY-100635. On the basis of all of their results, Reissig et al. (2005) concluded that 5-HT_{1A} receptor agonists are able to modulate the discriminative stimulus effects of LSD given at short times before testing, but that 5-HT_{1A} receptor stimulation appears to be a nonessential component of the LSD cue.

Winter et al. (2007) examined the stimulus properties of psilocybin in the rat using drug discrimination with an FR10 schedule of reinforcement with water. The psilocybin stimulus was partially blocked by the 5-HT_{2A} selective antagonist M100907, but not by the 5-HT_{1A}–selective antagonist WAY-100635. A selective 5-HT_{2C} antagonist SB-24084 had only modest effects, arguing against a contribution by 5-HT_{2C} receptors.

Winter et al. (2000b) trained rats to discriminate 5-MeO-DMT from saline, or (−)-DOM from saline. In rats trained with 5-MeO-DMT, the 5-HT_{1A} antagonists pindolol and WAY-100635 both produced significant attenuation of the 5-MeO-DMT stimulus, but the nonselective 5-HT_{2A} antagonist pirenperone produced an intermediate degree of antagonism. In rats trained with (−)-DOM, pirenperone but not WAY-100635 blocked the stimulus. In rats trained with 5-MeO-DMT the stimulus fully generalized to the 5-HT_{1A} agonist 8-OH-DPAT and only partially to (−)-DOM. After additional similar experiments, the authors concluded that the 5-MeO-DMT stimulus is mediated primarily by agonist activity at 5-HT_{1A} receptors, but that it produces a compound stimulus that includes an element mediated by 5-HT_{2A} agonist action.

Gatch et al. (2009) trained male Sprague-Dawley rats under an FR10 food-reinforced paradigm to discriminate DMT (5 mg/kg) from saline and then tested the ability of LSD, *R*-(−)-DOM, (+)-methamphetamine, and racemic MDMA to substitute in these rats. DMT also was evaluated for drug-appropriate responding in rats trained to discriminate LSD, DOM, MDMA, or (+)-methamphetamine from saline. LSD, DOM, and MDMA all fully substituted in DMT-trained rats, but (+)-methamphetamine failed to substitute. DMT, LSD, and MDMA all fully substituted in DOM-trained rats. Again, however, (+)-methamphetamine failed to substitute in these rats. In rats trained to discriminate LSD from saline, only DOM fully substituted, and DMT and MDMA only partially substituted. Again (+)-methamphetamine did not substitute in LSD-trained rats. When rats were trained to discriminate MDMA from saline, LSD and (+)-methamphetamine fully substituted, but DMT and DOM only partially substituted. In rats trained to discriminate (+)-methamphetamine from saline, only MDMA fully substituted. DOM, LSD, and MDMA produced full substitution in DMT-trained rats, but DMT produced full substitution only in DOM-trained rats. After a detailed analysis of stimulus generalization between the various drugs, the authors concluded that DMT produces a discriminative stimulus that is similar to, but not completely overlapping with, LSD, DOM, and MDMA. DMT, DOM, and LSD were psychedelics lacking psychostimulant effects.

Drug discrimination has also been used to determine that LSD has time-dependent pharmacology. That is, when LSD was given 30 minutes prior to training the stimulus was, as expected, mediated by activation of the 5-HT_{2A} receptor. However, when LSD was administered 90 minutes prior to training, the cue was found to be mediated by activation of the dopamine D_4 receptor (Marona-Lewicka et al., 2005, 2009, 2011; Marona-Lewicka and Nichols, 2007, 2011). Thus, drug discrimination has been used to define a temporal switch in the nature of the interoceptive cue for LSD, from predominant activation of the 5-HT_{2A} receptor at short times

before training to a primary action by stimulation of dopamine D_2 receptors at later times after administration. These experiments were described in greater detail in the earlier section on the possible role of other receptors.

2. Effects on Locomotor Activity. Although not specific for psychedelics, studies of effects on locomotor and open field behavior are simple to implement and are often the first animal model to study. Krebs-Thomson et al. (2006) reported that 5-MeO-DMT decreased locomotor activity, investigatory behavior, and center duration and disrupted prepulse inhibition (PPI) of startle in rats. All of these effects were blocked by pretreatment with the 5-HT_{1A} antagonist WAY-100635. Pretreatment with the 5-HT_{2A} selective antagonist M100907 failed to attenuate any of these effects, whereas the 5-HT_{2C} antagonist SER-082 [(+)-*cis* 4,5,7*a*,8,9,10,11,11*a*-octahydro-7*H*-10-methylindolo [1,7-*bc*][2,6]-naphthyridine] reduced only the 5-MeO-DMT–induced disruption of PPI. In earlier studies, this laboratory had reported that decreased locomotion, investigatory behavior, and center behavior induced by phenethylamine hallucinogens were mediated solely by activation of 5-HT_{2A} receptors, whereas the decrease in locomotion produced by LSD was attenuated by a 5-HT_{1A} antagonist and the decrease in investigatory behavior was antagonized by a 5-HT_{2A} antagonist (see references in Krebs-Thomson et al., 2006). There are numerous studies that report effects of psychedelics on motor activity, usually in the mouse, but because this test is not specific, no additional reports will be reviewed here.

3. Prepulse Inhibition. PPI of acoustic startle refers to the phenomenon that occurs when a weak prestimulus attenuates the reaction to a subsequent ASR. PPI serves as an operational measure of sensorimotor gating, and psychedelics affect PPI not only in rodents (Sipes and Geyer, 1994; Johansson et al., 1995; Padich et al., 1996) but also in humans (Vollenweider et al., 2007). Sipes and Geyer (1994) first reported that the selective 5-HT_{2A} antagonist ketanserin blocked the disruptive effects of DOI-induced PPI. In a later study, dose-response studies showed that the selective 5-HT_{2A} antagonist, M100907, but not the 5-HT_{2C} antagonist SER-082, prevented the disruption of PPI induced by DOI (Sipes and Geyer, 1995). These results support the hypothesis that the 5-HT_{2A} receptor is involved in the modulation of sensorimotor gating. In a subsequent study, infusion of DOI (1.0–5.0 μg/0.5 μl) into the ventral pallidum (VP) disrupted PPI without having effects on startle reactivity (Sipes and Geyer, 1997). By contrast, DOI infusions into the nucleus accumbens, an adjacent area also expressing 5-HT_{2A} receptors, had no effect on PPI or startle reactivity. Infusion of M100907 into the VP increased PPI by itself and attenuated the PPI-disruptive effects of systemically administered DOI. The authors concluded that 5-HT_{2A} receptors within the VP are important for the modulation of PPI, presumably through interactions at intrinsic GABAergic or cholinergic interneurons.

Similarly, Ouagazzal et al. (2001) demonstrated that the decrease in PPI induced by LSD in rats also was mediated by 5-HT_{2A} receptors. The disruption of PPI induced by LSD in Sprague-Dawley rats was completely blocked by pretreatment with the highly selective 5-HT_{2A} antagonist M100907. Pretreatment with a 5-HT_{2C} antagonist, a 5-$HT_{2B/2C}$ antagonist, a 5-HT_{1A} antagonist, a 5-HT_6 antagonist, or the dopamine D_2 antagonist haloperidol all failed to affect the LSD-induced effect on PPI.

Halberstadt and Geyer (2010) also characterized the effect of LSD on the PPI in rats and compared it with the nonhallucinogenic ergoline lisuride. It had previously been shown that lisuride did not evoke the head twitch in mice (González-Maeso et al., 2007). Although lisuride was found to decrease PPI, this effect was not blocked by the 5-HT_{2A} antagonist MDL11939 or the 5-HT_{1A} antagonist WAY-100635, but was blocked by the dopamine $D_{2/3}$ antagonist raclopride. Thus, lisuride disruption of the PPI is mediated by dopamine $D_{2/3}$ receptors. By contrast, the disruption of PPI by LSD was significantly attenuated by MDL11939, again illustrating that the disruption of PPI induced by psychedelics is mediated by activation of the 5-HT_{2A} receptor.

Wischhof et al. (2012) investigated interactions between 5-HT_{2A} receptors and mGlu2/3 receptors in the rat PPI and ASR. Rats were randomly injected with either vehicle or the mGlu2/3 agonist LY379268 (0.5 mg/kg, i.p.) 20 minutes prior to DOI (3 mg/kg, s.c.) or vehicle. Ten minutes after the second injection, rats were placed into the PPI chambers. DOI reduced PPI and ASR in Wistar, but not in Lister Hooded rats. Pretreatment of Wistar rats with LY379268 (1 mg/kg) attenuated the DOI-induced PPI disruption and reduction of ASR magnitude. LY379268 had no effect on PPI when given alone and only slightly increased magnitude of the ASR. Their data are consistent with the notion of functionally antagonistic interactions between 5-HT_{2A} and mGlu2/3 receptors that might regulate sensorimotor gating mechanisms.

B. Mouse Models

Although mice have become more popular for studying the action of psychedelics in the past decade or so, their physiology and pharmacological responses are probably not as similar to humans as are those of rats. Mice, however, have the advantage of being significantly less expensive to purchase and maintain than rats or other higher mammals; perhaps more importantly, the ability to create transgenic mouse lines represents a significant advantage over other mammalian models.

The first test of a psychedelic in the mouse was reported by Woolley (1955), in which mice administered

LSD predominantly walked backward in a prone position, as if backing up on an inclined plane. Although that observation proved not to be particularly informative, two mouse models have, however, been useful in dissecting the neuropharmacology of psychedelics: mouse HTR and mouse drug discrimination.

1. Head Twitch Response. Keller and Umbreit (1956) administered LSD intravenously to mice and reported "…a rapid and violent head shaking" that did not occur in normal mice. They indicated that it was easily observed, that independent observers could reliably detect the behavior, and that this HTR in mice "provided a suitable tool for the behavioral studies" (Keller and Umbreit, 1956). Subsequently, the mouse HTR was observed in mice after administration of 5-hydroxytryptophan, the biochemical precursor to serotonin (Corne et al., 1963). Corne and Pickering (1967), based on a study that involved several classic psychedelics that included DMT, LSD, mescaline, and psilocybin, as well as nonhallucinogenic compounds, proposed that the mouse HTR might correlate with the production of drug-induced hallucinations in humans. Subsequently, numerous studies have been reported using the HTR to assess potential psychedelic activity (e.g., Silva and Calil, 1975; Yamamoto and Ueki, 1981; Darmani et al., 1990b; Fantegrossi et al., 2005, 2015; González-Maeso et al., 2007; Fox et al., 2010; Halberstadt and Geyer, 2014; Nichols et al., 2015).

The head twitch in mice is operationally defined as a rapid rhythmic paroxysmal side-to-side rotational head movement that occurs after administration of serotonergic hallucinogens (Halberstadt and Geyer, 2011). Although it has most often been visually scored in real time, or scored from videos taken during the drug effect, Halberstadt and Geyer (2013a) recently developed an automated and relatively rapid method for assessing the mouse HTR. They first analyzed the kinematics of head twitches using high-speed video recordings, reporting that the HTR was highly rhythmic, occurring within a specific frequency range (mean head movement frequency of 90.3 Hz). On the basis of this analysis, they developed a system using a head-mounted magnet and a magnetometer coil, followed by extensive validation using video analysis and an observer blind to the treatment. This advancement allows the HTR to be used as a fairly rapid high-throughput assay that has eliminated the need for tedious visual scoring by an observer, and it gives reliable, unbiased, and reproducible results. Use of the mouse HTR to study psychedelics has recently been reviewed (Fantegrossi et al., 2008a; Canal and Morgan, 2012; Halberstadt and Geyer, 2013a; Hanks and González-Maeso, 2013).

The power of the HTR is the fact that mice do not require training and it is a response to activation of cortical serotonin 5-HT_{2A} receptors (Darmani et al., 1990a; Schreiber et al., 1995; Dursun and Handley, 1996; González-Maeso et al., 2003, 2007; Carbonaro et al., 2015). Mice null for the 5-HT_{2A} receptor gene fail to show a HTR in response to DOI (González-Maeso et al., 2003), and restoration of cortical 5-HT_{2A} expression rescued HTR behavior (González-Maeso et al., 2007). Furthermore, lisuride is an ergoline with 5-HT_{2A} agonist activity that is not hallucinogenic in humans and also fails to induce the HTR in mice (González-Maeso et al., 2003, 2007).

Tolerance and changes in receptor functional sensitivity can also develop in response to repeated administration of psychedelics (at least to DOI) and are reflected in the HTR assay. Darmani et al. (1992) administered a single 2.5 mg/kg injection of DOI. A challenge dose 24 hours later resulted in a significant 41% reduction in the total HTR score. Surprisingly, a challenge dose of DOI 48 hours later showed a significant 51% increase in the number of HTR. This supersensitivity persisted for up to 6 days after the first DOI injection but decreased over time, so that the HTR response had returned to control levels 8 days after the initial DOI injection. Chronic DOI administration also was examined by giving mice daily injections of 2.5 mg/kg DOI for 13 days. Daily HTR responses to DOI injections were reduced from 24 hours after the first injection, through day 5, when they returned to the value observed on day 1.

Dougherty and Aloyo (2011) examined mouse HTR responding after chronic DOI administration or the selective 5-HT_{2A} antagonist MDL11939. Mice were administered 1 mg/kg DOI every day for 8 days, or 2.95 mg/kg MDL11939 daily for either 4 or 8 days. Twenty-four hours after the last treatment, mice were challenged with 0.75 mg/kg DOI, and HTR behavior was scored. Densities of 5-HT_{2A} and 5-HT_{2C} receptors in the mouse whole cortex also were assessed 24 hours after the last drug treatment using competition binding with [^{3}H]ketanserin or [^{3}H]mesulergine, respectively. Eight days of DOI treatment led to a 41% reduction in B_{max} for 5-HT_{2A} receptors, with a slight but nonsignificant increase in K_d. Neither 4 nor 8 days of chronic treatment with MDL11939 had any significant effect on 5-HT_{2A} density or function. Mice showed tolerance to the behavioral effects of DOI that began 24 hours after the first dose of DOI and persisted to the end of the experiment. The significant reduction in HTR responses was consistent with the decreased density of cortical 5-HT_{2A} receptors. After chronic treatment with MDL11939, however, a challenge dose of 0.75 mg/kg DOI gave a HTR not significantly different from vehicle-treated mice. This result was also consistent with the lack of effect of chronic MDL11939 on cortical 5-HT_{2A} receptor density. The authors cite five earlier studies (Table 7 in Dougherty and Aloyo, 2011) that all reported similar findings, in which changes in 5-HT_{2A} receptor density were correlated with changes in the number of HTRs.

Qu et al. (2005) used PLA_2 as a measure of regional brain activation in SERT KO mice ($SERT^{-/-}$) and their

littermate controls (WT; SERT$^{+/+}$). Unanesthetized mice were injected intravenously with [^{3}H]AA and the regional coefficient of incorporation k^* of AA from plasma into phospholipids of individual brain regions was subsequently measured by quantitative autoradiography in 71 different brain regions. In WT mice, administration of DOI significantly increased k^* in 27 regions known to express 5-HT$_{2A/2C}$ receptors. By contrast, DOI did not increase k^* in any brain region of SERT($^{-/-}$) mice. Furthermore, the HTR was robust in WT mice after DOI administration but was markedly attenuated in SERT($^{-/-}$) mice. As Rioux et al. (1999) had previously shown, SERT($^{-/-}$) mice had a significant 32% reduction in the density of 5-HT$_{2A}$ receptors in cortical membranes. Thus, the results were interpreted to mean that elevated levels of synaptic 5-HT led to down-regulation of 5-HT$_{2A/2C}$–mediated PLA$_2$ signaling, as well as to marked and significant reduction in the HTR.

Although the mouse HTR is clearly mediated by activation of the 5-HT$_{2A}$ receptor, other neurotransmitter systems can modulate the response. With respect to the study of psychedelics, activation of the 5-HT$_{2C}$ receptor has been shown to mask the 5-HT$_{2A}$ receptor–induced head twitch in the rat (Vickers et al., 2001). Fantegrossi et al. (2010) noted that phenethylamine psychedelics typically have a biphasic dose-effect curve in the HTR assay. It is well known that phenethylamine psychedelics such as DOI have similar affinities and functional effects at both 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. Thus, Fantegrossi et al. (2010) proposed that the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors may functionally interact to produce the biphasic dose-response effect in the mouse HTR. That is, activation of the 5-HT$_{2A}$ receptor at low doses of drug might be responsible for the ascending limb of the dose-response curve, whereas activation of the 5-HT$_{2C}$ receptor by higher doses might be responsible for the descending limb of the curve. Their experimental results were consistent with this hypothesis. The 5-HT$_{2A}$ selective antagonist M100907 shifted the DOI dose response to the right but did not alter the descending limb of the curve. The 5-HT$_{2C}$–selective agonist Ro60-0175 [(*S*)-6-chloro-5-fluoro-1*H*-indole-2-propanamine] decreased the DOI-induced HTR in Swiss NIH mice, whereas the 5-HT$_{2C}$–selective antagonist RS102221 [*N*-{5-[5-(2,4-dioxo-1,3,8-triazaspiro[4.5]dec-8-yl)pentanoyl]-2,4-dimethoxyphenyl}-4-(trifluoromethyl) benzenesulfonamide] produced a rightward shift in the descending limb of the dose-response curve to DOI, and had no effect on the ascending limb.

Surprisingly, and in contrast with these results, Canal et al. (2010) reported that the mouse HTR was significantly attenuated in 5-HT$_{2C}$ KO mice and was significantly reduced by pretreatment of WT mice with the 5-HT$_{2C}$ antagonists. Further research may be warranted to clarify this issue, but all investigators appear to agree that 5-HT$_{2C}$ receptors may play a modulatory role in the HTR.

Fantegrossi et al. (2006) demonstrated that 5-MeO-DIPT induced the head twitch in mice, and the effect was antagonized by pretreatment with the 5-HT$_{2A}$–selective antagonist M100907. The affinity of 5-MeO-DIPT was measured at rat 5-HT$_{1A}$, 5-HT$_{2A}$, and 5-HT$_{2C}$ receptors using antagonist radioligands, with K_i values determined to be 35 nM, 5620 nM, and 1700 nM, respectively. Thus, despite the higher affinity for 5-MeO-DIPT at the 5-HT$_{1A}$ receptor, the head twitch was mediated through the 5-HT$_{2A}$ receptor.

Although much more has been discussed earlier about the role of glutamate in the actions of psychedelics, it is known that glutamate systems are also important in the mouse HTR. Moreno et al. (2011) found that the DOI- or LSD-elicited HTR was completely abolished in mGluR2-KO mice. They also carried out [^{3}H]ketanserin saturation binding experiments in frontal cortex membranes from KO mice and found no decrease in K_d or B_{max}, compared with WT mice.

By contrast, Kłodzinska et al. (2002) showed that 30-minute pretreatment of mice with the mGlu2/3 agonists LY354740 and LY379268 significantly decreased (by 64%–70%) head twitches induced by 2.5 mg/kg DOI intraperitoneally. Activation of mGlu2/3 receptors, which are localized presynaptically and serve as autoreceptors, would therefore be expected to suppress glutamate release from terminals. Their findings parallel an earlier study by Gewirtz and Marek (2000), who also had shown that a 30-minute pretreatment with the mGlu2/3 agonist LY354740 suppressed 5.0 mg/kg DOI–induced HTR in the mouse. In addition, these researchers also demonstrated that the mGlu2/3 antagonist LY341495 enhanced the frequency of the DOI-induced HTR. These studies all show that a functional mGlu2/3 receptor system is required for expression of the mouse HTR. An earlier section discussed in more detail what is currently known about the role of glutamate in the actions of psychedelics.

2. Drug Discrimination. Although drug discrimination has been used for studies of psychedelics in rats for several decades, more recently it has been applied to studies in mice. Although mice are thought not to be as similar to humans as rats, their care and maintenance is more economical than rats. In addition, transgenic mice have been developed with targeted mutations of specific genes, which serve as powerful tools to study mechanisms of drug action. The reader is again referred to the review of drug discrimination in animals by Winter (2009).

The first report of drug discrimination studies of a psychedelic in mice was by Smith et al. (2003). They trained C57BL/6J mice in a two-lever paradigm to discriminate racemic DOI from saline on a 30-second VI schedule of reinforcement. Mice readily acquired the DOI-saline discrimination task, and the investigators report that the most significant difference between rats and mice was that mice required a dose of 2.5 mg/kg for

acquisition of the discrimination, whereas rats required only 0.75 mg/kg in their earlier studies. Mice also had markedly higher response rates than rats when responding on either the saline or training drug lever. The DOI stimulus had a rapid onset of slightly more than 5 minutes and had begun to decay by 60 minutes postinjection, which was more rapid than in rats, suggesting that mice metabolize DOI more quickly than rats. Both LSD and *R*-(−)-DOB completely substituted in these mice, and the DOI stimulus was blocked by the 5-HT_{2A}–selective antagonist M100907. Surprisingly, Smith et al. (2003) also found that the DOI stimulus was partially blocked by the 5-$HT_{2B/2C}$ antagonist SB-206553 and was blocked by the selective 5-HT_{2C} antagonist SB-242084. They interpreted these results to indicate that the discriminative stimulus properties of DOI in mice have a 5-HT_{2C}–mediated component, whereas the cue is mediated solely by the 5-HT_{2A} receptor in rats.

Winter et al. (2005) examined the stimulus properties of LSD in C57BL/6 mice. Using a left or right nose-poke operant on a FR10 water reinforced task, mice were trained with 0.17 or 0.30 mg/kg LSD subcutaneously or saline (15-minute pretreatment). Only 6 of 16 mice could be trained at the low dose of LSD, but 11 of 16 could be trained at the higher dose. In mice trained at the lower dose of LSD (0.17 mg/kg), stimulus control was present for at least 30 minutes but then rapidly declined over the following 30 minutes. When mice were administered the lower dose of LSD in combination with the selective 5-HT_{2A} antagonist M100907, LSD-appropriate responding was reduced to approximately 50%, accompanied by a decrease in response rate. M100907 when given alone also had a significant rate-suppressant effect compared with vehicle. In mice trained at the higher dose of LSD, full generalization of the LSD stimulus occurred to *R*-(−)-DOM. The authors suggest that "certain non-5-HT_{2A}–mediated elements in the compound stimulus induced by LSD may be more salient in the mouse than in the rat" (Winter et al., 2005).

Similarly, Benneyworth et al. (2005) examined the discriminative stimulus properties of LSD in C57BL/6J mice using a 30-second VI schedule of reinforcement. After initial experiments revealed a very short duration of action for LSD, they used 0.45 mg/kg subcutaneously, with a preinjection interval of 10 minutes and 15-minute training sessions. The response rate also decreased significantly with higher doses of LSD. *R*-(−)-DOB fully substituted in LSD-trained mice, with a much longer duration of action than LSD. Substitution tests with the 5-HT_{1A} agonist 8-OH-DPAT also produced a dose-dependent increase in LSD lever selection, with the highest dose giving 60% LSD lever selection. Similar to the results reported by Winter et al. (2005), the selective 5-HT_{2A} antagonist M100907 gave only a partial blockade, down to 44% LSD lever selection. By contrast, M100907 completely blocked LSD lever selection in substitution tests with *R*-(−)-DOB. The 5-HT_{1A} receptor–selective antagonist WAY-100635 also partially blocked the LSD stimulus. The authors note that the stimulus time course for both LSD and *R*-(−)-DOB is consistent with them having a more rapid pharmacokinetic profile in mice than in rats. Based on the partial block of the LSD stimulus by M100907, the partial substitution of 8-OH-DPAT, and the partial blockade of the LSD stimulus by WAY-100635, the authors hypothesize that the LSD stimulus has a significant 5-HT_{1A} receptor component in mice, whereas the LSD stimulus appears to be solely mediated by 5-HT_{2A} receptor activation in rats.

Krall et al. (2008) investigated stimulus control by LSD in C57BL/6 mice that were homozygous for the SERT null mutation ($SERT^{-/-}$). They used a chamber with two snout-poke holes and an FR20 schedule of water reinforcement. They employed both a 0.17 and 0.30 mg/kg subcutaneous dose of LSD. Stimulus control could be achieved only in 3 of 13 SERT KO mice at the 0.17-mg/kg dose of LSD. Increasing the LSD training dose to 0.30 mg/kg led to 1 of 10 of the remaining mice reaching criterion (total mice reaching criterion at both doses was only 31%). By contrast, Krall et al. (2008) reported that 9 of 10 WT mice reached criterion at 0.30 mg/kg LSD. They note that when KO mice were trained to discriminate a visual stimulus, 85% of the mice exhibited operant behavior, whereas 100% of the WT mice reached criterion. Elevated synaptic levels of serotonin occur in the SERT KO mice, which would be expected to lead to receptor downregulation. Indeed, Li et al. (2000) showed that SERT KO mice have reduced densities of 5-HT_{1A} receptors, and Rioux et al. (1999) demonstrated reduced 5-HT_{2A} receptors in SERT KO mice. Both of these receptors would appear to be key components of the LSD cue. Interestingly, in a later study, Li et al. (2003) reported that 5-HT_{2A} receptor density was reduced only in the claustrum and ventral striatum of SERT KO mice, whereas Rioux et al. (1999) reported decreased expression of 5-HT_{2A} receptors also in the cortex.

3. Effects on Locomotor Activity. Although psychedelics can increase locomotor activity in mice, this test is not specific for them. Nonetheless, several laboratories have investigated the pharmacological basis for increased locomotor activity in mice after administration of psychedelics. Low to moderate doses of DOI (0.625–5 mg/kg) increased locomotor activity in C57BL/6 mice, measured as distance traveled, whereas higher doses (5–20 mg/kg) reduced it (Halberstadt et al., 2009). DOI administration produced an inverted U-shaped dose-response function in WT mice but had no effect on locomotor activity in 5-HT_{2A} KO mice. The hypoactivity seen after 10 mg/kg DOI in WT mice was prolonged in 5-HT_{2A} KO mice. Furthermore, pretreatment with the 5-$HT_{2C/2B}$ antagonist SER-082 attenuated the decrease

in locomotor activity induced by 10 mg/kg DOI. The 5-HT_{2C}–selective agonist WAY-161503 [(*R*)-8,9-dichloro-2,3,4,4*a*-tetrahydro-1*H*-pyrazino[1,2-*a*]quinoxalin-5(6*H*)-one] also reduced distance traveled by WT mice, and this effect was blocked by SER-082. The locomotor suppressant effect of WAY-161503 was enhanced in 5-HT_{2A} KO mice. These data were interpreted to mean that the decreased locomotor activity after 10 mg/kg DOI was mediated by activation of 5-HT_{2C} receptors. The authors suggest that 5-HT_{2A} and 5-HT_{2C} receptors exert opposing effects on locomotor activity, similar to their proposed role in the HTR, as discussed earlier. Numerous studies have provided evidence that 5-HT_{2A} and 5-HT_{2C} receptors exert functionally opposed activity (Bergqvist et al., 1999; Pozzi et al., 2002; Winstanley et al., 2004; Alex et al., 2005; Ramos et al., 2005; Bubar and Cunningham, 2006, 2007), especially with respect to CNS dopamine systems (Carli and Samanin, 1992; Ruotsalainen et al., 1997; Koskinen et al., 2000a,b; Passetti et al., 2003; Winstanley et al., 2003, 2004).

Based on literature indicating a functional interaction between the 5-HT_{2A} and mGlu5 receptors, Halberstadt et al. (2011b) tested the hypothesis that 5-HT_{2A} receptors are involved in the locomotor hyperactivity exhibited by mGlu5 receptor KO mice. They tested the effects of DOM, and the selective 5-HT_{2A} antagonist M100907, on locomotor activity in the mouse BPM in mGlu5 WT and KO mice on a C57 background. Male and female mGlu5 KO mice had increased locomotor activity and reduced locomotor habituation compared with WT animals during the last 40 minutes of testing. mGlu5 KO mice also had significantly more rearing than WT mice after vehicle treatment. The mGlu5 negative allosteric modulator MPEP (30 mg/kg) also significantly increased locomotor activity in WT mice, measured as distance traveled, during the first 50 minutes of the 1-hour test session, but had no effect on activity in mGlu5 KO animals. DOM (0.5. mg/kg) increased locomotor activity in WT mice, but only during the third 10-minute block of testing, whereas DOM significantly and markedly increased locomotor activity in all six 10-minute blocks of testing in mGlu5 KO mice. Locomotor hyperactivity in mGlu5 KO mice was significantly attenuated by 1.0 mg/kg M100907, and M100907 completely blocked the hyperactivity induced by MPEP. One possible explanation offered by Halberstadt et al. (2011b) is that the loss of mGlu5 signaling may lead to increased 5-HT release, which activates the 5-HT_{2A} receptor (see Stachowicz et al., 2007). The DOM potentiation of activity in mGlu5 KO mice could also be attributed to enhanced sensitivity of 5HT_{2A} receptors in these mice, and the authors suggest that the mGlu5 receptor may regulate responses to 5-HT_{2A} activation. Thus, they conclude that the mGlu5 receptor acts to attenuate 5-HT_{2A} receptor–induced hyperactivity, and either the loss of the mGlu5 receptor, or a negative allosteric regulator of mGlu5, essentially unmasks the influence of the 5-HT_{2A} receptor.

Halberstadt et al. (2013) tested a series of phenylalkylamine psychedelics in C57BL/6J mice using the BPM to determine whether they increased locomotor activity by activating the 5-HT_{2A} receptor. Low doses of mescaline, 2,5-dimethoxy-4-ethylamphetamine (DOEt), 2,5-dimethoxy-4-n-propylamphetamine (DOPr), 2,4,5-trimethoxyamphetamine (TMA-2), and 4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine (TCB-2) increased locomotor activity, whereas a nonhallucinogenic phenethylamine DOTB did not alter activity at any dose tested. The selective 5-HT_{2A} antagonist M100907 blocked the locomotor hyperactivity induced by mescaline and TCB-2. Notably, mescaline and TCB-2 did not increase locomotor activity in 5-HT_{2A} KO mice. Their results confirm that low doses of serotonergic psychedelics increase locomotor activity through activation of the 5-HT_{2A} receptor.

C. Rabbit Models

The rabbit has not been widely used as an animal model to study the effects of psychedelics, but a number of experiments in this species have been carried out over the past decade, primarily in the laboratory of the late John A. Harvey. These studies have examined the rabbit head-bobbing response, as well as associative learning. Aloyo et al. (2001) first described the production of head bobs by DOI in the rabbit. They found that daily injections of the 5-HT_{2A} agonists DOI, LSD, and the antagonist 2-bromo-lysergic acid-*N,N*-diethylamide (BOL) led to decreased cortical 5-HT_{2A} receptor density but had no effect on density of cortical 5-HT_{2C} receptors. Daily treatment with the highly selective 5-HT_{2A} antagonist MDL11939 surprisingly increased 5-HT_{2A} receptor density by 60%, with no effect on 5-HT_{2C} receptor levels. DOI-elicited head bobs were decreased by prior chronic treatment with DOI, LSD, or BOL, whereas DOI-induced head bobs increased after chronic treatment with MDL11939, consistent with the idea that head bob behavior in rabbits is mediated by the 5-HT_{2A} receptor.

Dave et al. (2002) reported that systemic administration of DOI to New Zealand white rabbits dose-dependently elicited head movements (vertical down-up head bobs) and body shakes (a paroxysmal shudder of the head, neck, and trunk combined, similar to wet dog shakes in rodents). They reported that head bobs were mediated by 5-HT_{2A} receptor activation, whereas body shakes were mediated by activity at the 5-HT_{2C} receptor. The same workers carried out experiments to determine whether the two behaviors were mediated by a central or peripheral action (Dave et al., 2004b). They found that pretreatment with xylamidine, a peripherally acting 5-$HT_{2A/2C}$ antagonist, had no effect on DOI-elicited head bobs, even at a high dose. By contrast, systemic xylamidine significantly

attenuated DOI-elicited body shakes. Intracerebroventricular administration of DOI significantly increased head bobs, but not the number of body shakes. Intracerebroventricular administration of either ketanserin or xylamidine significantly attenuated DOI-elicited head bobs but had no effect on DOI-elicited body shakes. These data were interpreted to mean that head bob behavior was mediated by central 5-HT_{2A} receptors, whereas body shakes were mediated by 5-HT_{2C} receptors located either peripherally, or else in brain areas not accessible to infusions of DOI into the lateral ventricle.

In a subsequent study, Dave et al. (2004a) examined the role of the hippocampus in $5HT_{2A}$ receptor–mediated head bobs in the rabbit. Rabbits received bilateral injections of DOI into the CA1 region of the hippocampus after either saline pretreatment or hippocampal administration of MDL11939, the selective 5-HT_{2A} antagonist. Head bobs induced by intrahippocampal DOI were blocked by prior treatment with hippocampal MDL11939. Three different cohorts of animals were injected chronically, once a day for 8 days, with MDL11939. In the first cohort, density and affinity of 5-HT_{2A} and 5-HT_{2C} receptors in the hippocampus was determined by radioligand binding studies. In the second group, brains were examined by quantitative autoradiography using [^{125}I]DOI. The third group was implanted with bilateral cannulas into the hippocampus. Radioligand binding studies in the first cohort found that chronic MDL11939 led to a significant 88% increase in receptor density with no change in K_d. In the second group, quantitative autoradiography revealed that animals chronically treated with MDL11939 had a significant 232% increase in receptor density in the CA1 field and a significant 231% increase in the dentate gyrus, compared with vehicle-injected controls. In the third group, after chronic MDL11939 administration, DOB was directly infused into the dorsal hippocampus 24 hours after the last MDL11939 injection. In this experiment, DOI elicited almost twice as many head bobs as in vehicle-treated control animals.

Harvey (2003) reviewed evidence showing that 5-HT_{2A} receptor activation can enhance associative learning. He focused on studies of classic conditioning of the rabbit nictitating membrane response (a component of the eyeblink) because it is acknowledged to provide a reliable measure of associative learning (see references in Harvey, 2003). The mPFC and hippocampal areas have a high density of 5-HT_{2A} receptors that are critically involved in acquisition of the rabbit eyeblink response during trace-conditioning procedures. LSD and DOM are both psychedelic 5-HT_{2A} agonists that enhance acquisition of the rabbit eyeblink response, and their effects are blocked by 5-HT_{2A} silent antagonists. Silent antagonists have no effect on the eyeblink response when given alone. Interestingly, MDL11939, ritanserin, and mianserin blocked the effect of LSD and DOM on the eyeblink response; however, when these drugs were given by themselves, they retarded learning, consistent with inverse agonist activity at the 5-HT_{2A} receptor, suggesting that the 5-HT_{2A} receptor that mediates associative learning is constitutively active.

In a study reported by Harvey et al. (2004), rabbits received eight daily injections of LSD, BOL, or MDL11939 followed 1 day, or 8 days later by eight daily 60-minute trace-conditioning sessions. Radioligand binding studies with [^{3}H]ketanserin were used to estimate 5-HT_{2A} receptor density in cortex of rabbits injected with MDL11939 for 8 days. Chronic MDL11939 administration led to a significant 62% increase in 5-HT_{2A} receptor binding in the frontal cortex at 24 hours after the last MDL11939 injection, but there was no change in K_d. Receptor density still remained at 48% above saline control 4 days after the last MDL11939 treatment, but it returned to basal levels between days 4 and 6. Chronic MDL11939 led to a significant enhancement of the conditioned response when animals were tested one day after the last MDL11939 injection. MDL11939-treated animals also achieved a higher rate of conditioned response acquisition compared with controls. Animals who began acquisition training 8 days after the last MDL11939 injection, at a time when 5-HT_{2A} receptor levels would have returned to baseline, demonstrated no significant difference from controls. Surprisingly, LSD and BOL, which produced 33% and 55% decreases in 5-HT_{2A} receptor density, respectively, had no effect on conditioned response acquisition.

Romano et al. (2006) examined the effect of serotonin depletion on eyeblink conditioning by bilateral intracerebroventricular administration of 5,7-dihydroxytryptamine. They achieved up to 85% and 90% depletion of serotonin in the cortex and hippocampus, respectively. Serotonin depletion failed to change the expression of 5-HT_{2A} receptors, however, and had no significant effect on acquisition of trace classic conditioning. Serotonin depletion likewise had no effect on the enhancement of learning produced by LSD or on the retardation of learning produced by MDL11939. It was concluded that acquisition was regulated by constitutive activity of the 5-HT_{2A} receptor.

In another study, Dave et al. (2007) were able to relate the production of head bobs to 5-HT_{2A} receptor density in the cortex. DOI was infused bilaterally into the mPFC. BOL and MDL11939 were injected subcutaneously prior to DOI administration to block acute effects of DOI or were given as eight daily injections for studies of chronic effects on 5-HT_{2A} receptor density. Receptor expression was estimated by radioligand competition binding using [^{3}H]ketanserin. The number of head bobs induced by DOI was significantly blocked by pretreatment with MDL11939 or BOL. Chronic administration of MDL11939 led to a significant increase in DOI-induced head bobs compared with vehicle pretreatment, with a significant 40% increase 24 hours after the first

MDL11939 injection and an 85% increase above controls after the eighth injection. A significant 30% increase in 5-HT_{2A} receptor density was seen in cortex 24 hours after the first MDL11939 injection, increasing to 81% above controls after the eighth injection. These increases roughly parallel the increases seen in DOI-induced head bobs after MDL11939 treatments. By contrast, chronic BOL significantly reduced the number of DOI-induced head bobs to 19%, 12%, and 11% of controls, respectively. Perhaps not surprisingly, 5-HT_{2A} receptor density was reduced by chronic BOL administration, with receptor density reduced by 40% 24 hours after the eighth injection. The authors found a high and significant correlation between the density of frontal cortical 5-HT_{2A} receptors and the elicitation of head bobs for animals given chronic MDL11939 or BOL.

In a later study, Schindler et al. (2012) reported that both the 5-HT_{2A} and dopamine D_1 receptors were required for rabbit head bob behavior. They carried out experiments similar to earlier ones from their laboratory, confirming that LSD and DOI elicit head bobs by 5-HT_{2A} activation, but also finding that SCH23390 [7-chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol], a selective D_1 dopamine antagonist could block head bobs induced by both LSD and DOI.

Finally, Schindler et al. (2013) reported that rabbit head bobs induced by DOI, but not LSD, were mediated by activation of PLC, the canonical signaling pathway coupled to the 5-HT_{2A} receptor. In this work, both DOI- and LSD-stimulated PI hydrolysis was measured as release of [^{3}H]inositol-4-phosphate in rabbit frontocortical tissue prisms. DOI-stimulated PI hydrolysis was blocked by the 5-HT_{2A}–selective antagonist ketanserin. The LSD signal was not blocked by ketanserin, however, but was antagonized by a 5-$HT_{2B/2C}$–selective antagonist, SB-206553. The selective 5-HT_{2C} antagonist SB-206553 also blocked LSD- but not DOI-stimulated PI hydrolysis. Preincubation of the frontocortical tissue with the PLC inhibitor U73122 significantly inhibited both DOI- and LSD-stimulated PI signals. As in earlier studies from this laboratory, bilateral infusion of either DOI or LSD into the mPFC dose-dependently increased the number of head bobs. Bilateral infusion of U73122 significantly reduced DOI-induced head bobs, but it surprisingly had no effect on head bobs in rabbits that had received an LSD infusion. These findings indicate that DOI-signaled through 5-HT_{2A} receptors, whereas LSD only signaled through 5-HT_{2C} receptors. The results of these experiments demonstrate that the two psychedelics can induce PI hydrolysis in the rabbit frontal cortex through PLC activation, but only DOI uses this signaling pathway to elicit head bobs.

D. Zebrafish Models

Zebrafish (*Danio rerio*) are emerging as a new non-mammalian organism for behavioral neuroscience research. They do have substantial physiologic and morphologic homology to humans, and they exhibit robust behavioral responses and are more economical for use than rodent models. Two studies have now been published describing the effects of psychedelics on zebrafish. Grossman et al. (2010) characterized the behavioral and endocrine effects of LSD on adult zebrafish. Behavioral paradigms used were novel tank, observation cylinder, light/dark box, open field, T-maze, social preference, and shoaling tests. The tests are all described in detail in Grossman et al. (2010). Video-tracking tools were used, and whole-body cortisol was measured. Low doses of 5–75 μg/l LSD in the tank water had no effect on zebrafish behavior, although 100 μg/l produced nonsignificant trends. The higher 250-μg/l concentration, however, had significant effects on nearly all behaviors (e.g., increasing top dwelling and reducing freezing in the novel tank and observation cylinder tests). LSD also evoked mild thigmotaxis (avoidance of the center of the tank) in the open field test, increased light behavior in the light/dark test, and reduced the number of arm entries and freezing in the T-maze and social preference tests, without affecting social preference. LSD increased interfish distance in group shoaling and elevated whole-body cortisol levels. LSD also significantly increased whole-body cortisol levels in the novel tank, light/dark box, and open field tests.

In a subsequent study, Kyzar et al. (2012) studied the effect of mescaline (5–20 mg/l) on zebrafish behavior in the novel tank test, open field, and shoaling tests. Mescaline dose-dependently increased top activity in the novel tank test, also reducing immobility and disrupting the patterning of swimming. At the highest dose tested (20 mg/l), mescaline markedly increased shoaling behavior but had no effect on whole-body cortisol levels, in contrast with the effects of LSD reported by Grossman et al. (2010). The pharmacology of LSD is more complex than mescaline, so it is not clear that all of the effects reported by Grossman et al. were due to 5-HT_{2A} receptor–mediated effects.

E. Drosophila melanogaster

The fruit fly (*D. melanogaster*) is the simplest animal model with a centralized brain that has been used to study the effect of a psychedelic. The similarity between mode of drug action, behavior, and gene response in *Drosophila* and mammalian systems make the fly an attractive system to study neuropharmacological processes relevant to human disease (Nichols, 2006). The fly has neurotransmitter receptors that drugs such as LSD, and more receptor-selective hallucinogens like DOI, directly target for their behavioral effects, including 5-HT_2, 5-HT_{1A}–like, and dopamine D_2–like receptors. Furthermore, the fly has glutamate and GABA systems that are known to be indirectly modulated by psychedelics in mammals that are important

for the behavioral effects. In the initial study reporting on the behavioral effects of LSD in the fly, Nichols et al. (2002) examined the effect of acute LSD on general activity, and the effects of LSD on the ability of the fly to follow a moving object (optomotor response). In that study, feeding was accomplished by starving the fly for 15–18 hours and then placing it on blotting paper saturated with a solution of LSD spiked with [^{3}H] glucose so as to be able to measure the amount of drug consumed after the experiments. With this method, a single bolus dose ranging from 200 to 1200 ng/fly was ingested within 1 to 2 minutes.

Flies carrying a mutation in the *white* gene (w^{1118}) demonstrated a progressive loss of activity and coordination over about 15 minutes after LSD administration, followed by a recovery beginning at about 60 minutes. The more severely affected flies became completely paralyzed at about 15–20 minutes after feeding. Ketanserin blocked the LSD-induced decrease in locomotor activity observed in the mutant fly strain. WT flies with red eyes, however, did not demonstrate any overt impairment of coordination or activity even after ingesting comparatively large amounts of LSD. The *white* gene encodes the protein for the tryptophan transporter (tryptophan is the biosynthetic precursor for serotonin) and it was later demonstrated by another group that *white* mutant flies have low levels of serotonin (Borycz et al., 2008). Therefore, it seems likely there is a compensatory upregulation of serotonin receptors in these flies that results from abnormally low levels of serotonin that confers the observed supersensitivity of w^{1118} flies to LSD.

Within the fly brain, there is a high density of serotonergic processes as well as 5-HT$_{1A}$–like receptors in the visual centers of the fly brain that mediate visual processing (Luo et al., 2012). One behavior mediated by these areas is the optomotor response, or the ability of a fly to track and follow a moving object. Feeding WT red-eyed flies LSD produced a profound disruption of this behavior without overtly affecting locomotor activity. Coadministration of 5-HT$_{1A}$ and 5-HT$_2$ receptor antagonists was able to rescue this LSD-induced deficit in behavior. At the molecular level, analysis of the brains of flies treated with LSD indicated that it induces expression of the immediate early gene homolog of *cFos*, *dfos* (*kayak*), similar to what has been observed in the mammalian brain (Nichols and Sanders-Bush, 2002; Nichols et al., 2002).

In subsequent studies, the Nichols group used a more receptor-selective psychedelic, DOI, to probe the role of the 5-HT$_2$ receptor in fly behavior (Nichols, 2006; Johnson et al., 2009, 2011). Rather than starving and feeding as performed earlier, drug was mixed with the food substrate and the flies were maintained on this for up to several days before testing. This feeding strategy was intended to allow the flies to achieve a steady-state level of drug. Different concentrations of drug were mixed into the food to perform dose-response experiments. DOI was found to increase locomotor activity slowly over several days, disrupt aspects of circadian behavior, and decrease aggressive interactions between two males when paired in an arena. In conditioned-stimulus olfactory learning, the psychedelic DOI was found significantly to disrupt short-term learning and memory (Johnson et al., 2011). LSD has been tested in flies for its effect on learning and memory, where it also was found to disrupt short-term memory (C. D. Nichols, personal communication). In experiments examining aspects of learning and long-term memory, DOI had no significant effect on acquisition but significantly disrupted consolidation and recall (Johnson et al., 2011).

F. Monkey Models

Although nonhuman primates might be the best animals to model the actions of psychedelics in humans, almost nothing has been published in the past decade using monkeys. Fantegrossi et al. (2004) reported on transient reinforcing effects of phenylisopropylamine and indoleamine psychedelics in rhesus monkeys. They investigated the capacity of several psychedelics to maintain self-administration in four rhesus monkeys with a history of MDMA self-administration. No dose of either DMT or psilocybin engendered significantly higher mean response rates than did contingent saline, although one monkey did exhibit transitory reinforcing effects after DMT and psilocybin. Similar results were obtained with mescaline and DOI, in which no dose tested engendered significantly higher mean rates than did contingent saline. Mescaline transiently maintained significant self-administration in three monkeys, but the apparent reinforcing effects of mescaline did not persist in any monkey. DOI was not self-administered by any subject. These results are consistent with a number of early experiments showing that classic psychedelics lack reinforcing properties, supporting the conclusion that these drugs do not lead to dependence or addiction.

Li et al. (2009) examined the discriminative stimulus effects of DOM, DPT, and 2,5-dimethoxy-4-*n*-propylthiophenethylamine (2C-T-7) in four adult rhesus monkeys. The nature of the cue was investigated using three antagonists: M100907, ketanserin, and ritanserin. Monkeys were trained to discriminate 0.32 mg/kg subcutaneous. DOM from vehicle, responding on a FR5 schedule of stimulus shock termination. DOM, 2C-T-7, and DPT all dose-dependently increased responding on the DOM-appropriate lever. M100907, ketanserin, and ritanserin each shifted the dose-response curves rightward in a parallel fashion. Potency of the antagonists was correlated with their affinity at 5-HT$_{2A}$ receptors. Their results provide evidence that the discriminative cue of the three psychedelics in rhesus monkeys is predominantly, if not exclusively, mediated by 5-HT$_{2A}$ receptors.

IX. Potential Therapeutic Value for Psychedelics

Clinical research with psychedelics essentially ended with the passage of the Controlled Substances Act of 1970. As pointed out in the *Introduction*, there were more than a thousand clinical articles discussing 40,000 patients, several dozen books, and six international conferences on psychedelic drug therapy (Grinspoon and Bakalar, 1979). There were serious attempts to employ LSD in various kinds of therapy, with major emphasis on treatment of alcoholism and other addictions (Bogenschutz, 2013), as well as issues related to death and dying (e.g., see Grof et al., 1973; Kurland, 1985). Other studies examined the use of psychedelics to treat anxiety and depression, schizophrenia, and even autism (e.g., Bender, 1966). A review of psychedelic-assisted therapy was recently published (Majić et al., 2015).

After a long hiatus in clinical research, one of the most exciting and encouraging recent developments in this field has been the reintroduction of human treatment studies with psychedelics. Studies discussed in this section will be seen by the reader to reinforce the early belief that psychedelics might represent an important new treatment modality for a variety of disorders. The fact that these new and positive clinical findings had to be postponed for several decades dramatically illustrates the destructive capacity of politics to hinder potentially significant medical advances.

It should be pointed out that the efficacy of a psychedelic in treating a particular condition could be attributed either to a psychologic effect or to actual physiologic adaptive changes in brain neurochemistry. Therefore, in some cases, the discussion will focus on possible receptor and neurochemical mechanisms that could serve as the explanation for efficacy. In other examples, there may be no evident explanation in known physiology, and efficacy may involve novel acute psychologic mechanisms, although ultimately one is still talking about neurochemistry. In any event, after this discussion of many new clinical findings, it is hoped that the reader will be convinced that further clinical research on psychedelics is clearly warranted.

A. Alleviation of Anxiety and Depression in Life-Threatening Illness

One of the most well documented therapeutic effects of LSD, first established in the 1960s, was alleviation of anxiety and depression in acutely ill patients. This research direction received its original impetus from observations by Chicago internist Eric Kast. Kast and Collins (1964) found that LSD had an analgesic effect at least comparable to opiates, but that the LSD analgesia outlasted its acute psychologic effects. Subsequent study revealed that patients treated with LSD had improved psychologic adjustment, were more responsive to their families and environments, and had enhanced ability to enjoy everyday life (Kast, 1966, 1970). Beginning in 1963, a group at the Spring Grove State Hospital in Maryland developed an extensive research program to study the value of psychedelic-assisted psychotherapy of patients with alcoholism or neuroses, patients who were addicted to narcotics, and patients who were dying of cancer (Pahnke et al., 1970a). With respect to treatment of dying cancer patients, this group found that about two-thirds of cancer patients who received LSD treatment had improved mood and reduced anxiety and fear of death (Pahnke et al., 1969, 1970b; Grof et al., 1973).

Building on the positive results from the Spring Grove group, Grob et al. (2011) recently used a crossover design and administered 0.2 mg/kg psilocybin versus niacin placebo to 12 patients with advanced-stage cancer with a diagnosis of anxiety related to their cancer. Grob et al. (2011) reported nonsignificant trends for benefits of psilocybin compared with placebo on measures of depression and anxiety. Compared with pretreatment baseline, however, the patients' Spielberger State-Trait Anxiety Inventory (STAI) trait anxiety subscale scores revealed a significant reduction in anxiety at 1 and 3 months after treatment. Similarly, the patients' Beck Depression Inventory (BDI) scores showed an improvement of mood that reached significance at 6 months compared with baseline. There was a trend for mood improvement that approached but did not reach significance.

These encouraging results in such a small study led to extension of this approach by two groups, one at Johns Hopkins University (JHU) and the other at New York University (NYU), in studies that were recently completed. These are two reasonably large, well powered phase 2 trials of psilocybin-assisted psychotherapy in patients suffering from cancer-related psychosocial distress (CRPD). These two studies represent the first well powered, properly designed, formal double-blind, placebo-controlled assessment of a psychedelic agent used for a medical treatment, using modern clinical approaches and assessment instruments. As such, they represent a modern watershed moment in clinical psychedelic research. Perhaps even more importantly, these studies report remarkable results of efficacy that are unprecedented for CRPD with any available conventional therapies. Unfortunately, neither study has yet been published, so complete details cannot be reviewed here, although I have seen the results. Nonetheless, a preliminary report of the JHU study results was presented at the 2015 Annual Meeting of the American Psychiatric Association; that presentation will serve as the basis for this summary (Griffiths, 2015).

The first of these trials of psilocybin-assisted psychotherapy for CRPD was completed by Roland Griffiths and his colleagues at JHU (Griffiths, 2015). In that study, 56 individuals were enrolled and randomized to

receive two treatments with psilocybin (high dose versus low dose) in a randomized, crossover design, and 51 participants completed at least one psilocybin session. All 51 participants had a potentially life-threatening cancer diagnosis, with 65% having recurrent or metastatic disease. All participants had a *DSM-IV* diagnosis [including adjustment disorder with anxiety; adjustment disorder with anxiety and depressed mood, chronic; dysthymic disorder; generalized anxiety disorder; major depressive disorder (MDD); or a duel diagnosis of generalized anxiety disorder and MDD, or generalized anxiety disorder and dysthymic disorder].

The study used a two-session, double-blind, crossover design to investigate the effects of a high psilocybin dose (22 mg/70 kg) with a low dose (1 mg/70 kg), both ingested orally, on a variety of outcome measures relevant to anxiety or depressive disorders likely caused or exacerbated by the cancer diagnosis. Expectancy is likely to be significantly operative in a standard drug versus placebo design when the drug being evaluated produces highly discriminable effects and participants and staff know the specific drug conditions to be tested. Therefore, a low dose of psilocybin was compared with a high dose of psilocybin and participants and guides were given instructions that obscured the range of possible drug conditions to be tested.

After enrollment and assessment of baseline measures and before the first psilocybin session, each participant met with study guides on two or more occasions to establish rapport and prepare the participant for the psilocybin sessions. After each psilocybin session, participants met with the study guides within a day or two after the session to discuss their experiences. The duration of each volunteer's participation was approximately 9 months.

Data assessments occurred as follows: immediately after study enrollment (baseline assessment), on psilocybin session day 1 (with multiple assessments across the day), approximately 5 weeks after psilocybin session one (postsession 1), on psilocybin session day 2 (multiple assessments across the day), approximately 5 weeks after psilocybin session 2 (postsession 2), and about 6 months after session 2 (6-month follow-up). Change in depressive symptoms was evaluated by the clinician-administered Hamilton Rating Scale for Depression (HAM-D) and the self-report BDI. Anxiety symptoms were assessed with the clinician-administered Hamilton Rating Scale for Anxiety (HAM-A) and the self-report Spielberger STAI.

For measures that were assessed in the two dose-sequence groups at baseline, postsession 1, postsession 2, and 6 months, the following planned comparisons most relevant to examining the effects of psilocybin dose were conducted: between-group comparisons at baseline, postsession 1, and postsession 2; and within-group comparisons of baseline versus postsession 1 in both dose-sequence groups, and postsession 1 versus postsession 2 in the low-dose first group. Therapeutically relevant outcome measures fulfilling the most conservative criteria for psilocybin effects all showed sustained effects at 6 months on the HAM-A, STAI, HAM-D, BDI, and several other scales. Between-group differences at 5 weeks after the first intervention (i.e., postsession 1 assessment) showed Cohen's d effect sizes of 1.30 for HAM-D scores, 0.81 for BDI scores, 1.23 for HAM-A scores, and 0.60 for STAI trait anxiety scores (Cohen's d is the difference between the two group means, divided by the pooled standard deviation; $d = 0.8$ is considered to be a large effect size). Effect sizes for within-group change scores in the low-dose first group (i.e., change between postsession 1 and postsession 2 assessments) were 1.33 for HAM-D, 0.69 for BDI, 1.10 for HAM-A, and 0.60 for STAI anxiety. These effects persisted through the 6-month follow-up period. Other outcome measures showing sustained effects at 6 months included Community Observer Ratings of Positive Changes in Attitudes and Behavior, Purpose in Life, Life Attitude Profile Revised (LAP-R) Coherence, and several measures of spirituality.

High-dose psilocybin showed a large effect size and a statistically significant advantage over low-dose psilocybin for reducing clinician-assessed and self-report measures of depression and anxiety. In addition to large effect size reductions in depression and anxiety, high-dose psilocybin produced significantly greater ratings of positive persisting effects on attitudes about life and self, social effects, and spirituality. These effects were generally sustained at the 6-month follow-up. Consistent with the positive changes, high-dose experiences were also rated as producing significantly greater personal meaning, spiritual significance, and increased well-being or life satisfaction than the low-dose experiences, with these differences sustained at 6 months. Furthermore, the immediate postsession mystical experience score was linearly correlated with therapeutic efficacy. Griffiths (2015) concluded that a single moderate to high dose of psilocybin, if given under supportive conditions to carefully screened and prepared participants, produced substantial and enduring decreases in anxiety and depression in patients with a life-threatening cancer diagnosis.

A very similar study was also just completed at NYU under the direction of Dr. Stephen Ross (S. Ross, personal communication), in which 29 participants with significant distress due to a cancer diagnosis were enrolled and randomized. The therapeutic approach and clinical setting were very similar to the one employed in the JHU study, the chief difference being the use of niacin as the placebo control in the NYU study, contrasted with the use of low-dose psilocybin, versus high-dose psilocybin in the JHU study. Eligible participants had a primary *DSM-IV* diagnosis (adjustment disorder with anxiety and depressed mood, chronic; adjustment disorder with anxiety, chronic; and generalized anxiety disorder), with more than

one-half of the participants in advanced stages of their illness. Participants were assigned to receive psilocybin (0.3 mg/kg) or niacin (250 mg) administered during two 8-hour treatment sessions.

Dramatic symptom reductions were observed, with large effect sizes comparable to the JHU study, and efficacy sustained at least out to 6 months after treatment. As with the JHU study, it was concluded that a single moderate dose (0.3 mg/kg) of psilocybin in conjunction with psychotherapy was safely administered to a cohort of patients with life-threatening cancer, leading to acute and sustained anxiolytic and antidepressant effects, with clinical benefit associated with intensity of the subjective mystical experience, improved attitudes toward disease progression and death, improved quality of life, and increased spiritual well-being.

Similarly, Gasser et al. (2014) employed LSD-assisted therapy in 12 patients with anxiety related to a diagnosis of life-threatening diseases. Patients received either 200 μg LSD (free base) or a 20 μg "active placebo" dose of LSD with an open-label crossover to 200 μg LSD after unblinding. There were no serious adverse effects associated with the treatment. Positive trends found using the STAI were sustained for 12 months after treatment. In a subsequent study, Gasser et al. (2015) followed up the same participants 12 months later to examine long-term effects on anxiety and explore subjective experiences and lasting psychologic effects. Nine of the original subjects participated. Gasser et al. (2015) found that the STAI state and trait scores did not increase after the end of the study. In semistructured interviews, seven of nine participants reported a sustained reduction in anxiety. None of the participants reported lasting negative effects. The authors concluded that LSD-assisted psychotherapy in patients with life-threatening illness demonstrated safety and positive stable treatment outcomes at long-term follow-up.

These studies, taken together, demonstrate remarkable efficacy in reducing the symptoms of CRPD. On the basis of the effect sizes, there is no currently available treatment that can produce such long-lasting and high therapeutic efficacy. Large-scale, multisite trials now need to be implemented to determine whether these results can be repeated in a larger cohort of patients.

Although these studies examined psychedelic-assisted therapy to treat depression and anxiety after a life-threatening cancer diagnosis, the question must be asked as to whether similar results could be obtained in a patient population that suffers from depression that does not result from a cancer diagnosis. In that regard, Osorio et al. (2015) recently reported that a single dose of ayahuasca had a significant antidepressant effect in patients with recurrent MDD. Osorio et al. conducted an open-label study of six volunteers with different severities of depressive symptoms. The average baseline HAM-D score was 17.56. One day after ayahuasca ingestion, there was a statistically significant 62% decrease in the mean HAM-D score. By day 7, there was a further drop in the score. Although the mean score increased by day 14 to a nonsignificant degree (due to one patient), it was again significantly below the baseline score by day 21. Scores on the Montgomery-Åsberg Depression Rating Scale showed similar effects, virtually paralleling the effects seen with the HAM-D.

This study was open label with a very small sample size, so it remains to be seen whether similar results could be obtained in a larger, randomized, double-blind, placebo-controlled trial. It should be noted, however, that at the present time, it seems unlikely that the U.S. Food and Drug Administration would approve clinical studies employing ayahuasca because of the lack of control in its preparation and its variable alkaloid content. Thus, studies of the potential antidepressant effects of a psychedelic in endogenous depression likely will have to focus on psilocybin or LSD, at least in the United States.

Evidence from animal studies suggests that some psychedelics may alleviate anxiety, in particular DOI, a nonselective 5-$HT_{2A/2C}$ agonist, which has been perhaps the most widely studied drug in this class. The four-plates test–retest paradigm in mice is an animal model of anxiety that is broadly sensitive not only to benzodiazepines but also to nonbenzodiazepine-type compounds. DOI was first shown to possess an anxiolytic effect in this model by Nic Dhonnchadha et al. (2003). Subsequently, Ripoll et al. (2005) assessed the ability of the four-plates to distinguish the anxiolytic effect of DOI from diazepam, alprazolam, paroxetine, and venlafaxine. In contrast with the other drugs, DOI was the only one able to restore the number of punished crossings to the same value seen in naïve saline-treated mice. These authors used the mouse hotplate test to insure that the effect was not due simply to analgesia produced by DOI. In a further study, Ripoll et al. (2006) used 5-HT_{2A}–selective and 5-$HT_{2B/C}$–selective antagonists to examine the receptor type involved in the anxiolytic effect of DOI. Only the 5-HT_{2A}–selective antagonist blocked the DOI-induced antipunishment effect, suggesting that the anxiolytic effect of DOI was mediated through the 5-HT_{2A} receptor. The authors discuss many possible mechanisms whereby 5-HT_{2A} receptor activation could affect other neurotransmitter systems known to be involved in anxiety, such as GABA or noradrenergic pathways, but the mechanism through which DOI exerts an anxiolytic effect remains to be elucidated.

Massé et al. (2007) investigated the role of the GABA system in the anxiolytic activity of DOI. They coadministered DOI along with $GABA_A$ and $GABA_B$ receptor agonists and antagonists, and investigated effects on the four-plates test of anxiety in mice. Low doses of DOI that had no effect alone in the four-plates test were significantly potentiated by alprazolam and diazepam,

but not by flumazenil. The $GABA_B$ agonist baclofen significantly decreased the anxiolytic effect of DOI. Their results, as well as a number of articles cited showing the expression of 5-HT_{2A} receptors on GABA neurons, led these authors to hypothesize that 5-HT_{2A} receptor activation on GABA neurons increases GABA release, which stimulates postsynaptic $GABA_A$ receptors. In a separate study, Masse et al. (2008) microinjected DOI into the mouse hippocampus, amygdala, or periaqueductal gray matter (PAG) and then immediately subjected the mice to the four-plates test to assess anxiety. They demonstrated that DOI produced an anxiolytic effect only when microinjected into the hippocampus, but not into the amygdala or PAG. Indeed, when injected into the amygdala or PAG, DOI appeared to have an anxiogenic effect in the four-plates test, decreasing the number of punished crossings.

B. Possible Use in Depression

The first report describing use of LSD in treating depression was in 1952 (Savage, 1952). There were no controls, and LSD was given in variable daily doses for 1 month. Savage concluded that improvement obtained using LSD therapy was no better than therapy without LSD but did state that therapeutically valuable insights into unconscious processes were gained. Since then, virtually nothing has been reported to suggest that psychedelics might have specific efficacy against endogenous depression. Nonetheless, studies cited in the previous section concerning treatment of anxiety and depression secondary to a cancer diagnosis do indicate that psychedelics may be effective in treating depression. A small, open-label study of ayahuasca in relieving depression reported by Osorio et al. (2015) is also suggestive. It remains to be proven whether psychedelics can be useful for treating MDD.

There is good reason to believe that psychedelics might have efficacy in treating depression, however. For example, it has been shown that cortical 5-HT_{2A} receptor expression is increased in postmortem samples of depressed and suicidal patients (Mendelson, 2000; Pandey et al., 2002; Shelton et al., 2009), whereas 5-HT_{2A} binding was decreased in the hippocampus of depressed patients (Sheline et al., 2004). Furthermore, it has been shown that medication-free depressed patients with high pessimistic attitudes have increased 5-HT_{2A} receptor binding in the PFC compared with healthy control subjects (Meyer et al., 2003; Bhagwagar et al., 2006; Meyer, 2012).

Frokjaer et al. (2008) used [^{18}F]altanserin PET to image serotonin 5-HT_{2A} receptor binding in the human brain of 83 healthy Danish volunteers. They also were administered a standardized personality questionnaire to assess the trait of neuroticism. Neuroticism was positively correlated with frontolimbic 5-HT_{2A} receptor binding, the correlation being primarily driven by two constituent traits of neuroticism: vulnerability and anxiety, with vulnerability displaying the strongest positive correlation. Within the frontolimbic region, the highest correlations were found in the entorhinal cortex, superior frontal cortex, PCC, and inferior frontal cortex. The authors hypothesize that because neuroticism is associated with the risk of developing depression, and with the high correlation between neuroticism and its constitutive trait of vulnerability, perhaps a high vulnerability score is also associated with risk of developing depression. Vulnerability was measured by questions that explored the subject's ability to cope with stress and critical situations, among others. The authors speculate that "high frontolimbic 5-HT_{2A} binding in healthy subjects with high neuroticism scores could be a genetically determined trait that might lead to a depressive state when a subject is exposed to stressful stimuli" (Frokjaer et al., 2008).

Borderline personality disorder (BPD) is a high-risk model for studying suicidal behavior and is a contributing factor in more than one-third of completed suicides (Soloff et al., 2007, and references therein); indeed, BPD is one of the most lethal psychiatric disorders. Soloff et al. (2007) used [^{18}F]altanserin, a high-affinity 5-HT_{2A} antagonist, to conduct PET imaging studies of 5-HT_{2A} receptor binding in 14 female impulsive BPD subjects with recurrent suicidal or self-injurious behaviors who were considered at high risk for future suicide. A control sample of 11 matched healthy subjects was used for comparison. Significantly increased 5-HT_{2A} binding was found for BPD patients in the hippocampus, medial temporal cortex, and occipital cortex compared with healthy controls. The most robust increase was seen in the hippocampus, especially among nondepressed BPD subjects. Increased binding is typically interpreted to mean that the 5-HT_{2A} receptors have been upregulated in response to decreased 5-HT neurotransmission. Although the authors present an extensive discussion of potential confounding factors and limitations to their study, it is nevertheless intriguing to speculate as to whether a psychedelic might prove useful in treating BPD.

The amygdala is a central brain structure involved in the neurocircuitry of emotion processing. Serotonin neurotransmission plays a key role in amygdala activity (see earlier section III.G. in this review on 5-HT_{2A} receptor expression in the amygdala) and may be implicated in the pathogenesis of depression. Kraehenmann et al. (2015) used BOLD fMRI to evaluate the effects of psilocybin (0.16 mg/kg, p.o.) on brain activity during emotion processing in 25 healthy, right-handed subjects, focusing on the amygdala as a region of interest (ROI). Subjects first completed a slightly modified version of the amygdala reactivity task, which comprised alternating blocks of emotional picture discrimination tasks. Shape discrimination tasks were interspersed within the picture discrimination task to serve as baseline tasks and to allow the amygdala responses to return to baseline.

Psilocybin significantly increased positive affect, but not negative affect or state anxiety. Psilocybin increased subjective reports of positive mood but did not increase anxiety. BOLD fMRI results revealed that psilocybin significantly attenuated right amygdala activation to both negative and neutral pictures; amygdala reactivity to negative and neutral stimuli was lower after psilocybin treatment than placebo. Psilocybin-induced attenuation of amygdala reactivity was significantly correlated with increase of positive mood. Attenuation of right amygdala reactivity in response to negative stimuli was associated with the psilocybin-induced increase in positive mood state. The authors conclude that their findings may be relevant to the normalization of amygdala hyperactivity and negative mood states seen in patients with major depression, and they suggest that psilocybin has the potential to normalize limbic hyperactivity in persons with depressed mood.

It has been recognized for some time that psychedelics (i.e., 5-HT_{2A} agonists) increase levels of cortical glutamate (Aghajanian and Marek, 1997, 1999b; Béïque et al., 2007; also see the earlier section on glutamate in this review). Vollenweider and Kometer (2010) suggested that indirect activation of glutamate networks by classic psychedelics may enhance neuroplasticity through stimulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–type glutamate receptors and subsequent increase in the level of BDNF. Serum BDNF levels are abnormally low in depressed individuals and treatment with antidepressants is known to normalize BDNF levels (Sen et al., 2008).

Using olfactory bulbectomy in rats as an animal model of depression, Buchborn et al. (2014) demonstrated that repeated LSD treatment reversed the marked deficits in active avoidance learning in this animal model. This finding was similar to an earlier article in which Grecksch et al., (1997) found reversal of these deficits by imipramine; Buchborn et al. (2014) conclude that LSD has an antidepressant-like effect in this model.

Trace fear conditioning in mice has previously been shown to be a hippocampus-dependent learning paradigm. Catlow et al. (2013) investigated the role of psilocybin on hippocampal neurogenesis and trace fear conditioning in rats. They found that a single low dose of psilocybin (0.1 mg/kg, once per week for 1 month) led to more rapid extinction of cued fear conditioning and increased neurogenesis compared with saline control. The low dose of psilocybin was also associated with a slight but nonsignificant trend toward an increase in hippocampal neurogenesis compared with vehicle control. Higher doses of psilocybin, or the 5-HT_{2A} antagonist ketanserin, had no effect on extinction of the conditioned fear response but significantly depressed hippocampal neurogenesis. The authors speculate that the 5-HT_{2A} receptor might serve as a target for conditions where a previously neutral set of stimuli is associated with noxious or life-threatening events, such as post-traumatic stress disorder (PTSD).

Depressed subjects need more intensely happy facial expressions to label happiness correctly and are slower in responding to positive but not to negative words in emotional go/no-go tasks. Taking into account an extensive literature indicating that serotonin 5-HT_{1A} and 5-HT_{2A} receptors are implicated in the pathophysiology of dysfunctional emotional biases, Kometer et al. (2012) examined for the first time the effects of psilocybin on facial recognition, goal-directed behavior, and mood state. Seventeen healthy human subjects on 4 separate days received placebo, psilocybin (215 μg/kg), the preferential 5-HT_{2A} antagonist ketanserin (50 mg), or psilocybin plus ketanserin. Mood states were assessed by self-report ratings, and behavioral and event-related potential measurements were used to quantify facial emotional recognition and goal-directed behavior toward emotional cues. Here, Kometer et al. (2012) assessed the ability to recognize the emotional state of another person from facial cues. They also quantified response selection and inhibition to emotional cues by behavioral and event-related EEG measurements in an emotional go/no-go task. In this test, 36 black-and-white photographs of the eye region of persons expressing different subtle emotional states were presented on a computer screen along with four words describing the states of the persons. Participants were instructed to choose the word that most accurately described the state of the person. The total number of correct recognitions was calculated for different valence categories. In the emotional go/no-go task, emotionally valenced words were presented in the middle of the computer screen. Participants were instructed by text appearing at the beginning of each block to press as rapidly as possible a response button when words of one valence category were presented (go cues) and withhold responses to words of another valence category (no-go cues). In the EEG analysis, event-related potentials were referenced to the average reference before N2 and P300 amplitudes were quantified against baseline activity.

Kometer et al. (2012) reported that psilocybin enhanced positive mood and attenuated recognition of negative facial expression. Psilocybin also increased goal-directed behavior toward positive compared with negative cues, facilitated positive but inhibited negative sequential emotional effects, and valence-dependently attenuated the P300 component. Ketanserin blocked the psilocybin-induced mood enhancement and decreased recognition of negative facial expression. This study demonstrated that psilocybin shifts the emotional bias across various psychologic domains and that activation of 5-HT_{2A} receptors is central in mood regulation and emotional face recognition in healthy subjects.

The strong effects observed of psilocybin on mood contrast with many other drugs that modulate serotonergic tone, which usually lack acute effects on mood. The serotonin 5-HT_{2A} receptor also appears to be crucially involved in the recognition of negative facial expressions, because ketanserin blocked psilocybin-induced attenuation in recognizing negative emotional states from the eye region of human faces. In the emotional go/no-go task, psilocybin enhanced the response bias toward positive relative to neutral and negative emotional stimuli, evidenced by the psilocybin-induced increase in reaction times to negative and neutral compared with positive stimuli.

In a subsequent study, Bernasconi et al. (2014) carried out electrical neuroimaging analyses on visual evoked potentials in response to facial expressions (fearful, happy, and neutral) under placebo and psilocybin treatment. The aim of the study was to identify neurophysiological modulation induced by psilocybin to emotional face processing. The experiment consisted of an EEG passive-viewing emotional face task, in which participants were instructed to determine the emotional valence of each face; no response was required. Stimuli were black-and-white images that were neutral with basic emotional expressions. They found a first time period of strength (i.e., Global Field Power) modulation of the 168- to 189-millisecond poststimulus interval, induced by psilocybin. They also identified a second time period of strength modulation of the 211- to 242-millisecond poststimulus interval. Source estimations over these two time periods revealed decreased activity in response to both neutral and fearful faces within limbic areas that included the amygdala and parahippocampal gyrus, the right temporal cortex over the 168- to 189-millisecond interval, and reduced activity in response to happy faces within limbic and right temporo-occipital brain areas over the 211- to 242-millisecond interval. Their results indicate a selective and temporally dissociable effect of psilocybin on the neuronal correlates of emotional face processing, consistent with a modulation of top-down control.

C. Obsessive-Compulsive Disorder

Obsessive-compulsive disorder (OCD) is a debilitating medical condition that is very difficult to treat and for which conventional therapies, such as selective serotonin reuptake inhibitors (SSRIs), are not highly efficacious. It has long been believed that serotonin systems are involved in OCD (Benkelfat et al., 1989), and the apparent efficacy of serotonergic psychedelics would point more specifically to involvement of the 5-HT_{2A} receptor. The earliest indication of efficacy for a psychedelic in treatment of OCD was reported by Jackson (1962), in which a patient suffering from depression and violent obsessive sexual thoughts experienced dramatic and permanent improvement after only two doses of LSD. Brandrup and Vanggaard (1977) report on the outcome of LSD treatment of a 30-year-old man suffering from a completely disabling OCD. The treatment took place over 15 months, and surprisingly, without any other therapy provided. While under the influence of LSD, the patient was simply left alone except for brief visits by the doctor or the nurse. At 3 years, the patient was completely symptom free and remained so at 12-year follow-up, the point at which the therapists published the case report.

In addition to these reports of LSD treatment, anecdotal accounts of OCD symptom reduction by consumption of *Psilocybe* mushrooms have also been published (Leonard and Rapoport, 1987; Moreno and Delgado, 1997). Hanes (1996) reported on a 27-year-old male patient with body dysmorphic disorder who spent up to 4 hours every day checking his appearance in the mirror. The intensity of his somatic distress markedly improved on occasions when he had ingested psilocybin mushrooms, noting that at those times, when he looked in the mirror, he no longer appeared deformed.

Following up on these reports, the effect of varying of doses of oral psilocybin (100, 200, or 300 μg/kg) was tested in a small proof-of-concept pilot study of nine subjects who suffered from OCD (Moreno et al., 2006). Subjects received up to four different doses, separated by 1 week. Symptom assessment with the Yale-Brown Obsessive Compulsive Scale was carried out at 4, 8, and 24 hours after treatment. Marked decreases in symptoms were seen in all subjects during one or more sessions (23%–100% reduction in Yale-Brown Obsessive Compulsive Scale score), and most subjects with symptom reduction experienced relief beyond the expected pharmacological life of psilocybin, and beyond the 24-hour assessment. Unfortunately, only one subject had achieved long-term remission at the 6-month follow-up. The authors suggest that their results warrant future studies using a traditional blinded, randomized, placebo-controlled trial to explore the efficacy and duration of effect from a more prolonged exposure to repeated doses of psilocybin in OCD patients.

Adams et al. (2005) compared cerebral 5-HT_{2A} receptor binding in 15 untreated OCD patients and 15 matched healthy controls using [^{18}F]altanserin PET imaging. Increased 5-HT_{2A} receptor binding was found in the caudate nuclei of untreated OCD patients, but there was no correlation between the severity of OCD symptoms and 5-HT_{2A} receptor binding. Compared with the healthy group, untreated OCD patients had significantly higher 5-HT_{2A} binding in both the left and right caudate nuclei. Eleven OCD patients were rescanned with PET after receiving a minimum of 12 weeks of daily treatment with an SSRI, and there was no longer a difference in receptor binding levels between the treated patients and the healthy controls. The investigators suggested that the upregulation of 5-HT_{2A} receptors in OCD patients may be a compensatory mechanism for a lack of serotonin in the feedback loop between the thalamus and OFC, the caudate nuclei, and the globus

pallidus. It is known that serotonergic psychedelics cause rapid downregulation of 5-HT_{2A} receptors, which might have an effect similar to SSRI treatment with respect to 5-HT_{2A} receptors.

Marble-burying behavior in mice is considered to be an animal model of OCD. Matsushima et al. (2009) studied the effect of psilocybin and the dried and powdered mycelium of a psilocybin-containing mushroom *Psilocybe argentipes* on marble-burying behavior in male mice. *P. argentipes* at a dose of 0.1–1 g/kg significantly reduced the number of buried marbles without reducing locomotor behavior. Pure psilocybin at a dose of 1.5 mg/kg also significantly reduced the number of buried marbles. This dose of psilocybin was higher than that calculated to be contained in the effective dose of *P. argentipes*, and the authors speculate that perhaps other tryptamine components of the mushrooms may also be involved in the effect.

These promising results provide support for more extensive controlled clinical trials of a psychedelic, either LSD or psilocybin, in OCD. The relative lack of efficacy for current therapies argues for more effort to be put into studies of the efficacy of psychedelics for this very-difficult- to-treat condition.

D. Treatment of Alcoholism or Nicotine Addiction

Canadian psychiatrists Humphrey Osmond and Abram Hoffer considered LSD for the treatment of alcoholism to be especially promising. Indeed, there were a number of publications suggesting that psychedelics could be useful in treating substance abuse (Chwelos et al., 1959; Smart et al., 1966; Hollister et al., 1969; Savage and McCabe, 1973). Unfortunately, early investigators did not employ rigorous clinical methods such as randomized controlled trials, outcome measures, and treatment settings, and thus those studies did not provide definitive results. Abuzzahab and Anderson (1971) reviewed studies of 1100 alcoholic patients treated with LSD in the period from 1953 to 1969 and came to a similar conclusion; however, they did find reports of improvement at 10 months for 75% of patients who received a single dose of LSD compared with only 44% of controls. For patients who received multiple doses of LSD, 58% were "improved" compared with 54% of the controls. Unfortunately, the social concerns engendered by widespread recreational use of LSD led to government restrictions that effectively ended legitimate medical research (Dyck, 2005).

Whether LSD was actually effective in treating alcoholism remained an open question for decades (Mangini, 1998), until the recent meta-analysis by Krebs and Johansen (2012), which concluded that a single dose of LSD, used in various treatment programs, was associated with a decrease in alcohol misuse. Their analysis was based on six eligible clinical trials from the period from 1943 to 2010, which included 325 adults that received a single (210–800 μg) dose of LSD and 211 adults that were used as controls.

Bogenschutz and colleagues at the University of New Mexico recently revisited this issue. These investigators carried out an open-label proof-of-concept trial of psilocybin treatment in a sample of 10 volunteers with a *DSM-IV* diagnosis of alcohol dependence (Bogenschutz et al., 2014). Treatment consisted of psychosocial treatment only, psychosocial treatment coupled with psilocybin, and post-treatment follow-up. There was no increase in abstinence during the first month of psychosocial treatment only, but there was a significant increase in abstinence after subjects had received psilocybin. The strength of the psilocybin experience was a predictor of drinking behavior improvement, craving, and self-efficacy. No significant treatment-related adverse events were reported.

Remarkably, a recent study has shown that therapeutic approaches using psilocybin can also be highly effective in helping long-time cigarette users quit smoking. Johnson et al. (2014) carried out an open-label pilot study using psilocybin as an adjunct to psychotherapy in a structured 15-week smoking cessation program. Subjects were 15 healthy nicotine-dependent smokers who had made multiple unsuccessful attempts to quit smoking. Subjects received weekly cognitive-behavioral therapy, with a psilocybin treatment session at week 5. Weekly therapy sessions continued, with a second dose of psilocybin at week 7 and a third optional dose at week 13. At the 6-month follow-up, 12 of 15 subjects (80%) showed abstinence. This smoking cessation rate exceeds the typical (<35%) rates observed in other behavioral or pharmacological therapies. These very promising results certainly argue for further investigation of this approach.

In a follow-up to the report by Johnson et al. (2014), Garcia-Romeu et al. (2014) reported that abstainers scored significantly higher on a measure of psilocybin-occasioned mystical experience. There were no significant differences found between groups in general intensity of drug effect, suggesting that mystical-type subjective effects, rather than overall intensity of drug effect, were responsible for smoking cessation. Smoking cessation outcomes were significantly correlated with measures of mystical experience on drug session days. The authors propose a mediating role for mystical experience in psychedelic-facilitated addiction treatment.

In a longitudinal study of 25,622 individuals with a history of substance involvement, Hendricks et al. (2014) found that use of psychedelics predicted a reduced likelihood of noncompliance with legal requirements that included alcohol and other drug use. The authors concluded that psychedelic use may promote alcohol and other drug abstinence as well as prosocial behavior in a population with high rates of recidivism.

E. Cluster Headaches

Cluster headache is a devastating idiopathic pain syndrome. It is the most severe type of headache,

characterized by unilateral orbital or periorbital pain, accompanied by ipsilateral autonomic features in the nose, eyes, and face, with attacks lasting on average about 90 minutes. Cluster attacks can be acute or episodic, although the episodic form is most common (May, 2005). The most effective treatments have been 100% oxygen or subcutaneous sumatriptan (Becker, 2013). Recent evidence, however, has indicated that psychedelics may be more effective therapies for aborting acute attacks than conventional treatments. Online interviews of 53 cluster headache patients who had used either psilocybin or LSD to treat their condition found that 22 of 26 psilocybin users reported that psilocybin aborted attacks, and 25 of 48 psilocybin users and 7 of 8 LSD users reported cluster period termination. Extension of the remission period for attacks was reported by 18 of 19 psilocybin users and 4 of 5 LSD users (Sewell et al., 2006).

Surprisingly, a nonhallucinogenic LSD derivative, BOL-148 (2-bromo-LSD), was also found to be an effective treatment of cluster headaches (Karst et al., 2010). Three single oral doses of 30 μg/kg BOL-148 in five sufferers could either break a cluster headache cycle or considerably improve the frequency and intensity of attacks. The lack of psychedelic activity for BOL-148 clearly indicates that therapeutic efficacy in cluster headaches cannot be related to psychoactive effects, which presumably are manifested through the 5-HT_{2A} receptor, but the mechanism of action remains unknown. Early reports examined the pharmacology of BOL compared with LSD, although not much conclusive was found; however, it does appear that there must be some overlap between the pharmacology of BOL and LSD. For example, when 1 mg BOL was given three times a day for 5 days to 10 human volunteers, the effect of 1 μg/kg LSD was significantly attenuated (Isbell et al., 1959). If a single dose of 2–4 mg BOL was given along with LSD, there was no effect on the LSD response. When 3 mg/d BOL was given for 2 days prior to LSD, a trend was observed but not a significant block of the LSD effect. Early in vitro studies showed that the "antiserotonin" effect of BOL was at least comparable to that of LSD (Cerletti and Doepfner, 1958), so downregulation of the 5-HT_{2A} receptor by BOL might be expected to block the effects of LSD. At the 5-HT_{2C} receptor, however, BOL is a silent antagonist and did not cause receptor downregulation at the choroid plexus 5-HT_{2C} receptor, whereas the inverse agonist minanserin led to reduced 5-HT_{2C} receptor density. A detailed analysis of the receptor binding profile for BOL, along with its functional effects, might shed light on the basis for the therapeutic effects of BOL against cluster headaches.

F. Autism

During the very early years, when LSD was considered to be somewhat of a miracle drug, the possibility was investigated that psychedelics might be useful in ameliorating the symptoms of autistic spectrum disorders. Between 1959 and 1974, a number of studies were reported on the use of LSD to treat children with autism. Sigafoos et al. (2007) reviewed these reports. Unfortunately, the vast majority of those studies had serious methodological flaws. Typically, after the drug was administered, the children were simply observed and their reactions were recorded in a narrative format. Thus, the resulting data were for the most part qualitative and were presented in a form that was highly subjective, potentially biased by observer expectations, and of unknown reliability and validity. Furthermore, when enthusiastic investigators obtained neutral or negative findings, they often were cast in a more positive light than was warranted. Despite a good number of independent studies, it remains impossible to determine whether LSD had any therapeutic value for children with autism. When judged by today's standards of randomized controlled trials, or a properly controlled and systematically replicated single-case study, most of the early autism/LSD studies were so flawed as to be little better than anecdotal.

Although a rationale for treating autism with LSD might seem obscure on the surface, there is a potential scientific basis for such treatment. Murphy et al. (2006) used single-photon emission computed tomography (SPECT) imaging with a 5-HT_{2A}–selective antagonist [^{123}I]-radioligand (^{123}I-5-I-R91150) to compare cortical density of 5-HT_{2A} receptors in 10 healthy adult subjects and 8 adults with Asperger's syndrome. They found that the Asperger's patients had a significant reduction in cortical 5-HT_{2A} binding in the total, anterior, and posterior cingulate; bilaterally in the frontal and superior temporal lobes; and in the left parietal lobe. They reported that reduced receptor binding was significantly related to abnormal social communication. It therefore seems possible that the use of a 5-HT_{2A} agonist might improve functioning, at least acutely.

G. Cognitive Function

There are no clinical studies of the effect of psychedelics on memory or cognitive function, but there is a reasonable basis to believe they might have a beneficial effect on memory or cognitive deficits, at least acutely. It is known that 5-HT_{2A} receptors have a role in cognitive function of working memory that involves actions at both excitatory and inhibitory elements within local circuitry (Williams et al., 2002). A frequent polymorphism of the gene that encodes for the 5-HT_{2A} receptor is known that attenuates its function and this polymorphism has been shown to have an effect on memory. More specifically, de Quervain et al. (2003) examined a polymorphism that predicts an amino acid substitution of His to Tyr at residue 452 (H452Y) of the 5-HT_{2A} receptor. Heterozygous (His/Tyr) carriers show a blunted response when the receptor is pharmacologically stimulated.

de Quervain et al. investigated the effects of the H452Y polymorphism on human memory. Memory testing and genotyping was done in a total of 349 subjects composed of two independent populations of either university students or age-matched employees/trainees who were not studying at the university and did not have a university degree. After genotyping, 279 participants were found to be His/His and 70 were His/Tyr. All subjects underwent cognitive assessment over 2 days. The His/Tyr subjects showed a 21% poorer memory performance compared with His/His subjects.

Wagner et al. (2008) followed up on the finding by de Quervain et al. and reported that the H452Y variant of the gene encoding the 5-HT_{2A} receptor is associated with the consolidation of episodic memory in humans. Carriers of the H452Y allele showed poorer verbal delayed recall and recognition, whereas immediate recall and other measure of attentional and executive function were not affected by the H452Y genotype. They essentially replicated the findings of de Quervain et al. (2003), noting that their effect size for recall after 30 minutes was similar to the effect size observed by de Quervain et al. for 5-minute recall and for recall 24 hours later. Wagner et al. (2008) suggest that these results may foster further search for memory-enhancing agents to treat mild cognitive impairment, and one can infer that this recommendation might well apply to psychedelic agents, but perhaps at subintoxicating doses.

Substantial evidence has also accumulated from postmortem human brain studies, clinical pharmacology, animal studies, and PET brain imaging in humans to point to dysfunction of the serotonergic system in Alzheimer's disease (AD) (see review by Meltzer et al., 1998). In particular, a consistent postmortem finding in AD is a marked reduction in the density of 5-HT_{2A} receptors (Reynolds et al., 1984; Bowen et al., 1989; Cheng et al., 1991; Lai et al., 2005).

Using PET with the 5-HT_{2A}–selective antagonist [^{18}F]altanserin, Meltzer et al. (1999) measured 5-HT_{2A} receptor density in 11 elderly AD patients compared with 10 age-matched healthy controls. The patients suffered from depression or AD, with three of the AD patients also having concurrent depression. Meltzer et al. (1999) reported finding no reduction in [^{18}F] altanserin binding in depressed patients, and no effect of depression on binding in the AD group. By contrast, the AD patients had significantly lower 5-HT_{2A} binding potential in several brain regions, compared with controls. These regions included the ACC, PFC, lateral temporal cortex, amygdala-hippocampal complex, and sensorimotor cortex.

Nair and Gudelsky (2004), using in vivo microdialysis experiments in rats, reported that DOI (given intraperitoneally) significantly increased extracellular ACh in both the PFC and dorsal hippocampus. This increase was attenuated if rats were pretreated with a 5-HT_2–nonselective antagonist. Although the 5-HT_{2C}–selective agonist MK-212 [6-chloro-2-(1-piperazinyl)pyrazine] significantly enhanced release of ACh in both brain areas, the 5-$HT_{2A/2C}$ agonist mescaline produced a 2-fold ACh increase only in the PFC. Intracortical, but not intrahippocampal, infusion of DOI significantly enhanced the release of ACh in the cortex.

More recently, Hasselbalch et al. (2008) used [^{18}F] altanserin PET to assess cerebral 5-HT_{2A} receptor density in 16 patients with mild cognitive impairment of the amnestic type, compared with 17 age and sex-matched controls. Patients with mild cognitive impairment are considered at increased risk of developing AD, and their inclusion criteria were deliberately aimed at including patients with symptoms of very early AD. Indeed, at 1-year follow-up, six of their patients had progressed clinically and fulfilled AD criteria. This study revealed a significant 20%–30% global reduction of 5-HT_{2A} binding in most neocortical areas. These widespread reductions in 5-HT_{2A} density may point to serotonergic dysfunction in prodromal AD.

H. Creativity

Since the introduction of LSD into popular culture, there has been a great deal of speculation as to whether psychedelics might acutely improve creativity. Certainly, there was a veritable explosion of new music, art, and fashion during the 1960s, which were characterized as "psychedelic." Probably few would argue that the use of psychedelics was not a major factor driving that revolution; even today, if the adjective "psychedelic" is applied to something, for most people it immediately brings to mind ideas of bright and colorful objects, swirling patterns, and so forth. Sessa (2008) wrote an essay about much of the thinking and reported on creativity from that early period of time.

Unfortunately, from a science perspective, there has been no well done, controlled study to determine whether psychedelics actually do improve creativity. The closest the published literature comes is an early study by Harman et al. (1966). Harman et al. recruited 27 male participants who were in various professional occupations, including engineers, physicists, mathematicians, architects, a furniture designer, and a commercial artist. Subjects were instructed to select one or more problems that required a creative solution. A number of subjects had worked for weeks or months on their chosen projects without being able to find a satisfactory solution. Various psychologic tests and tests of creativity were administered before and after the drug sessions, which involved administration of 200 mg mescaline. Participants were prepared by presession interviews and instructions regarding how to approach the drug sessions. The results reported are rather remarkable. Of the 44 problems brought by the subjects, 20 had new avenues opened for further investigation, 1 was a developmental model that received authorization

to test the solution, a working model had been completed for 2, 6 had a solution that had been accepted for construction or production, and 10 had partial solutions that were being developed further or being applied in practice. No solution was obtained only for four of the problems. Although lacking detailed specifics as well as a follow-up, this early report does suggest that psychedelics might acutely improve creativity.

In 1991, tech journalist Denise Caruso attended SIGGRAPH, the largest gathering of computer graphics professionals in the world. Apparently hearing that the use of psychedelics had played a foundational role in the development of computer graphics and software, she took a survey of 180 professionals in the field who admitted they had taken psychedelics and that they were important to their work. She published her story in the August 4, 1991 edition of the *San Francisco Examiner*. The late Apple cofounder Steve Jobs was open about the fact he had experimented with LSD while in college and said that taking LSD was a profound experience, and "one of the two or three most important things I have done in my life."

Another incidence of psychedelic drug–induced creativity from the scientific community comes from Nobel Prize–winning chemist Dr. Kary Mullis, the inventor of PCR, who is quoted as saying "Would I have invented PCR if I hadn't taken LSD? I seriously doubt it… I could sit on a DNA molecule and watch the polymers go by. I learnt that partly on psychedelic drugs."

Nobel Prize winner Francis Crick, who discovered the double-helix structure of the DNA molecule, is known to have used LSD on occasion. Although there have been allegations that he conceived the idea of the double-helix while on LSD, the story is false. His biographer has pointed out that although Crick was given LSD on several occasions from 1967 onward, his major breakthrough discoveries occurred prior to that date.

I. 3,4-Methylenedioxymethamphetamine in Post-Traumatic Stress Disorder

Although MDMA is not, strictly speaking, a classic psychedelic, it is a unique psychoactive agent that, like the psychedelics, was placed into Schedule 1 of the Controlled Substances Act. Thus, although numerous investigators have reported on its effects in animals, no well designed clinical studies of its medical potential had been carried out for many years. However, Mithoefer et al. (2011) recently studied the potential of MDMA, coupled with psychotherapy, to have therapeutic benefit in patients with PTSD. Twenty subjects with chronic PTSD refractory to both psychotherapy and psychopharmacology were enrolled in a randomized placebo-controlled pilot study. Clinical response was defined as a >30% reduction from baseline in total severity score on the Clinician-Administered PTSD Scale.

In stage 1 (the initial double-blind group), the clinical response was 83.3% (10 of 12) in the MDMA group versus 25% (2 of 8) in the placebo group. Likewise, 10 participants in the MDMA group no longer met *DSM-IV* criteria for PTSD compared with only 2 in the placebo group. In stage 2 (a second open-label administration that was offered to the initial placebo group), the clinical response rate was 100% in the seven subjects, six of whom had failed to respond to placebo and one of whom had relapsed after an initial placebo response. A decrease in Clinician-Administered PTSD Scale scores from baseline was significantly greater for the MDMA group than for the placebo group. The rate of clinical response in the active treatment groups was 85% compared with 25% in the placebo group.

J. Use as Ocular Hypotensives for Glaucoma

It has been known for some time that several serotonin receptor types are expressed in ocular tissues of the human eye (Martin et al., 1992), and May et al. (2003) demonstrated that 5-HT_2 receptors were involved in local control of intraocular pressure (IOP) in cynomolgus monkeys. The nonselective 5-$HT_{2A/2C}$ agonist *R*-DOI also was shown to have a dose-dependent reducing effect on IOP in the cynomolgus monkey (May et al., 2003). Sharif and Senchyna (2006) used RT-PCR to demonstrate that human ocular tissues expressed mRNA for the 5-HT_{2A} and 5-HT_{2B} receptors, with greatest abundance in the retina, ciliary body, ciliary epithelium, choroid, conjunctiva, and iris. Sharif et al. (2006) also reported that human trabecular meshwork cells expressed robust mRNA signals for 5-HT_{2A} and 5-HT_{2B} receptors. A number of 5-HT_2 agonists were demonstrated to stimulate PI turnover and Ca^{2+} mobilization in human trabecular meshwork cells. These effects were blocked completely by the 5-HT_{2A}–selective antagonist M100907 but were only weakly antagonized by selective 5-HT_{2B} or 5-HT_{2C} antagonists. Both *R*-DOI and α-methylserotonin were shown to lower IOP in ocular hypertensive cynomolgus monkeys by 34% and 31%, respectively.

Gabelt et al. (2005) also examined the effects of *R*-DOI on IOP and aqueous humor dynamics in normotensive cynomolgus monkeys. Monkeys were treated topically once daily with four 5-ml drops of 0.5% *R*-DOI in one eye and vehicle in the other eye. The 6-hour IOP was determined before drug treatment and on the third day of treatment. Aqueous humor formation was measured between 3 and 8 hours after the third dose, and aqueous humor flow was measured 3.5 hours after the fourth or fifth dose. After the third dose of *R*-DOI, IOP was significantly decreased by 1.4 to 4.7 mm Hg over 6 hours. Aqueous humor flow was increased by 13% in treated compared with control eyes. These effects were attributed to an increase in uveoscleral outflow, an important component of aqueous humor dynamics that contributes to the maintenance of IOP.

May et al. (2006) discovered indazole-based 5-HT_{2A} agonists with poor CNS activity and solution stability

superior to conventional tryptamine-based 5-HT_2 agonists such as serotonin and AMS. They identified one compound, 1-((*S*)-2-aminopropyl)-1*H*-indazol-6-ol), with an EC_{50} of 43 nM and E_{max} of 89% that lowered intraocular pressure by 33% in conscious ocular hypertensive monkeys.

Feng et al. (2007) also identified novel 5-HT_{2A} agonists with ocular hypotensive activity. They replaced the 4-bromine atom of DOB analogs with polar groups to lower their lipophilicity and thus to reduce potential CNS effects. Three compounds were selected for further testing based on IC_{50} values of 0.28, 0.38, and 0.7 nM for [^{125}I]DOI displacement from the rat cerebral cortex homogenate. All three compounds were potent partial agonists in an intracellular calcium mobilization assay in rat vascular smooth muscle cells. After topical administration of 300-μg doses in cynomolgus monkeys, they observed maximum IOP reductions of 36.8%, 27%, and 24%, respectively.

These studies demonstrate that selective 5-HT_{2A} agonists may have application when topically applied to reduce IOP in glaucoma. The caveat here, which drove much of the more recent drug development efforts, was to identify potent agonists that would not penetrate the CNS and thus would lack psychedelic activity. Clearly, highly 5-HT_{2A} receptor–specific agonists would be most desirable, but thus far no such ligand has been identified.

K. Tissue Regeneration

Psychedelics may also have potential to stimulate tissue regeneration. Arvanian et al. (2006) carried out a study in which rats received a staggered double hemisection (DH) at postnatal day two (P2) of the left hemicord at T11 and the right hemicord at T12. A second group had a complete transection at T11 (CT), with a third group serving as a sham-operated control. Drug treatments consisted of neurotrophin-3 (NT-3) and LSD given either alone or in combination. Drugs were administered intrathecally above the lesion during surgery and again subcutaneously at P4, P6, P8, and P10. The frequency of rearing in an open field test and hindlimb kicks during swimming were then used to assess motor function, and both DH and CT rats showed severe impairment. DH rats treated with the combination of NT-3 and LSD showed significantly more kicks during swimming than untreated DH or CT rats or treated CT rats. Improvement began as early as P9 and lasted for the duration of the testing. Rearing frequency also improved with treatment, beginning only in the third postnatal week, when it normally develops, and reached sham values by P40. The authors suggest that the combination treatment of NT-3 with LSD may be a promising strategy for facilitating recovery from moderate spinal cord injury (SCI). The possibility that LSD might promote neuronal regrowth in SCIs is very exciting.

Lee et al. (2007) presented evidence that 5-HT_{2A} receptors are upregulated after contusional SCI in rats. They studied the monosynaptic connection between primary muscle afferents and α motoneurons using the Hoffman reflex (H-reflex). The H-reflex is elicited by electrical stimulation of a peripheral nerve that innervates the muscle from which the reflex is recorded. The stimulus generates a short-latency M-wave by direct stimulation of the motor axons innervating the muscle, as well as a long latency H-wave (a measure of the α motoneurons activated by Ia afferents). The authors had previously observed (Lee et al., 2005) increased H-reflex amplitude 4 weeks after contusional SCIs of different severity. In addition, they also noted that the increased H-reflex amplitude was positively correlated with serotonin immunoreactivity around the motoneurons involved in the reflex. DOI had previously been shown by Miller et al. (1996) to restore excitability of extensor motoneurons that is abolished after acute spinalization in the cat. In addition, no serotonergic innervation of the lumbar-sacral spinal cord remains after a CT (Lee et al., 2005), because completely transected animals showed no 5-HT immunoreactivity at the level of the rostral dorsolateral nucleus.

Thus, Lee et al. (2007) measured H-reflex amplitude after a standardized incomplete contusive SCI in rats and measured the H-wave/M-wave ratio. To study the role of 5-HT_2 receptors in the response, they carried out experiments in which they administered either intrathecal DOI or a 5-HT_{2A} antagonist. A mild contusive SCI was created at T8, and slides containing the rostral dorsolateral nucleus were used for 5-HT immunohistochemistry. 5-HT_{2A} receptor immunohistochemistry was performed on spinal cord sections adjacent to those used for the 5-HT immunohistochemistry. Four weeks after a mild SCI, the H-wave/M-wave ratio was significantly increased by 93 nM, but not 31 nM of DOI. Mild SCI animals had significantly more 5-HT_{2A} receptor immunoreactivity 4 weeks after SCI than did uninjured controls. Lee et al. (2007) concluded that upregulation of the 5-HT_{2A} receptor "is likely to be involved in the enhanced H-reflex that develops after clinically relevant incomplete conclusive SCI." They note that their results are consistent with other studies providing evidence for increased expression of 5-HT_2 receptors and increased sensitivity to serotonergic drugs by spinal motoneurons distal to an SCI. They further speculate that 5-HT_2 receptor modulation of enhanced recruitment of motoneurons in response to afferent input may contribute to locomotor recovery after an incomplete SCI. If these results can be translated to humans, it suggests that administration of a 5-HT_{2A} agonist at the site of a contusional SCI, possibly by intrathecal administration, might promote recovery from the injury.

The most significant limiting factor for survival after liver surgery and transplantation of a partial graft is the

ability of the remnant liver to regenerate (Furrer et al., 2011, and references therein). Serotonin, derived from platelets, is thought to be involved in liver regeneration after major tissue loss. Platelets are major carriers of serotonin in the blood, and 5-$HT_{2A/2C}$ serotonin agonist DOI completely restored liver proliferation in thrombocytopenic mice (Lesurtel et al., 2006). Partial (70%) hepatectomy was performed in mice, in which platelet function was inhibited pharmacologically or platelets were depleted. When mice were treated with a 5-HT_{2A} antagonist, hepatocyte proliferation was reduced compared with vehicle-treated controls. A 5-HT_{2B} receptor antagonist also reduced hepatocytes. The authors suggest that 5-HT_{2A} and 5-HT_{2B} receptor subtypes mediate serotonin-dependent liver regeneration.

Furrer et al. (2011) identified markers of hepatocyte proliferation 48 hours after mouse hepatectomy, as well as the mitotic index at 4 days. Both markers were dramatically decreased in cell proliferation in 2-year-old mice, and the mitotic index also was significantly decreased in old compared with young (7- to 8-week-old) mice. Upregulation of the 5-HT_{2A} receptor was seen after hepatectomy in young mice, whereas this upregulation was absent in old mice, possibly reflecting regenerative impairment. Pretreating old mice with DOI prior to hepatectomy increased the weight of the liver remnant compared with untreated animals, significantly improved hepatocyte proliferation, and converted animal survival from 48% to 86%. Treating control animals with DOI alone did not affect 5-HT_{2A} receptor expression. Their results demonstrate that 5-HT_{2A} receptor activation by DOI restores deficient regeneration of old livers. Furrer et al. (2011) also reported that old animals had characteristic changes of pseudocapillarization, with loss of fenestration, but that young animals had a thin sinusoidal lining containing many fenestrae. DOI had no significant effect in young animals but led to increased numbers of fenestrae in old animals.

Furrer et al. (2011) also observed significantly lower portal blood flow at baseline in old compared with young mice, and DOI improved portal flow and increased microperfusion in old livers. Electron microscopy studies demonstrated deficient platelet adhesion in old livers after hepatectomy, which was improved by DOI administration. Mechanistic studies revealed that DOI increased interleukin (IL)-6 at 48 hours after hepatectomy, but the strongest effect of DOI was to increase serum levels of vascular endothelial growth factor (VEGF). Thus, application of anti-VEGF antibodies blunted the proliferative effect of DOI, and administering exogenous VEGF enhanced liver regeneration to levels seen in DOI-treated mice. Furrer et al. (2011) conclude that liver regeneration is impaired in old mice due to a deficiency in the fenestration of hepatic sinusoids. Administering DOI ameliorates this defect through a pathway that involves VEGF, which regulates opening of endothelial fenestrae, improving microcirculation and enabling normal regenerative response after liver injury. In essence, 5-HT_{2A} agonists can restore the capacity of old livers to regenerate.

L. Effects on Immune Response

Although serotonin itself has long been established to be involved in inflammation and inflammatory processes, the role of psychedelics and their primary target, the serotonin 5-HT_{2A} receptor, in these processes was unknown until recently. In 2008, Charles Nichols discovered that psychedelics produced powerful anti-inflammatory effects against tumor necrosis factor (TNF)-α–mediated inflammatory processes in several cell types, including primary aortic smooth muscle cells, through activation of 5-HT_{2A} receptors (Yu et al., 2008). Whereas several psychedelic drugs, including LSD, demonstrated potent anti-inflammatory effects, the drug *R*-DOI was extraordinarily potent at blocking inflammation. TNF-α–induced proinflammatory markers that were inhibited by *R*-DOI included the expression of genes encoding for cell adhesion molecules (intracellular adhesion molecule-1 and vascular cell adhesion molecule-1), the inflammatory cytokine IL-6, activity of the enzyme nitric oxide synthase, and activation and nuclear translocation of nuclear factor κB. IC_{50} concentrations for *R*-DOI inhibition were very low (in the range of only 10–20 pM), and significant inhibition could be observed even if *R*-DOI was added several hours after TNF-α treatment.

Following up on these promising results, the same group tested the ability of *R*-DOI to block the inflammatory effects of TNF-α in the whole animal (Nau et al., 2013). Saline, *R*-DOI (0.01, 0.1, or 0.3 μg/kg), and TNF-α (10 mg/kg) were administered intraperitoneally to C57BL/6J mice, with *R*-DOI given 30 minutes prior to TNF-α. The highest dose of *R*-DOI administered in that study (0.3 mg/kg) is the lowest dose that can be behaviorally detected by mice (Smith et al., 2003). Five hours after TNF-α injection, animals were euthanized and various tissues were removed for analysis of the expression of genes for cell adhesion markers (intracellular adhesion molecule-1 and vascular cell adhesion molecule-1), cytokines (IL-6 and IL-1b), and chemokines (monocyte chemotactic protein-1 and chemokine (C-X3-C motif) ligand 1 *Cx3cl1*). *R*-DOI was found to block TNF-α–induced increases of these inflammatory markers in the vasculature (aortic arch). In the intestine, the lowest amount of drug (0.01 mg/kg) produced a maximal anti-inflammatory effect and nearly completely blocked the expression of all proinflammatory markers examined. *R*-DOI was also able to block dose-dependently and completely TNF-α–induced levels of circulating IL-6 in the blood. Importantly, antagonist studies using the highly selective 5-HT_{2A} receptor antagonist M100907 demonstrated that the anti-inflammatory effects of *R*-DOI in the whole animal

were mediated by activation of the 5-HT_{2A} receptor, as they were in the earlier cell culture studies.

To test the potential efficacy of *R*-DOI to treat a human inflammatory disease, the Nichols group (Nau et al., 2015) assessed the effects of *R*-DOI in a mouse model of allergic asthma. In this model, BALB/c mice were sensitized with chicken egg white protein [ovalbumin (OVA)] to generate an IgE allergic response. To induce asthma-like symptoms after sensitization, the mice were then exposed to nebulized OVA (nose-only). This treatment results in the mice developing pulmonary inflammation, airways hyperresponsiveness, eosinophilia, and mucus hyperproduction, each representative of hallmark symptoms of human allergic asthma. Remarkably, *R*-DOI at doses as low as 0.01 mg/kg administered nose-only prior to OVA administration prevented the symptoms of allergic asthma from developing, including inflammation and eosinophilia. In mechanistic studies, cells of the lung were further analyzed by both gene expression and flow cytometry. Some, but not all, proinflammatory cytokines were found to be repressed in the *R*-DOI pretreated animals. For example, expression of IL-4, a proinflammatory cytokine implicated in asthma was unaffected by *R*-DOI, whereas expression levels of others including IL-5, IL-13, and granulocyte-macrophage colony-stimulating factor were all significantly reduced. These results suggested that unlike conventional anti-inflammatory treatments like steroids, which simply repress the immune system, *R*-DOI selectively targets only certain key components relevant to the pathology.

The nature of proinflammatory markers inhibited by *R*-DOI suggested effects on T-helper Th2 cells and/or innate immune cells, each of which is known to express 5-HT_{2A} receptors. Flow cytometric analysis indicated that not only was *R*-DOI leading to a reduction in overall Th2 cells, but that these cells were also producing fewer proinflammatory cytokines. In addition to its effect on Th2 cells, DOI also has been demonstrated to have a suppressive effect on spleen and blood peripheral $CD8^+$ cells (Davydova et al., 2010) and the recruitment of eosinophils (Kang et al., 2013).

It remains to be determined whether the effects of *R*-DOI on these immune cells, and on its ability to prevent the development of asthma, involves blockade of TNF-α signaling. Together, each of these studies indicates that agonism of 5-HT_{2A} receptors may be a novel small molecule steroid-sparing therapeutic strategy to treat inflammatory diseases that include asthma, atherosclerosis, and inflammatory bowel disease. Importantly, the doses of *R*-DOI necessary to produce these therapeutic effects are orders of magnitude lower than those necessary to influence behaviors.

Genetic variations in the 5-HT_{2A} receptor may also be associated with rheumatoid arthritis (RA). Previous studies had shown an association between the rs6313 (T102C polymorphism) of the 5-HT_{2A} receptor gene and certain diseases with musculoskeletal manifestations (Kling et al., 2008, and references therein). To examine this issue further, Kling et al. (2008) analyzed a Northern Swedish study population of 292 patients with RA. For further analysis of additional single nucleotide polymorphisms (SNPs), they used the Epidemiologic Investigation of Rheumatoid Arthritis study population of 2168 patients with RA. Their control group was composed of 524 subjects drawn from three Swedish population-based medical biobanks. In the study population from Northern Sweden, the frequency distribution of rs6313 (T102C) genotypes differed significantly between the RA patients and the controls (P = 0.006). They tested the same variation in the larger Epidemiologic Investigation of Rheumatoid Arthritis study population and also performed genotyping for seven more SNPs along the gene sequence. The rs6313 (T102C) single marker association with RA was replicated in this larger study population. Haplotype analysis with the inclusion of four of the SNPs revealed strong evidence for association of variations in the 5-HT_{2A} receptor gene with RA, with both protective and susceptible haplotypes that associated with a significantly decreased or increased risk of developing RA. Although this report made no reference to the effect of a 5-HT_{2A} agonist or antagonist in RA, taken together with the studies discussed just above it seems possible that an agonist ligand for the 5-HT_{2A} receptor might have some therapeutic efficacy in RA.

In mice, administration of 1 mg/kg DOI led to immune response suppression and reduction of spleen and peripheral blood CD8(+) T cell counts, with the cytotoxic/suppressor function (Davydova et al., 2010). The effect was blocked by administration of the 5-HT_{2A}–selective antagonist ketanserin. These data also demonstrate the mediation of the serotonin 5-HT_{2A} receptor in the immune response.

Szabo (2015) recently proposed that the psychedelic tryptamines *N,N*-DMT and 5-MeO-DMT may have immunomodulatory effects mediated through the σ-1 receptor. Pretreatment of human primary monocyte-derived dendritic cells with 100 μM DMT or 5-MeO-DMT was able significantly to attenuate the production of proinflammatory cytokines after treatment of the cells with bacterial lipopolysaccharide or high molecular weight polyinosinic/polycytidylic acid. Szabo (2015) concluded that dimethyltryptamines may serve as important regulators of both innate and adaptive immunity.

M. Effects on Cell Differentiation and Growth

Serotonin is an important modulator of cell differentiation and growth. The receptors most closely linked to these processes are the serotonin 5-HT_2 receptors. Furthermore, activation of 5-HT_2 receptors in multiple cell types influences actin cytoskeletal structure and dynamics, which influences processes including cell

motility and axon growth. The most infamous example of 5-HT_2 receptor activation having an impact on cell growth is the potentially fatal hyperplasia associated with the cardiac valvulopathy induced by fenfluramine, which was a component of the diet drug fenfluramine/phentermine. Here, fenfluramine and its active metabolite norfenfluramine activated 5-HT_{2B} receptors in heart valve and cardiac tissues, causing them to proliferate and develop heart valve disease and cardiac fibrosis. Although all known classic psychedelics produce their behavioral effects through activation of 5-HT_{2A} receptors, they also have significant 5-HT_{2B} receptor agonist activity at the doses necessary for behavioral effects. Activation of 5-HT_{2A} receptors directly in certain tissues including vascular, placental, and cancers can also have a proliferative effect (Sonier et al., 2005; Göoz et al., 2006; Liu and Fanburg, 2008; Chen et al., 2014), suggesting that the classic psychedelics may also have an effect on these processes. The molecular mechanisms underlying the proliferative effects largely appear to involve activation of ERK/MAPK signaling downstream from the receptor.

The 5-HT_{2A} receptor has been identified as a mitogenic receptor in human placental choriocarcinoma cell lines (and in a rat glomerular mesangial cell line) as coupled to several mitogenic signal transduction pathways and potentially oncogenic for its transforming properties in transfected NIH3T3 cells (see references in Sonier et al., 2006). Thus, Sonier et al. (2006) examined 5-HT_{2A} receptor expression in human breast cancer cell line MCF-7. They tested the effect of 5-HT and DOI on MCF-7 cell proliferation using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell proliferation assays. Their results showed that 5-HT_{2A} receptor mRNA and protein are expressed in MCF-7 cells, and that 5-HT has a positive proliferative effect on these cells, at least partly through stimulation of 5-HT_{2A} receptors. A dose-response effect was determined for both 5-HT and DOI, revealing that 5-HT stimulated MCF-7 proliferation up to 52.2% at a concentration of 10 μM. DOI had a maximum proliferative effect of 34.4% at a concentration of 10 μM.

Hirai et al. (2010) evaluated the involvement of 5-HT receptor subtypes in mouse MC3T3-E1 osteoblasts. Both DOI and 5-HT increased proliferative activity of MC3T3-E1 cells in a concentration-dependent manner, an effect that was blocked by ketanserin. DOI-induced cell proliferation and phosphorylation of ERK1 and ERK2 was blocked by PD98059 [2-(2-amino-3-methoxyphenyl)-4*H*-1-benzopyran-4-one] and U0126 [1,4-diamino-2,3-dicyano-1,4-*bis*[2-aminophenylthio]butadiene], selective inhibitors of mitogen-activated protein kinase kinase (MEK). The results suggest that the 5-HT_{2A} receptor may be functionally expressed to regulate mechanisms underlying osteoblast cell proliferation, at least in part through activation of ERK/MAPK pathways in these cells.

Oufkir et al. (2010) evaluated the effect of DOI on cell viability, cell cycle progression, and activation of the MEK-ERK1/2 and the Janus kinase 2–signal transducer and activator of transcription 3 signaling pathways in trophoblast-like BeWo and JEG-3 cancer cell lines. JEG-3 and BeWo cells are human placental choriocarcinoma cell lines that have been widely used as in vitro models for the placenta. Oufkir et al. (2010) found that activation of the 5-HT_{2A} receptor by DOI promoted viability of BeWo and JEG-3 cells. Although DOI is a nonselective 5-$HT_{2A/2C}$ agonist, these effects were blocked by pretreating the cells with ketanserin, a 5-HT_{2A}–selective antagonist. DOI treatment significantly increased both MEK1/2 and ERK1/2 phosphorylation in a time-dependent fashion. DOI also promoted interaction between Janus kinase 2 and the 5-HT_{2A} receptor. The work of Oufkir et al. (2010) suggests that 5-HT_{2A} receptors may be involved in regulation of placentation and may play a role in the pathophysiology of pregnancy disorders associated with alteration in placental development.

X. Models of Psychosis

Halberstadt and Geyer (2013b) recently reviewed the topic of serotonergic hallucinogens as translational models relevant to schizophrenia. In their review, they note the many early groups that studied the effects of LSD, mescaline, and psilocybin, who concluded that these drugs produced mental states that resembled the earliest phases of schizophrenia. Although the similarities between the effects of serotonergic psychedelics and schizophrenia are not as great as were initially believed, a number of animal models have nevertheless been developed to facilitate understanding of the role that 5-HT plays in schizophrenia, to help characterize interactions between 5-HT and other neurotransmitter systems, and to identify novel pharmacotherapies. The four widely used animal behavioral models are startle habituation, PPI, HTRs in rodents, and deficits in temporal processing (interval timing). Each of these models is extensively discussed in this review, and it is pointed out that the 5-HT_{2A} receptor plays a fundamental role in each of them and is also known to be an important target for atypical antipsychotic drugs.

To provide a bit more specific detail here, the actions of psychedelics such as DOI, psilocybin, and LSD have been considered to model, to a certain extent, some of the positive symptoms of schizophrenia (see reviews by Vollenweider et al., 1998; Geyer et al., 2001; Vollenweider, 2001; Vollenweider and Geyer, 2001). Schizophrenia patients with predominantly negative symptoms exhibit reduced prefrontal activation compared with patients without negative symptoms. For example, using [^{18}F]fludeoxyglucose (FDG) PET, it was found that schizophrenia subjects with predominantly negative symptoms have a lower glucose metabolic rate

than subjects with predominantly positive symptoms, particularly in frontal, temporal, and cerebellar circuitry (Potkin et al., 2002). By contrast, schizophrenia patients with positive symptomatology have increased frontal metabolic rate of glucose. Soyka et al. (2005) used [^{18}F]FDG PET in 10 unmedicated schizophrenia patients with vivid positive symptoms. Schizophrenia patients had higher, but nonsignificant, regional metabolic rates of glucose in almost all quantified regions compared with controls, but the right/left frontal-occipital metabolic ratio was significantly higher for the schizophrenia patients, indicating a hypermetabolic pattern.

PPI of the ASR has been used as a measure of sensorimotor gating and is considered an example of mechanisms that limit sensory information overflow, facilitate selective attention, and enable efficient processing of relevant information (Vollenweider et al., 2007). Reductions in PPI have been consistently shown in schizophrenia; in rats, DOI disrupts PPI, an effect that could be blocked by M100907 (see references in Vollenweider et al., 2007). In this study, 16 subjects received placebo or 115, 215, or 315 μg/kg psilocybin at 4-week intervals in a randomized and counterbalanced order. PPI at interstimulus intervals (ISIs) of 30, 60, 120, 240, and 2000 milliseconds was measured 90 and 165 minutes after psilocybin administration. Acoustic startle stimuli were presented through headphones, and the eyeblink component of the ASR was measured with an electromyography startle system. Subjective effects of drug and placebo were assessed using the 5D-ASC, and effects of psilocybin on sustained attention were assessed using the FAIR test. Psilocybin was found to reduce PPI dose-dependently at short (30-millisecond) ISIs, had no effect at medium (60-millisecond) ISIs, and increased PPI at long (120- to 2000-millisecond) ISIs, without affecting startle reactivity or habituation. Psilocybin impaired attentional performance, reducing the FAIR attentional performance capacity score P as well as score Q indexing the number of attentively made decisions relative to the total decisions, and the attentional continuity performance score C. The reduction in the P and C scores was significant after the low, medium, or high dose, whereas the reduction in the Q score was significant only after the peak effect of the high dose of psilocybin. Psilocybin-induced impairments in sustained attention performance were positively correlated with reduced PPI at the 30-millisecond ISI, but not with the concomitant increases in PPI observed at long ISIs. Psilocybin also produced a decrease in PPI at short ISIs that is correlated with impaired attention and consistent with deficient PPI in schizophrenia.

In another investigation of effects of psychedelics on PPI, 15 healthy volunteers were enrolled in a double-blind crossover study with two doses of DMT and (*S*)-ketamine (Heekeren et al., 2007). A low dose of DMT fumarate (0.15 or 0.2 mg/kg) was given as an intravenous bolus over 5 minutes, followed 1 minute later by a continuous infusion of 0.01125 or 0.015 mg/kg per minute over 84 minutes. A high dose of DMT (0.2 or 0.3 mg/kg bolus) was followed by continuous infusion of 0.015 or 0.02 mg/kg per minute. For ketamine, a low dose was a bolus injection over 5 minutes of 0.1 or 0.15 mg/kg, followed by continuous infusion of 0.0066 or 0.01 mg/kg per minute over 54 minutes, followed by a continuous infusion of 75% of the previous dose over 30 minutes. The high dose of ketamine was a bolus injection of 0.15 or 0.2 mg/kg, then continuous infusion with 0.01 or 0.015 mg/kg per minute over 54 minutes, followed by continuous infusion at a rate of 75% of the previous dose over 30 minutes. The psychologic effects of both drugs were fully developed within 15 minutes and were kept relatively constant over the following 75 minutes (see additional details in Gouzoulis-Mayfrank et al., 2005). Both drugs dose-dependently induced symptoms resembling the positive symptoms of schizophrenia, but positive formal thought disorder and inappropriate affect were stronger after DMT. Phenomena resembling negative symptoms of schizophrenia were stronger after *S*-ketamine. Acoustic startle was then measured and, surprisingly, there was no significant difference in PPI between baseline and DMT. Both *S*-ketamine doses marginally or significantly increased PPI compared with baseline at the 100-millisecond, but not at the 240-millisecond, lead interval. These results do not support the hypothesis of sensorimotor gating deficits in humans after administration of hallucinogens, although the authors note methodological limitations, including the small sample size.

XI. Use as Tools to Study Brain Function and Connectivity

The advent of powerful brain imaging technologies such as fMRI, PET, and MEG has allowed rapid advances in our understanding of the areas and functions of the brain responsible for a variety of behavioral and cognitive tasks. Based on the convergence of ideas about the substrates of consciousness and the recognition that psychedelics target the most important of these, it should be no surprise that psychedelics, in particular agonist ligands for the 5-HT$_{2A}$ receptor, can be powerful tools to study how the brain works.

The field of cognitive neuroscience is addressing the challenge of attempting to understand consciousness and how it arises within the brain. Surely the psychedelics, one of the most potent drug classes known that alter consciousness, should play an increasingly important role in that investigation. We know now that there is no "seat" of consciousness, and that consciousness is not a property of a single brain location but more likely arises as the result of dynamic interactions among widely distributed groups of neurons that integrate a

very large number of sensory inputs and motor responses occurring in parallel. Edelman (1989) emphasized that consciousness concerns the rapid integration of signals from a great variety of modalities and submodalities to create a unified, coherent scene or idea. When one considers all of the key brain areas noted in this review that either express or are directly affected by 5-HT_{2A} agonist interactions, it should be no surprise that the psychopharmacology of psychedelics is so complex.

Even with the development of modern brain scanning technologies, however, the overall action of psychedelics in the brain is far from being understood. Psychedelics act as agonists or partial agonists at serotonin 5-HT_{2A}, 5-HT_{2C}, and 5-HT_{1A} receptors, with downstream effects on glutamate, GABA, and dopamine, among others, in a multitude of loci in the brain (Vollenweider and Geyer, 2001). Although it is the scientific consensus that activation of brain 5-HT_{2A} receptors is the central event in the action of psychedelics (Nichols, 2004; Halberstadt, 2015), that statement of what outwardly might seem to be a simple pharmacology fails to completely capture the overall consequences of that action within the dynamic interacting human brain.

Brain imaging studies have begun to offer some preliminary answers, but a tremendous amount of science remains to carried out before (or if) we can begin to understand the complex psychopharmacology that occurs in the intact human brain after administration of a psychedelic such as LSD. All that being said, however, a few studies have begun to characterize neurobiological effects of psychedelics.

In an early study, Hermle et al. (1992) employed ^{99m}Tc-hexamethylpropyleneaminoxime SPECT to measure regional cerebral blood flow (CBF) in 12 healthy male subjects after administration of a relatively large 500-mg dose of mescaline sulfate. The functional brain imaging showed an increase in regional CBF in both anterior regions and an even more pronounced increase in right anterior cortical regions, indicating a pattern of hyperfrontality. The authors note, however, that it was not known how CBF correlated with neuronal activity. Acute neurometabolic effects of psilocybin in humans also were initially measured on the regional cerebral metabolic rate of glucose (CMRglu) in humans using [^{18}F]FDG PET in the laboratories of Vollenweider et al. (1997a,b) and Gouzoulis-Mayfrank et al. (1999).

Correlational analysis between normalized metabolic activity and psychologic dimensions of the APZ questionnaire revealed that the severity of "oceanic boundlessness" (OB, one factor of the APZ questionnaire) induced by psilocybin correlated positively with CMRglu bilaterally in the frontomedial superior, frontolateral, and left inferolateral PFC and ACC, as well as bilaterally in the inferior parietal and occipitomedial cortex (Vollenweider, 2001). Negative correlations were found between OB and CMRglu bilaterally in the hippocampus and caudate nucleus, and left amygdala and ventral striatum. The severity of "anxious ego dissolution" positively correlated with CMRglu in the thalamus and left temporomedial gyrus and negatively correlated with CMRglu bilaterally in the OFC and adjacent ACC. Thus, anxious ego dissolution and the associated thought disorder appear to depend mainly on thalamic overactivity and orbitofrontal underactivity.

In the study by Vollenweider et al. (1997b), effective doses of psilocybin produced a global increase of CMRglu, with significant and most marked increases in the frontomedial and frontolateral cortex (24.3%), ACC (24.9%), and temporomedial cortex (25.3%). In the study by Gouzoulis-Mayfrank et al. (1999), psilocybin increased regional cerebral glucose metabolism in distinct right hemispheric frontotemporal cortical regions, particularly in the ACC, consistent with the findings of Vollenweider et al. (1997b). These results indicate that psilocybin leads to increased cortical activation.

The spatial resolution of ayahuasca-induced changes in brain electrical activity was investigated in 18 volunteers using low-resolution electromagnetic tomography (Riba et al., 2004). Analyses of EEG sources showed changes in current density in the ACC, but even more so in the PCC. These electrophysiological findings have been replicated in a MEG study of psilocybin use (Muthukumaraswamy et al., 2013).

To examine the neural correlates of acute ayahuasca effects, Riba et al. (2006) used single-photon emission tomography to study regional CBF after acute administration of ayahuasca to 15 healthy volunteers. Ayahuasca administration led to bilateral activation of the anterior insula/inferior frontal gyrus, with greater intensity seen in the right hemisphere. Additional increased blood perfusion was observed in the frontomedial wall of the right hemisphere, with the largest cluster of suprathreshold voxels located in the ACC/medial frontal gyrus. No significant decreases in regional CBF were observed anywhere in the brain. These effects were consistent with the earlier SPECT study by Hermle et al. (1992) and with data from studies on psilocybin that employed [^{18}F]FDG PET (Vollenweider et al., 1997b).

Evidence from animal and human studies suggests that psychedelics disrupt information processing in cortico–striato–thalamocortical (CSTC) feedback loops that have been implicated in sensory and sensorimotor gating of internal and external information to the cortex (Vollenweider and Geyer, 2001; Geyer and Vollenweider, 2008). It is widely believed that the thalamocortical system is essential for conscious activity (Edelman, 2003), and that thalamocortical interactions play a special role in the integration of distributed neural activity across wide cortical regions and in the generation of conscious experience (Tononi and Edelman, 1998). The so-called CSTC model of information processing proposes that deficits in early information processing may underlie alterations in perception,

cognition, and the sense of self seen in psychedelic-induced ASC (Vollenweider and Geyer, 2001). This model suggests that the inability to filter, inhibit, and screen out exteroceptive and interoceptive stimuli may lead to sensory overload and a breakdown of cognitive integrity, leading to hallucinations and alterations of ego functioning. Deficits in preattentive sensorimotor gating have been reported repeatedly after psilocybin and LSD administration (Vollenweider et al., 2007; Quednow et al., 2012; Schmid et al., 2015) and have been related to alterations in cognitive functioning (Vollenweider et al., 2007; Quednow et al., 2012). The CSTC model proposes that the thalamus plays a key role within CSTC feedback loops for gating external and internal information to the cortex, and therefore is crucially involved in the regulation of the level of awareness and attention (Vollenweider et al., 2007; Geyer and Vollenweider, 2008).

This view is consistent with the information integration theory of consciousness (Tononi, 2004), which proposes that the thalamus and thalamocortical system play a key role in integrating information and ensuing consciousness. Psychedelics may alter thalamocortical transmission by stimulating 5-HT_{2A} receptors located in several key components of the CSTC loops (Vollenweider et al., 2007; Geyer and Vollenweider, 2008). This interpretation is consistent with the neuroimaging studies showing that oral administration of psilocybin, mescaline, and DMT alter neuronal activity during their peak effects in the frontomedial and frontolateral cortices ("hyperfrontality"), basal ganglia, and thalamus (Hermle et al., 1992, 1998; Gouzoulis-Mayfrank et al., 1999; Riba et al., 2006), variously correlating with different dimensions of psychedelic states (Vollenweider and Geyer, 2001).

A recent fMRI study, however, reported an overall decrease of brain activity in the medial frontal cortex after intravenous administration of psilocybin (Carhart-Harris et al., 2012). Notably, the reported decrease correlates with a suppression of the DMN (Buckner et al., 2008). Subjective observations support what might be described as a loosening of the "sense of self," or a loss of ego structure, with feelings of unity and oneness with others and the world (Dittrich, 1998; Griffiths et al., 2006). As will be discussed shortly, the fMRI data from this study have been reanalyzed at least twice to develop further hypotheses about the psychedelic state.

Interpretation of fMRI data is not straightforward, however, because the hemodynamic changes that the imaging signals depend on, such as the BOLD response, are not a direct measure of neuronal activity but arise from the relationship between hemodynamic changes and underlying neuronal activity (Logothetis, 2008). The BOLD signal measures local fluctuations in deoxyhemoglobin concentrations, and some degree of controversy remains as to what the BOLD signal actually represents (Viswanathan and Freeman, 2007). It is known that cerebral vasoconstrictors can cause an increase in deoxyhemoglobin concentration, resulting in a decrease in the BOLD baseline resting signal (Mulderink et al., 2002). Thus, using hemodynamic neuroimaging signals as proxy measures for neuronal activity makes off-target effects, including nonhallucinogenic and peripheral actions mediated by other receptor subtypes, an important consideration if a drug has vasoactive properties in addition to its effects on neurons.

Early autoradiographic studies by Jueptner and Weiller (1995) indicated that perisynaptic activity, representing primarily input and local processing in the cortex, rather than cell bodies, accounts for the major part of cortical metabolic energy demands. Consistent with that finding, simultaneous measurement of intracortical activity and fMRI in the nonhuman primate has demonstrated that local field potential (LFP) generated by a local neuronal network is more closely related to the BOLD signal than multiunit activity (MUA) of the same network (Logothetis et al., 2001).

In his review on the underpinnings of the BOLD fMRI signal, Logothetis (2003) concentrated on one aspect of what is a complex issue, namely the type of neural activity that plays a dominant role in the generation of the imaging signal capitalizing on the BOLD contrast mechanism. LFPs, the low frequency range of the mean extracellular field potentials, mostly represent slow events reflecting cooperative activity in neural populations. Afterpotentials, with a duration on the order of tens of milliseconds, most likely contribute to the generation of LFP signals. LFPs represent slow waveforms that include synaptic potentials, afterpotentials of somatodendritic spikes, and voltage-gated membrane oscillations and reflect the input of a particular cortical area, as well as its local intracortical processing, including the activity of both excitatory and inhibitory interneurons. Logothetis (2003) initially applied linear systems analysis to examine the relationship of the BOLD fMRI signal to the different types of neural activities and found that the BOLD response "indisputably and directly reflects a local increase in neural activity assessed by the mean extracellular field potential (mEFP) signal."

Rauch et al. (2008) also explored the relationship between the BOLD signal and LFPs and MUA. They simultaneously recorded BOLD and electrophysiological activity in primary visual cortex V1 of anesthetized monkeys while inducing a dissociation of MUA from LFP activity by local injections of the 5-HT_{1A} agonist BP554. Infusion of BP554 (1-[3-(3,4-methylenedioxyphenoxy) propyl]-4-phenyl-piperazine) into the visual cortex reduced MUA reliably without affecting either LFP or BOLD. BP554 presumably activated 5-HT_{1A} receptors located on the axon hillock in layer 3 and 5 pyramidal neurons, hyperpolarizing the cells so that they could reach a spiking

threshold only under a much heavier synaptic load. Rauch et al. (2008) reported that infusion of BP554 had no effect on either LFP or the BOLD signal, but did significantly decrease MUA, and they concluded that the BOLD signal is well predicted by LFP.

Viswanathan and Freeman (2007) used a dual microelectrode arrangement to make simultaneous colocalized extracellular measurements of tissue oxygen and neural activity in the cortex of the cat primary visual cortex to determine whether the BOLD signal reflected mainly neuronal input (synaptic activity) or neuronal output (spiking activity). They report that changes in tissue oxygen were more closely coupled with LFPs than with neuronal spikes. Their results suggest that the BOLD fMRI signal primarily reflects neuronal input, rather than neuronal output (spiking activity).

As shown in their earlier study (Puig et al., 2003), systemic DOI evoked a dramatic increase in the firing rate of a subpopulation of PFC pyramidal neurons. Celada et al. (2008) extended their earlier approach by showing the effect of DOI on burst firing and LFPs, in which firing rate was increased by DOI. Yet DOI markedly reduced the amplitude of low frequency (3 to 4 Hz) oscillations in the mPFC, an effect that was completely blocked by M100907 but was not attenuated by thalamic lesions, supporting an intracortical origin for the effects of DOI. They demonstrate that DOI disrupts cortical activity by reducing low frequency oscillations (3 to 4 Hz) and by desynchronizing pyramidal discharge from active phases of slow oscillations. Slow oscillatory activity reflects alternating periods of activity and silence ("up" and "down" states) of cortico–thalamo–cortical networks that result from synchronized changes in membrane potential and synaptic activity of neuronal ensembles.

Wood et al. (2012) compared the effect of DOI with amphetamine and MK-801 on PFC neuronal activity in freely moving rats. They implanted microelectrode arrays in male rats and measured neuronal activity in the OFC and ACC. Their study was the first to investigate the effects of a psychedelic on cortical neurophysiology in awake animals. They analyzed neuronal population activity, LFP power, and correlations between spike-discharge power and LFP power. DOI (1 mg/kg) significantly reduced population activity in OFC compared with baseline, with larger doses of DOI producing greater population suppression. DOI (3–5 mg/kg) suppressed population activity in the ACC in a similar fashion, although the 1-mg/kg dose failed to have an effect. The 3-mg/kg dose of DOI significantly decreased low (30–55 Hz) and high (55–80 Hz) γ power in the OFC. A similar trend was observed in the ACC. The reduced γ in both OFC and ACC power is an effect that disrupts spike timing. The lowest dose of DOI drove weak and sporadic increases in ACC single-unit activity, which did not modulate population activity, but larger doses of DOI predominantly decreased activity of individual ACC units in a sustained fashion. The data of Wood et al. (2012) indicate that a common effect of psychedelics in the PFC may be to reduce the spontaneous coupling of single-unit activity with γ oscillations. The integrity of cortical networks depends on maintaining a delicate balance of inhibitory and excitatory neurotransmission. Thus, excitation or inhibition produced by these drugs at any node in a neuronal network could potentially disrupt the activity of that network, leading to perceptual disorganization and cognitive effects. Disruption of spontaneous γ power may be indicative of, or cause, disrupted network signaling. Taken together, these data demonstrate that psychedelics decouple single-unit discharge from rhythmic oscillation in the OFC and ACC, thereby decreasing coordination of neuronal populations.

Functional connectivity analysis, using independent component analysis, of the data from a 2012 study (Carhart-Harris et al., 2012), revealed increased DMN and task positive network (TPN) functional connectivity, therefore showing a decrease in DMN-TPN orthogonality after psilocybin (Carhart-Harris et al., 2013). Carhart-Harris et al. propose that increased DMN-TPN coupling in the presence of preserved thalamocortical connectivity is related to a state in which arousal is preserved but the distinction between inner thought and external focus becomes blurred.

To directly assess the effects of a psychedelic drug on neural activity in the human brain, Muthukumaraswamy et al. (2013) performed MEG to record broadband (1–100 Hz) neural activity directly in 15 healthy volunteers with prior experience with psychedelics who were given 2 mg psilocybin intravenously. Subjective effects began within seconds, allowing the capture of the transition from normal waking consciousness to the psychedelic state. Approximately 6 minutes after the infusion, participants performed a visuomotor task designed to elicit stimulus-induced γ band oscillations in the primary visual and motor cortex. Five minutes of resting MEG was recorded; the participant was then infused with psilocybin over 60 seconds and 5 minutes of resting MEG was recorded immediately after infusion. The authors reported decreased oscillatory power across a broad frequency range after psilocybin, mainly localized to association cortices, with marked decreases in areas of the DMN such as the PCC. They recovered 11 functional brain networks, 7 of which showed postpsilocybin infusion decreases in oscillatory power in the frequency bands from which they were derived. A further four networks were identified that did not pass the significance criterion, but activity in these networks was consistently decreased by psilocybin.

Muthukumaraswamy et al. (2013) set up a model in which drug effects could be accounted for by differential gain (or excitability) of each of four cell types: inhibitory interneurons, superficial pyramidal cells, spiny stellate

cells, and deep pyramidal cells. Their model showed a psilocybin-dependent increase in excitability of deep-layer pyramidal cells. Excitation of deep-layer pyramidal cells is consistent with decreased brain activity and oscillatory power because the net effect of feedback connections is thought to be inhibitory. Participants performed the visuomotor task reasonably well but were marginally slower after psilocybin than placebo. The authors conducted exploratory correlations between resting postpsilocybin infusion decreases in α power extracted from a PCC mask, because PCC α power decreases were especially marked. Two items from their subjective state questionnaire showed positive significant correlations with α power decreases in the PCC ("I experienced a disintegration of my 'self' or 'ego'" and "the experience had a supernatural quality"). Positive correlations also were found between deep-layer pyramidal cell excitation and the magnitude of the decreases in PCC α power. Muthukumaraswamy et al. (2013) observed a broadband desynchronization of cortical oscillatory rhythms after psilocybin infusions and decreased brain network integrity. The fact that the decreases occurred in all of the frequency bands suggested a general collapse of the normal rhythmic structure of cortical activity, consistent with the suggestion by Carhart-Harris et al. (2012) that psychedelics disorganize spontaneous brain activity.

The MEG source localizations they found were consistent with previous fMRI results (Carhart-Harris et al., 2012) including the PCC, precuneus, superior and middle frontal gyri, ACC, and supramarginal and precentral gyri, which represent hub areas of the association cortex rather than primary sensory cortex. The PCC showed especially marked effects, which correlated with the drug's subjective effects. The pharmacologically driven suppression of all observed networks suggested a general disorganization of network-level activity during resting conditions consistent with EEG studies that found decreased cortical synchrony after ingestion of the psychedelic plant brew ayahuasca (Riba et al., 2004). They propose that the marked α power decreases observed with psilocybin were likely due to interference with the intrinsic α oscillations of deep-layer pyramidal neurons via stimulation of the 5-HT_{2A} receptor.

Riga et al. (2014) examined the effect of systemic 5-MeO-DMT on cortical function in rats using single-unit and LFP recordings and also assessed regional brain activity by BOLD fMRI. Their studies were carried out in chloral hydrate–anesthetized rats pretreated with the selective MAO-A inhibitor clorgyline to prevent the metabolism of 5-MeO-DMT and to mimic the effects of ayahuasca. They found that 5-MeO-DMT disrupted mPFC activity, increasing and decreasing the discharge of 51% and 35% of the recorded pyramidal neurons, respectively, and reducing (−31%) the power of low frequency cortical oscillations (LFCOs); overall, 5-MeO-DMT increased pyramidal cell firing rate to 215% of baseline. The latter effect depended on both 5-HT_{1A} and 5-HT_{2A} receptor activation because when WAY-100635 and M100907 were administered together, the decrease in LFCOs induced by 5-MeO-DMT in the mPFC was reversed. However, if WAY-100635 and M100907 were administered separately, neither antagonist blocked the effect of 5-MeO-DMT on LFCOs. This finding is consistent with behavioral studies showing that indoleamines can have a significant behavioral component mediated by activation of 5-HT_{1A} receptors (Winter et al., 2000b; Krebs-Thomson et al., 2006; Halberstadt and Geyer, 2011). The mGlu2/3 agonist LY379268 also fully reversed the effect of 5-MeO-DMT on LFCOs.

The BOLD responses in visual cortex V1 and mPFC observed by Riga et al. (2014) were also decreased by 5-MeO-DMT. Simultaneous recordings in the mPFC and V1 indicated that 5-MeO-DMT concurrently reduced the amplitude of LFCOs similarly in both areas. In parallel with the effect on pyramidal discharge, 5-MeO-DMT significantly reduced the amplitude of LFCOs in the mPFC. Thus, 5-MeO-DMT markedly reduced LFCOs in the mPFC and V1, an action potentially related to its psychedelic activity. It evoked a disrupted activity state characterized by altered pyramidal neuron discharge/pattern and reduced intensity of LFCOs. Riga et al. (2014) suggest that 5-MeO-DMT–evoked alterations in PFC activity likely lead to secondary changes in several brain networks.

Riga et al. (2014) indicate that the reduction in BOLD signal appears paradoxical, given the overall increase in pyramidal discharge rate produced by 5-MeO-DMT and the relationship between neuronal discharge, energy consumption, and blood flow, noting that some studies suggest a better correlation with oscillatory activity rather than spiking activity (Logothetis, 2003; Viswanathan and Freeman, 2007). They conclude that reductions in LFCOs appear to be a common signature of psychedelic drugs but note that further work is required to understand the relationship between BOLD signal and neuronal activity.

This study has relevance to clinical studies of psilocybin. Although the salient features of psilocybin intoxication are blocked by the selective 5-HT_{2A} antagonist ketanserin (Vollenweider et al., 1998), psilocin (the active form derived in vivo by dephosphorylation of psilocybin) also has significant agonist activity at the 5-HT_{1A} receptor (e.g., Blair et al., 2000). It is unknown to what extent intravenous administration of psilocybin might produce effects on neuronal function through activation of 5-HT_{1A} receptors.

Tagliazucchi et al. (2014) carried out a reanalysis of the previously published data from Carhart-Harris et al. (2012). Their new analyses were prompted by a view that more sensitive and specific indices might help to develop a better understanding of the neurobiology of conscious states, and specifically that measures that

include variance over time might be especially informative. They note that the brain has been described as a system resting in (or near) a critical point or transition zone between states of order and disorder (see references in Tagliazucchi et al., 2014). They therefore tested the hypothesis that the brain can explore a maximal repertoire of its possible dynamic states in this critical zone, asking the question as to whether changes in spontaneous brain activity produced by psilocybin were consistent with displacement from this critical point, possibly moving toward a more entropic or supercritical state (Carhart-Harris et al., 2014). To test this hypothesis, the authors focused on variability in activity and functional connectivity parameters over time and presented empirical data that tested the hypothesis that brain activity becomes less ordered in the psychedelic state, with enhancement of the repertoire of possible states. The power spectrum density of the spectral content of spontaneous BOLD fluctuations can be characterized by a single parameter α, which condenses the scaling behavior and is demonstrative of the long-range temporal correlations of any given signal. Both BOLD signal variance and total spectral power measures showed increased variability after psilocybin, both in the temporal and spectral domain, with peaks in the ACC and bilateral hippocampus.

Tagliazucchi et al. (2014) selected four regions of interest for BOLD variance and computed the evolution of their variance. Although these ROIs were selected precisely because they had fluctuations in their activity and corresponding increased variance, their analysis provided additional information about when those increases occurred. A homogeneous increase in signal variance was observed during the first 3 minutes of psilocybin infusion, corresponding to the subjects' reports that the most intense psychologic effects occurred within the first 3 minutes of the psilocybin infusion. The authors also studied the variance of intrahippocampal connectivity over time and found that variance was higher in both hippocampi after psilocybin. After psilocybin, diffuse widespread decreases in LFPs and the scaling exponent α were observed in frontal and parietal regions, with no changes after placebo. To further characterize the BOLD fluctuations, the authors transformed whole brain signals into a spatiotemporal point process and extracted the rate of events and the average separation between two events. They observed a rate increase and interval decrease after psilocybin in the parietal and frontal regions, but no differences after placebo. Brain networks associated with these changes were computed from the overlap of the statistical significance maps with a set of well established resting state networks. For LFPs and α, significant overlaps were detected in the default mode, control, and attention networks, whereas all sensory networks remained unaffected. For the point-process rate and point-process interval, the changes were local and confined to the DMN.

Tagliazucchi et al. (2014) also evaluated the entropy of the distribution of connectivity states in the network comprised by two ACC ROIs and the bilateral hippocampi. An entropy increase was found when comparing the results between the periods before and after psilocybin infusion, but no change was seen before and after placebo. Cross-hemispheric connections between hippocampal and ACC ROIs were also observed after psilocybin administration. Thus, the psilocybin state is characterized by a larger repertoire of states (i.e., novel motifs that are exclusive to the psychedelic state). These states were found to be among the most interconnected states possible.

The resting state networks that exhibited the most significant changes correspond to higher brain systems such as the DMN, executive control, and attention networks, and not primary sensory and motor networks. The authors note that the increased amplitude fluctuations in the hippocampus are particularly intriguing considering early depth EEG studies that recorded similar abnormalities in hippocampal activity after LSD and mescaline (Schwarz et al., 1956; Monroe and Heath, 1961). The increased repertoire of metastable states observed with psilocybin may be a mechanism by which these phenomena occur. Tagliazucchi et al. (2014) suggest that altered interhemispheric communication may also be an important component of the mechanism of action of psychedelics. A primary action of psilocybin may be to cause a generalized desynchrony and loss of oscillatory power in higher-level cortical regions, probably resulting from activation of serotonin 5-HT_{2A} receptors expressed on deep-later pyramidal neurons.

Scherf and Angenstein (2015) simultaneously measured generated field EPSPs and BOLD response in the CA1 region of the rat hippocampus during electrical stimulation of the contralateral CA3 region. Consecutive stimulations with low-intensity stimulation trains resulted in clear postsynaptic responses of CA1 pyramidal cells, but no significant BOLD response. No positive correlation was found between the electrophysiologic parameters of CA1 pyramidal cell activity and the BOLD response. Consequently, postsynaptic activity of pyramidal cells, the most abundant neurons in the CA1, is not directly linked to the measured BOLD response.

Palhano-Fontes et al. (2015) used fMRI to inspect the DMN after ayahuasca administration to 10 healthy volunteers who were regular users of ayahuasca. Subjects underwent fMRI sessions before and 40 minutes after ayahuasca intake. Ayahuasca caused a significant decrease in activity throughout most parts of the DMN, including its most consistent hubs: the PCC/precuneus and the mPFC. Their results are consistent with the notion that the ASC induced by ayahuasca is linked to the modulation of activity and connectivity of the DMN. Functional connectivity fMRI maps were constructed

before and after ayahuasca administration, and a comparison of the maps revealed a significant functional connectivity decrease within the PCC/precuneus after ayahuasca administration, with most of the contribution for the observed connectivity decrease driven by the PCC. Although similar to the changes observed after psilocybin, the changes induced by ayahuasca did not result in a significantly reduced coupling between the PCC and mPFC, as observed after intravenous psilocybin administration (Carhart-Harris et al., 2012). Overall, these results support the conclusion that the effects of ayahuasca are associated with diminished DMN activation and decreased functional connectivity of the PCC/precuneus. They conclude that the ASC induced by ayahuasca is linked to the modulation of the activity and connectivity of the DMN.

Carhart-Harris et al. (2014) consider the psychedelic state to be an exemplar of a primitive or primary state of consciousness that preceded the development of modern, adult, human, normal waking consciousness. On the basis of neuroimaging data with psilocybin, they argue that the defining feature of "primary states" is elevated entropy in certain aspects of brain function, such as the repertoire of functional connectivity motifs that form and fragment across time. They also propose that entry into primary states depends on the collapse of the normally highly organized activity within the DMN and a decoupling between the DMN and the medial temporal lobes that are normally significantly coupled. The functional centrality of the DMN is not shared by other brain networks, implying that as the highest level of a functional hierarchy, the DMN serves as a central orchestrator or conductor of global brain function. Carhart-Harris et al. (2014) previously found a highly significant positive correlation between the magnitude of α power decreases in the PCC after psilocybin and the ratings of the item "I experienced a disintegration of my 'self' or 'ego.'" It is a central hypothesis of their article that psychedelics induce a primitive state of consciousness. They present a comprehensive model in which psychedelics 1) activate the 5-HT_{2A} receptor, 2) depolarize deep-layer pyramidal neurons, 3) desynchronize cortical activity, 4) "disintegrate" brain networks, 5) increase network metastability, and 6) increase the repertoire of connectivity motifs within a limbic/paralimbic network. The net effect of these processes is an increase in system entropy as the system enters criticality proper. Specifically, it is proposed that psychedelics work by dismantling reinforced patterns of negative thought and behavior by breaking down the stable spatiotemporal patterns of brain activity upon which they rest.

The fMRI studies and their various analyses and reanalyses by Carhart-Harris and colleagues are at odds with the earlier SPECT and PET imaging studies and may reflect different methodologies. In particular, psilocybin is not typically administered to humans by injection, nor are *Psilocybe* mushrooms generally taken in routes other than by mouth. In the Hermle et al. (1992), Vollenweider et al. (1997a,b), and Gouzoulis-Mayfrank et al. (1999) studies cited earlier, the drug was administered orally, but psilocybin was administered intravenously in the studies by Carhart-Harris et al. Subjective reports from the two different routes of administration indicate profound differences in the speed of onset, as well as the intensity of the subjective effects. Psilocybin given orally generally takes about 40 minutes to begin to manifest its effect, with a duration of action lasting 4–6 hours. By contrast, subjective effects of 2 mg psilocybin given as an intravenous injection over 60 seconds begin at the injection period, reach a sustained peak after 4 minutes, and subside completely after 45–60 minutes (Carhart-Harris et al., 2011). Vascular responses resulting from the agonist activity of psilocin at 5-HT_1 family receptors are likely to be more pronounced after intravenous drug administration. Oral administration of psilocybin followed by repeated fMRI scans would lead to perspective on the temporally related changes in blood flow/brain activation and the effects of different doses could be examined. In any event, differences in neuronal activity indices (metabolic rate of glucose, CBF, or BOLD), and differences in the intensity, dynamics, and content of psilocybin-induced symptoms could potentially account for these apparent discrepancies.

Psilocybin (2 mg/kg, i.v.) administered to rats evoked phMRI signal increases in a number of regions, including olfactory and limbic areas and elements of the visual system (Spain et al., 2015). LFP amplitude in response to sensory stimuli was decreased by psilocin administration, concurrently with enhanced CBF. These results suggest that the hemodynamic signal changes underlying phMRI responses reflect changes in both neuronal activity and neurovascular coupling. Thus, phMRI studies cannot be interpreted solely in terms of the drug effect on neurons. A further potential confound in BOLD signal interpretation in the case of psilocin results from its combined neuronal and vascular effects. Both the 5-HT_{2A} and 5-HT_{1A} receptors can mediate vasoconstriction, and negative BOLD signals may occur in the presence of increased neuronal signaling (Angenstein et al., 2009).

The alteration in neurovascular coupling reported by Spain et al. (2015) might explain, in part, the apparent discrepancy between fMRI and PET findings of decreased CBF (Carhart-Harris et al., 2012) and increased glucose metabolism (Vollenweider et al., 1997b; Gouzoulis-Mayfrank et al., 1999) in human studies with psilocybin and related drugs.

Most recently, Carhart-Harris et al. (2016) used arterial spin labeling (ASL), BOLD, and MEG to image brain activity in 20 healthy human subjects administered 75 μg LSD intravenously. Visual cortex desynchronization and expanded V1 functional connectivity were found to be correlated with visual hallucinations.

Dysregulation of high-level regions and networks correlated with profound changes in consciousness. The peak phase of subjective effects of LSD surprisingly occurred at about 100 minutes after intravenous administration, whereas the subjective effects occurred within minutes after intravenous administration in their studies with psilocybin. All subjects reported closed-eye visual hallucinations and marked changes of consciousness. Decreased parahippocampus resting state functional connectivity correlated with ego dissolution and altered meaning. Principal findings included the following: 1) increased visual cortex CBF, resting state functional connectivity (RSFC), and decreased α power, predicting the intensity of visual hallucinations; and 2) decreased DMN "integrity", parahippocampus-RSC/PCC resting state functional connectivity and δ and α power (e.g., in the PCC), all predicting profound changes in consciousness, characterized by ego dissolution. Results more broadly revealed that resting state BOLD functional connectivity and MEG measures (but less so CBF) possess considerable power to predict the psychologic effects of LSD. Strong relationships were found between the different imaging measures, particularly between changes in BOLD RSFC (e.g., network "disintegration" and "desegregation") and changes (decreases) in oscillatory power. Surprisingly, although the effects of LSD on the visual system were pronounced, they did not correlate with its effects on consciousness. Consistent with their previous psilocybin research, a significant relationship was found between decreased PCC α (and δ) power and ego dissolution. An especially strong relationship was found between parahippocampus and RSC/PCC decoupling and ego dissolution. Taken together with their earlier studies (Carhart-Harris et al. (2011; 2012)), it appears that psychedelics destabilize and disintegrate normally well established brain networks and reduce the degree of segregation between them.

The mechanisms of long-term effects of one or several psychedelic experiences are even less well understood. The initial agonist action on serotonin receptors does not explain the long-term effects seen 14 months after these experiences with "positive changes in attitudes, mood, life satisfaction, behavior, and altruism/social effects" (Griffiths et al., 2011). After this "sense of self," reassembles at the end of the psilocybin experience, there appears to be a chance of abandoning habits and repetitive thoughts that no longer serve a useful purpose for the person. One open study found that long-term positive effects may persist for decades (Doblin, 1991) and it seems possible that long-term adaptation through changes in gene expression also may occur that can be therapeutic (e.g., see Nichols and Sanders-Bush, 2002).

XII. Conclusion and Outlook

Dr. Albert Hofmann, the natural products chemist who accidently discovered the effects of LSD in 1943 while working at the Sandoz Laboratories in Basel, Switzerland, wrote an autobiographical account of his discovery titled *LSD: My Problem Child* (Hofmann, 1979b). In his book, Hofmann talks about the potential of LSD, which he had hoped would be a promising new tool for psychiatry, but also expresses dismay at the social turbulence that ensued when "LSD was swept up in the huge wave of an inebriant mania that began to spread over the Western world, above all the United States…" (Hofmann, 1979b). Hofmann died in 2008 at the age of 102 years; but in his later years, he was delighted to see that real science had begun to take a thorough approach to unraveling the psychopharmacological mysteries of LSD, which he had always believed would eventually prove to be a miracle drug for psychiatry.

With that in mind, if the positive therapeutic effects of psychedelics continue to be validated by additional well designed clinical studies, it opens up a whole new dimension of medical research. If psilocybin or LSD can acutely abolish depression or anxiety after one or only a few treatments, the question must be asked, "How does that occur?" There are many who believe that such improvement must be related to neurochemical effects, or neuroadaptation, and refuse to believe that the mystical experience may be relevant. Yet both modern and older studies consistently find that those who experience the most profound mystical experiences invariably receive the greatest symptom improvement. Of course, as reductionists, it is understood that the mystical experience must have neurochemical correlates. Even so, understanding what they are, how and why they occur, and how they lead to therapeutic improvement should shed light on the underlying deficits in brain function that lead to these disorders in the first place. Before-and-after brain imaging studies of patients with depression, anxiety, or addictive disorders will show how brain connectivity has changed as a result of psychedelic treatment. To understand these disorders at the present time with standard state-of-the-art approaches involves a sort of "fishing expedition," searching for biomarkers that might be clues to the basis of the underlying disorder. Genome-wide association studies plow through many thousands or hundreds of thousands of genes, searching for candidates that might be the underlying causes of affective disorders. One generally cannot do prospective studies, to compare the brain function of the normal patient prior to the onset of his or her disease, and then examine it again after therapeutic improvement. Rather, one begins with a patient who is already sick, and then if therapeutic improvement occurs, usually over a long period of time, one tries to understand how it happened. By contrast, some of the recent treatments of anxiety and depression with psilocybin or LSD are so dramatic, and happen so quickly, that there must be some overt measureable changes in brain function or connectivity

that correlate with therapeutic improvement. Learning what these are is the next big challenge, a process that promises to completely revolutionize the way we approach discovering better treatments for a host of human psychiatric disorders.

Considering the most recent scientific and clinical developments in understanding the actions of psychedelics, a statement made in 1980 by Dr. Stanislav Grof seems particularly relevant today: "It does not seem to be an exaggeration to say that psychedelics, used responsibly and with proper caution, would be for psychiatry what the microscope is for biology and medicine or the telescope is for astronomy. These tools make it possible to study important processes that under normal circumstances are not available for direct observation" (Grof, 1980).

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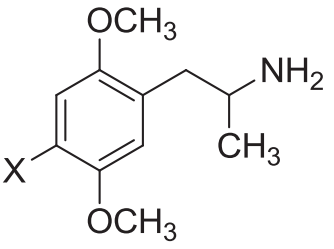
Correction to: “Psychedelics”

In the above article [Nichols D (2016) *Pharmacol Rev* **68**:264–355], a portion of Figure 4 was omitted. The corrected Figure 4 is provided below. The XHTML and PDF versions of the article have been replaced.



X = CH_3; DOM
X = Br; DOB
X = I; DOI