Autoradiographic Approaches to Studying Hallucinogens or Other Drugs

Nathan M. Appel

INTRODUCTION

The technique of autoradiographic localization of neurotransmitter and drug binding sites, often referred to as receptor autoradiography, has become a frequently used approach to investigate drug effects in the central nervous system (CNS). The technique enables the researcher to reveal the anatomic locus of actions of drugs and can be used to evaluate consequences of drug treatment. It is a rare issue of *Current Contents* in which the keyword "autoradiography" does not yield relevant citations.

This chapter provides brief descriptions of the theory and practice of in vitro and in vivo receptor autoradiography, provides examples of how this approach has been used to study hallucinogens and other drugs in experimental animals, describes how the technique of receptor autoradiography has been extended to study hallucinogens in a clinical setting using positron emission tomography (PET), and describes a novel autoradiographic approach for assessing fatty acid incorporation that is a promising research and clinical tool for studying the response to and effects of hallucinogens and other classes of drugs or stimuli.

There are many excellent review articles and books that address the theoretical basis, practical aspects, and limitations of the receptor autoradiographic technique. For the reader who is interested in a more comprehensive treatment of the subject, the chapters on receptor autoradiography by Kuhar are an excellent "first read" (Kuhar 1985; Kuhar and Unnerstall 1990).

RECEPTOR AUTORADIOGRAPHY

Receptor autoradiography is essentially a receptor binding assay. The assay is performed on an intact, instead of homogenized, tissue sample. Thus, the anatomic integrity of the tissue under investigation is preserved.

Binding of the radiotracer follows the law of mass action. Radiotracer binding is saturable and stereospecific and displays nanomolar affinities. For a particular radiotracer, the optimum incubation time (ligand association) and the optimum rinse times (separation of bound drug from free) are determined. Thus, binding kinetics can be determined, as can orders of potency of competing drugs, sensitivity to ions, nucleotides temperature, and other factors.

In practice, the differences between the techniques are manifested in how the tissue of interest is radiolabeled and how the quantity of bound radiolabel in tissue is determined. To perform in vitro receptor autoradiography, tissues are labeled by incubating slide-mounted tissue slices (microtome sections) instead of homogenized tissue with a radiolabeled drug. Nonspecific binding is detected by incubating tissues with the radiolabeled drug in the presence of excess (typically thousandfold) unlabeled ("cold") competing drugs. For in vivo receptor autoradiography, tissues are labeled by injecting test animals with a radiolabeled drug. By using in vivo receptor autoradiography, nonspecific binding can be determined in animals treated with excess unlabeled competing drug prior to injection of the radiotracer. An alternative method for illustrating specific binding using the in vivo approach is to express binding as the ratio of radioactivity in a region of interest and another area where few receptors are known to exist, for example, the ratio of spiperone-labeled dopamine (DA) receptors in caudate putamen and cerebellum (Kuhar 1985; figure 1).

With receptor autoradiography, the binding of radiotracer is quantified by determining the intensity of the image that results from exposing the radiolabeled tissue to a photosensitive medium. In contrast, for a homogenate receptor binding assay, the radioactivity accumulated on a filter or pelleted at the bottom of a test tube is counted. The photosensitive medium is typically photographic emulsion. It can be directly applied to the radiolabeled tissue, apposed to the radiolabeled tissue in the form of an emulsion-coated coverslip, or apposed to the radiolabeled tissue in the form of commercially available autoradiographic film.

An exciting new development is filmless autoradiography in which autoradiographic images are acquired by apposing radiolabeled specimens to screens coated with a storage phosphor. A latent image of the pattern of labeling forms within the storage phosphor and is visualized by scanning the screen with a laser that allows the image to be

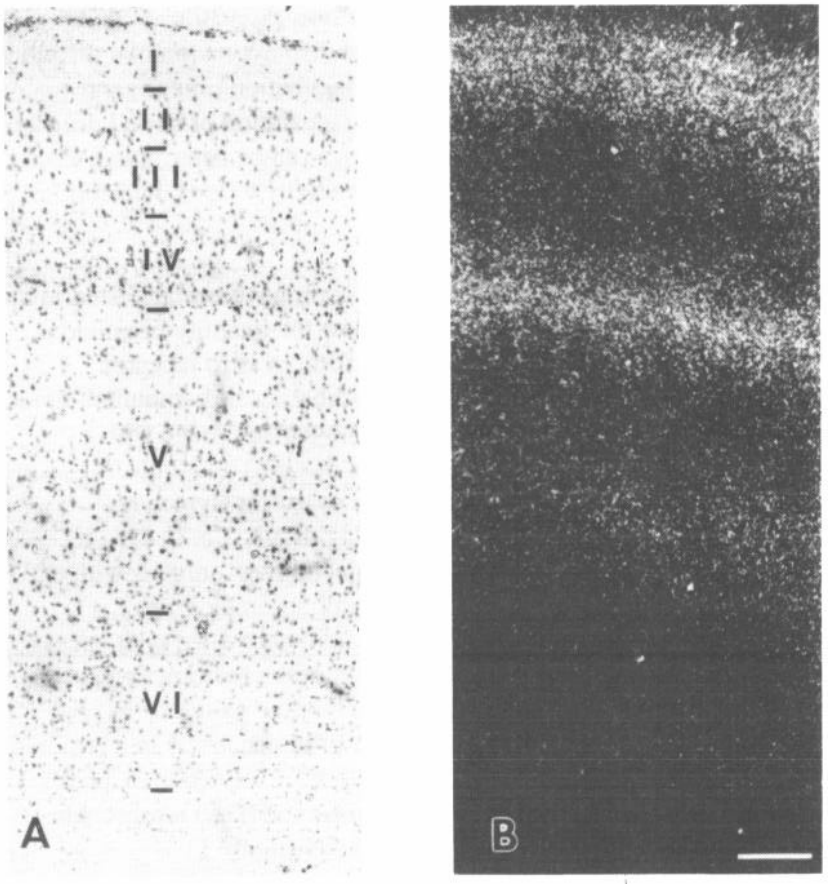

FIGURE 1. *Distribution of [^{125}I] DOI-labeled serotonin, (5-HT$_2$) receptors in cerebral cortex. A: Bright-field photomicrograph of a Nissl-stained horizontal section through parietal cortex. Lamination is annotated with Roman numerals and delineated by the short black horizontal bars. B: Dark-field photomicrograph of the "coverslip" autoradiograph, generated by the section shown in A (same magnification), depicting ([^{125}I]DOI) 1-(2,5-dimethoxy-4-[^{125}I]iodophenyl)-2-aminopropane) binding in cerebral cortex. Note that silver grains are most concentrated in laminae I and IV. Magnification bar (lower right-hand corner) = 200 μm.*

SOURCE: Appel et al. 1990*a*

detected electronically using a photomultiplier. The image then is stored on a computer for subsequent analysis (Appel et al. 1991; Kuhar et al. 1991). The image pattern (autoradiographic distribution) depicts the spatial distribution of binding sites in the tissue.

Regardless of the photographic medium used, the optical density or intensity of the image is related to the quantity of bound drug and, by extension, of binding sites in a particular region of interest. Thus, the principal advantage that receptor autoradiography provides over homogenate binding assays is increased anatomic resolution. For example, using a homogenate binding assay, it can be determined that binding of a certain drug is greater in the cerebral cortex than in the cerebellum. Using autoradiography, it can be said with certainty that binding is greater in cerebral cortex layer V than layer VI but less than in layers I or IV (e.g., figure 1). For an exact quantitative assessment of regional binding, regional optical densities can be measured by densitometry and compared with the optical density produced by calibrated radioactive standards coexposed with the experimental material. Several computer-assisted digital image analysis systems are available commercially to facilitate quantification of autoradiographs.

Another advantage of receptor autoradiography over homogenate binding assays is increased sensitivity. In a homogenate binding assay, the ability to detect binding is limited if the density of a particular binding site or the specific activity of a labeled drug is low. Using autoradiography, one can increase the duration the photosensitive medium is exposed to the radiolabeled tissue to overcome these impediments.

IN VITRO RECEPTOR AUTORADIOGRAPHY TO STUDY POTENTIAL SITES OF ACTION OF HALLUCINOGENS

The exact criteria that categorize a substance as a hallucinogen remain unresolved (Szára, this volume). However, three classes of compounds constitute the majority of what typically are referred to as hallucinogenic drugs (see chapters by Glennon; Pfaff et al.; and Jacob and Shulgin, this volume). They are indolealkylamines (e.g., dimethyltryptamine [DMT]), ergolines (e.g., lysergic acid diethylamide [LSD]), and phenylalkylamines (e.g., 2,5-dimethoxy-4-methylamphetamine [DOM]). They have in common a high affinity for serotonin$_2$ (5-HIT$_2$) receptors. Drug discrimination studies, other behavioral tests, and homogenate receptor binding assays have related hallucinogenic potency to binding affinity of

5-HT_2 receptor agonists (Glennon and Rosecrans 1982; Glennon et al. 1984; Heym and Jacobs 1987, 1988; Titeler et al. 1988). Moreover, a significant positive correlation has been demonstrated between 5-HT_2 binding affinities of drugs and their human hallucinogenic potencies (Glennon et al. 1984). In consideration of these data, drugs that label 5-HT^2 receptors would, by extension, label at least some sites in the brain where hallucinogenic effects of these drugs may be manifested.

In an early study in which binding sites for a hallucinogen were demonstrated, autoradiographic distribution of 5-HT binding sites was demonstrated using [^{3}H]LSD. However, unlabeled serotonin (5-HT) was unable to displace [^{3}H]LSD labeling in all areas (Meibach et al., 1980). The recognition of subtypes of 5-HT receptors, as distinguished by differential binding of [^{3}H]5-HT, [^{3}H]LSD, and [^{3}H]spiroperidol (spiperone), would point the way toward using more selective ligands to study the now apparent different classes of 5-HT receptors (Peroutka and Snyder 1979). Thus, anatomic distribution of 5-HT_2 receptors has been studied autoradiographically using radioligands such as [^{3}H]spiperone (Altar et al. 1985; De Souza 1986; Palacios et al. 1981; Pazos et al. 1985) and [^{3}H]ketanserin (Fishette et al. 1987; Pazos et al. 1985; Slater and Patel 1983). However, both these radioligands are antagonists: [^{3}H]spiperone potently labels DA type 2 (D_2) receptors (Niznik et al. 1985; Stefanini et al. 1980), and [^{3}H]ketanserin labels nonserotonergic sites in striatum (Leysen et al. 1987).

The autoradiographic distribution of 5-HT_2 receptors also has been studied using another radioiodinated ligand, [^{125}I]-LSD (Altar et al. 1986; De Souza 1986; Engel et al. 1984; Nakada et al. 1984). However, because [^{125}I]-LSD binds D_2 receptors with nanomolar affinity (Engel et al. 1984; Hartig et al. 1985*a*), one must perform [^{125}I]-LSD autoradiography for 5-HT_2 receptors in the presence of unlabeled D_2 ligands to ensure that only 5-HT_2 receptors are being visualized. There is another congener of LSD, *N*1-methyl-2-[^{125}I]-LSD, that is more selective for 5-HT_2 receptors than D_2 receptors. It also has been used to autoradiographically localize 5-HT_2 receptors (Hoffman et al. 1987).

The hallucinogenic potency of drugs appears to be related to their 5-HT_2 receptor binding affinities and their being agonists at this binding site (Glennon and Rosecrans 1982; Glennon et al. 1984; Heym and Jacobs 1988; Titeler et al. 1988). However, evidence has been presented suggesting that 5-HT_2 receptors exist in two states-one state having high affinity for agonists and one state having low affinity for agonists-and

that antagonists (such as ketanserin and spiperone) do not discriminate between these two states. Moreover, the density of the high-affinity state of 5-HT_2 receptors is only a small fraction of the total 5-HT_2 receptor binding sites (Lyon et al. 1987; Teitler et al. 1990). Thus, to examine autoradiographically the distribution of the high-affinity state of the receptor, and by extension a hallucinogen binding site, would require an agonist 5-HT_2 receptor radioligand with high specific activity so that it could be detected in spite of the low density of these sites. A drug satisfying these criteria is [^{125}I]DOI. It is a potent agonist at 5-HT_2 receptors and has high specific activity (~2200 curies per millimole [Ci/mmol]) (Glennon et al. 1988; Johnson et al. 1987; Teitler et al. 1990). These pharmacological and radiochemical properties make [^{125}I]DOI well suited for studying this state of 5-HT_2 receptors.

[^{125}I]DOI has proven to be an excellent ligand to autoradiographically label 5-HT_2 receptors (Appel et al. 1990*b*; McKenna and Saavedra 1987; McKenna et al. 1989). Patterns of autoradiographs of [^{125}I]DOI binding to 5-HT_2 receptors were similar to those seen when 5-HT_2 receptors were labeled with [^{3}H]ketanserin or [^{125}I-LSD (Appel et al. 1990*a*; McKenna et al. 1989). High densities of [^{125}I]DOI labeling were present in the olfactory bulb, anterior regions of cerebral cortex, claustrum, caudate putamen, globus pallidus, ventral pallidum, islands of Calleja, mammillary nuclei, and inferior olive. Binding in the hippocampus, thalamus, and hypothalamus generally was sparse (see figures 1 and 2). The choroid plexus, a site rich in 5-HT_2 receptors, had a high density of [^{125}I]DOI binding sites, but [^{3}H]ketanserin binding in this region was low (figure 2). [^{125}I]DOI binding on autoradiographs also displayed appropriate pharmacology for agonist-labeled 5-HT_2 receptors, in that it was inhibited by guanyl nucleotides but not adenosine triphosphate (Appel et al. 1990*a*; Battaglia et al. 1984; Lyon et al. 1987).

Such data allow speculation on possible circuits mediating hallucinogenic effects of drugs. For example, the serotonergic innervation of the brain is widespread and not uniform (Kosofsky and Molliver 1987; Steinbusch 1981). In particular, the cerebral cortex displays a distinctive, highly organized laminar pattern of innervation (for review, see Audet et al. 1989). Similarly, [^{125}I]DOI binding in the cerebral cortex has a laminar pattern. [^{125}I]DOI binding was especially enriched in layer IV in this study, with additional relative enrichment of binding sites also seen corresponding to layers I and V (see figure 1). On the other hand, [^{125}I]DOI binding in the claustrum was especially dense, but the serotonergic innervation of this brain region was not (Steinbusch 1981).

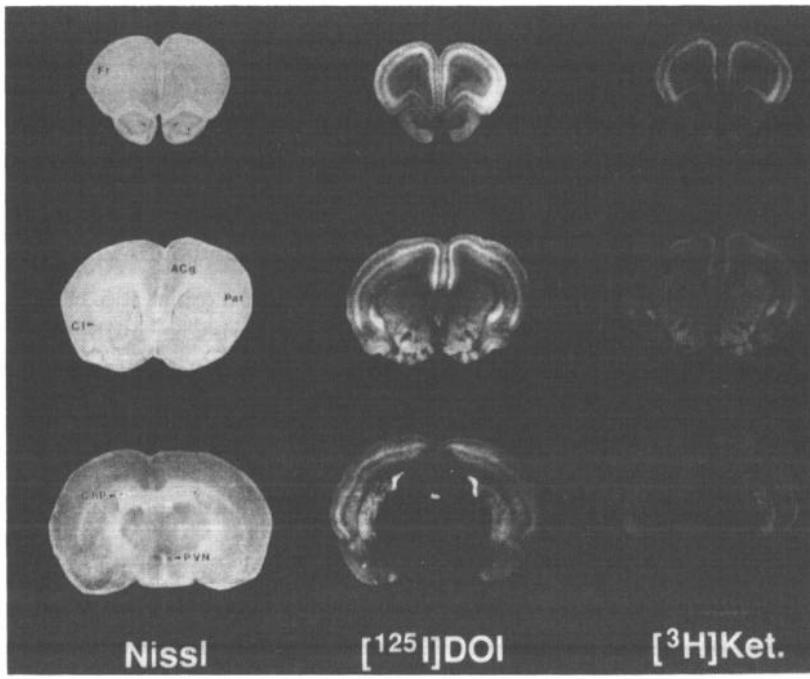

FIGURE 2. *Autoradiographs comparing the distribution of [^{125}I]DOI- and [^{3}H]ketanserin-labeled 5HT$_2$ receptors in coronal sections of rat brain. Left-hand column: Nissl-stained sections sampled adjacent to sections used for autoradiography and annotated to denote anatomical structures. Center and right-hand columns: Distribution of 5-HT$_2$ binding sites as revealed using [^{125}I]DOI and [^{3}H]ketanserin, respectively. These images were generated by using the autoradiographs (Ultrofilm ^{3}H) as photographic negatives; thus, lighter areas in individual autoradiographs correspond to brain regions displaying higher densities of binding. For [^{125}I]DOI autoradiographs, tissues were labeled with 200 pM [^{125}I]DOI. For [^{3}H]ketanserin autoradiographs, tissues were labeled with 2 nM [^{3}H]ketanserin.*

KEY: ACg = anterior cingulate cortex; ChP = choroid plexus; C1 = claustrum; Fr = frontal cortex; Par = parietal cortex; PVN = paraventricular nucleus.

SOURCE: Appel et al. 1990*a*

Similar enrichment of [^{3}H]ketanserin- and [^{125}I]-LSD-labeled 5-HT$_2$ receptors has been noted in the claustrum (Appel et al. 1990*a*; McKenna et al. 1989). McKenna and colleagues (1989) suggested that the claustrum may play an important, but as yet unrecognized, role in mediating the effects of hallucinogens via its extensive connections with the cerebral cortex and the limbic system (Pearson et al. 1982; Wilhite et al. 1986).

Relatively high densities of [^{125}I]DOI binding sites also have been noted in mammillary nuclei, which are richly innervated by serotonergic afferents (Steinbusch 1981). Similar to the claustrum, mammillary nuclei may play an important, but as yet unrecognized, role in mediating effects of hallucinogens via their extensive connections with the limbic system (Bleier and Byne 1985; MacLean 1985). Likewise, the ventral pallidum, which displays high levels of [^{125}I]DOI binding sites and is richly innervated by serotonergic afferents, may mediate effects of hallucinogenic 5-HT$_2$ agonists via its extensive connections with the limbic system and basal ganglia (Heimer et al. 1985; Steinbusch 1981).

The choroid plexus, a site rich in 5-HT$_2$ receptors, has a high density of [^{125}I]DOI binding sites (Appel et al. 1990*a*; McKenna et al. 1989). Competition studies on autoradiographs demonstrated that [^{125}I]DOI was binding at 5-HT$_2$ receptors (figure 3). Using [^{125}I]DOI autoradiography, the distribution of 5-HT^2 receptors now can be demonstrated in the brain outside of the choroid plexus (figure 4). In situ hybridization histochemistry studies have demonstrated that 5-HT$_2$ receptor messenger ribonucleic acid mRNA) is widespread in rat brain (Hoffman and Mezey 1989; Molineaux et al. 1989). Such observations take on added significance in the context of accumulating evidence that hallucinogenic drugs may be acting at 5-HT$_{1C}$ *receptors* (Burris et al.1991; Sanders-Bush, this volume; Sanders-Bush and Breeding 1991; Titeler et al. 1988).

IN VITRO RECEPTOR AUTORADIOGRAPHY TO STUDY EFFECTS OF HALLUCINOGENS ON STRUCTURAL INTEGRITY OF NEURONS

Receptor autoradiography need not be limited to studying neurotransmitter or drug binding sites. A candidate for receptor autoradiography essentially requires a saturable and specific binding at a biologically relevant site and the availability of a selective ligand with

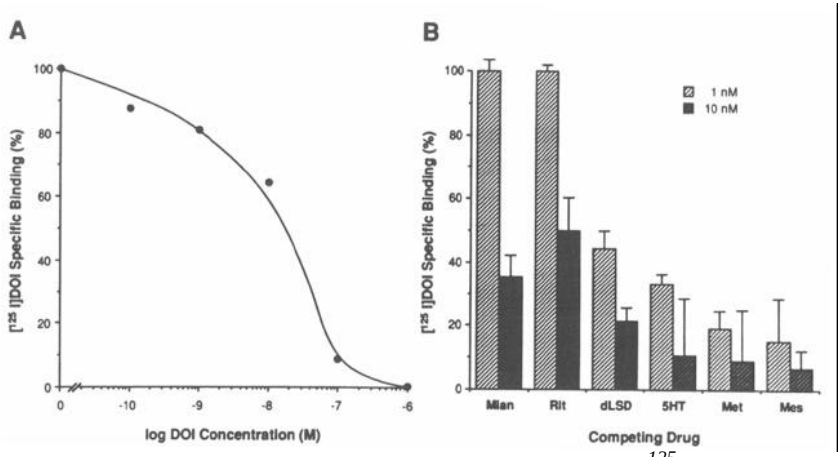

FIGURE 3. *Pharmacological characterization of [^{125}I]DOI binding to 5-HT$_{1C}$ receptors in rat choroid plexus. Slide-mounted sections (2/slide, 10 µm thick) of rat brain were labeled with 200 pM [^{125} I]DOI in the presence of spiperone (100 nM, to block [^{125}I]DOI binding to 5-HT$_2$ receptors) and varying concentrations of drugs. Nonspecific binding was determined in the presence of 1 µM mianserin. A: Displacement of [^{125}I]DOI binding to 5-HT$_{1C}$ receptors in rat choroid plexus by (±)DOI. Median effective concentration (IC$_{50}$) is approximately 12 nM. B: Potencies of various drugs that displace [^{125}I]DOI binding to 5-HT$_{1C}$ receptors in rat choroid plexus.*

SOURCE: Appel et al. 1990*a*

sufficient specific activity to facilitate its detection. To date, several cellular components in the brain have been studied using principles of receptor autoradiography. They include ion channels, neurotransmitter reuptake sites, second messenger molecules, metabolic enzymes, and hexose transporters, among others (Appel and De Souza, in press; Kuhar and De Souza 1989).

Detection of nonneurotransmitter binding sites by autoradiography can be used as an approach for studying the anatomic locus of structural or functional consequences of drug treatment. One approach is to assess changes in the density of presynaptic markers as an index of the neurotoxic potential of a drug. For example, in vitro assessment of

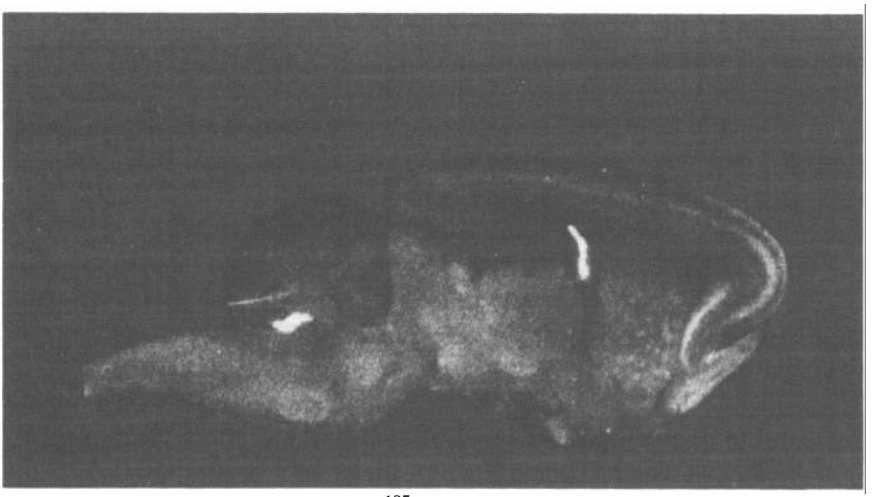

FIGURE 4. *Distribution of [^{125}I]DOI-labeled 5-HT$_{1C}$ receptors in a sagittal section of rat brain. The tissue was incubated in 200 pM [^{125}I]DOI in the presence of 100 nM spiperone to block [^{125}I]DOI binding to 5-HT$_2$ receptors. Under this condition the fractional occupancy of [^{125}I]DOI at 5-HT^2 receptors is <0.1 percent. This image was generated by using the autoradiograph (Ultrofilm ^{3}H) as a photographic negative; thus, lighter areas correspond to brain regions displaying higher densities of binding.*

monoamine uptake sites can be used as an index of brain monoamine neuron structural integrity following a drug treatment. The validity of this approach has been established (D'Amato et al. 1987; De Souza and Kuyatt 1987; Javitch et al. 1985). In vitro autoradiography of 5-HT uptake sites as labeled by [^{3}H]paroxetine can serve as an index of structural integrity of brain serotonergic axons following fenfluramine treatment. Serotonin uptake sites are highly concentrated on 5-HT-containing nerve terminals (Kuhar and Aghajanian 1973); thus, a decrease in the density of [^{3}H]paroxetine binding would reflect loss of 5-HT axons following drug treatment. By extension, the same approach can be used to examine drug effects on catecholamine axons by looking for alterations in density and distribution of catecholamine uptake sites as labeled by [^{3}H]mazindol.

The ring-substituted phelylalkylamines 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymeth-amphetamine (MDMA) have received much notoriety recently because of their use as recreational

drugs. (For a comprehensive review, see Asghar and De Souza 1989.) Although these compounds differ in structure by only a single methyl group, evidence suggests that, whereas MDA is hallucinogenic, MDMA is not (Glennon 1989; Nichols and Oberlender 1989). Evidence has been presented that MDA and MDMA are neurotoxic and cause diffuse and prolonged decreases in brain 5-HT markers (Battaglia et al. 1987; Commins et al. 1987; Insel et al. 1989; Ricuarte et al. 1985).

Effects of these compounds on serotonergic neurons have been assessed using immunohistochemistry (O'Hearn et al. 1988). Widespread loss of 5-HT-like immunoreactivity was observed in the cerebral cortex, hippocampus, caudate putamen, hypothalamus, and other forebrain areas. Decreases in immunostaining appeared to be more profound following MDA treatment than following MDMA treatment. In addition, some remaining 5-HT-like immunoreactive axons displayed morphology reminiscent of that seen following treatment with the 5-HT neurotoxin 5,7-dihydroxytryptamine. Such axons appeared swollen, fragmented, and intensely stained. They suggested also that, in particular, axons derived from the dorsal raphe nucleus, which are morphologically distinct from axons derived from the median raphe nucleus (Kosofsky and Molliver 1987), were selectively vulnerable to these drugs. Serotonin-like immunoreactive cell bodies did not appear to be affected. When treated tissues were immunostained for tyrosine hydroxylase, a marker of monoaminergic neurons, immunostaining appeared unaffected by treatment with either drug. O'Hearn and colleagues (1988) thus concluded that MDA, a purported hallucinogen, and MDMA were exerting neurotoxic effects on dorsal raphe-derived serotonergic axons.

Although these immunohistochemical data strongly supported the conclusion that MDA and MDMA were neurotoxic, a shortfall of interpreting immunohistochemical data is that detection techniques depend on the presence of the antigen (in this case, 5-HT). Thus, if drug treatment causes depletion of the antigen of interest, lack of or decreased immunostaining could mistakenly be interpreted as an actual loss of axons. Furthermore, immunohistochemistry is limited from the perspective that related changes in staining are difficult to assess quantitatively.

To address this question with respect to MDA and MDMA, [^{3}H]paroxetine autoradiography was used to assess the potential neurotoxic effects of these drugs by examining changes in a presynaptic marker, 5-HT uptake sites (Battaglia et al. 1991; De Souza and Kuyatt

1987). Both the hallucinogen MDA and MDMA caused widespread decreases in regional [^{3}H]paroxetine binding that correlated with loss of 5-HT-like immunoreactivity noted by O'Hearn and coworkers (1988).

Furthermore, regional decreases in [^{3}H]paroxetine binding in MDMA-treated rats were greater 2 weeks after, rather than immediately after, cessation of drug treatment. This suggested that there was a continuing degenerative process occurring during this time interval (Battaglia et al. 1991).

To confirm the neurochemical specificity of this effect, Battaglia and colleagues (199 1) performed parallel experiments using [^{3}H]mazindol to assess the structural integrity of catecholaminergic nerve terminals. [^{3}H]Mazindol is a marker for DA and norepinephrine (NE) uptake sites (Javitch et al. 1985). [^{3}H]Mazindol binding was unaffected by either MDA or MDMA treatment, confirming the specificity of the neurotoxic effect of these drugs to 5-HT monoaminergic neurons.

Such autoradiographic assays also have been used to investigate the neurotoxic potential of other drugs structurally derived from phenylalkylamines. Regional decreases in 5-HT-uptake site density have been noted following treatment with the CNS stimulant methamphetamine using [^{3}H]cyanoimipramine binding as a marker (Kovachich et al. 1989). Fenfluramine is a substituted phenylalkylamine derivative structurally similar to MDA and MDMA. Unlike methamphetamine, MDA, or MDMA, it is devoid of undesirable psychostimulant effects. Racemic fenfluramine and its dextrorotary (D) isomer are used therapeutically as anorectics to treat obesity (McTavish and Heel 1992; Rowland and Carlton 1986). Short-term administration of racemic fenfluramine at high doses results in a pattern of 5-HT immunoreactivity similar to that seen with MDA, suggesting neurotoxic effects of this drug on 5-HT axons using this treatment protocol (Appel et al. 1989; Molliver and Molliver 1990).

This hypothesis was supported by autoradiographic studies using [^{3}H]paroxetine and [^{3}H]mazindol as markers for 5-HT and catecholamine uptake sites, respectively (Appel et al. 1990*b*). Decreased paroxetine binding parallels decreases in 5-HT-like immunostained axons following an identical fenfluramine treatment protocol. Catechol-aminergic axons as assessed by [^{3}H]mazindol autoradiography were unaffected. These data suggest that as a class, phenylalkylamine derivatives may have increased potential for neurotoxic side effects regardless of their being

hallucinogenic and may reinforce the utility of autoradiographic approaches as a tool for studying such effects of drug treatment.

PRECLINICAL AND CLINICAL APPLICATIONS OF IN VIVO RECEPTOR AUTORADIOGRAPHY IN THE STUDY OF HALLUCINOGENS

As discussed earlier, receptor autoradiographic technique lends itself to being applied in vivo. The obvious advantage is that assessment can be made in situ in a living subject. Furthermore, using tracer drugs labeled with positron-emitting isotopes such as ^{11}C, ^{18}F, ^{15}O, and ^{13}N, dynamic mapping studies can be accomplished in animal or, potentially, human subjects using PET. In PET, the spatial distribution of positron-emitting isotopes in the brain or any other body region is detected using a circular array of detectors that surrounds the subject. Precise spatial localization is realized by accepting as the signal pairs of simultaneously emitted 511-kiloelectron volt (keV) gamma rays that are detected 180" apart from each other. The gamma rays are the result of positron annihilation that occurs when a positron interacts with a negatively charged electron to form a short-lived positronium that is subsequently converted into electromagnetic radiation in the form of two photons (gamma rays) (Frost 1990; Ter-Pogossian 1985). With high probability, the gamma rays represent the product of a single disintegrating positron. An obvious advantage of PET over postmortem (in vitro) receptor autoradiography is that it permits measurements several times from the same subject. Thus, dynamic studies during, as well as longitudinal studies following, hallucinogen administration are possible.

Several preconditions limit whether a drug is useful as an in vivo marker. As a minimum, the tracer drug cannot be toxic to the host. Other basic requirements summarized by Kung (1990) are that the radiotracer:

- Be labeled with a short-lived isotope and be quickly prepared (minutes);
- Possess high specific activity;
- Be able to penetrate the intact blood-brain barrier following intravenous (IV) injection;
- Be relatively resistant to in vivo metabolism;

- Have a high affinity for the binding site of interest (nM K_d);
- Possess a high target-to-nontarget ratio after IV injection; and
- Can be modeled to quantitate receptor density based on kinetic information obtained by imaging.

For the most part, in vivo studies performed with radiotracers in experimental animals and having the potential for improving understanding of hallucinogens have been focused not so much toward imaging binding sites in that setting as toward determining whether a particular tracer is of potential clinical use. For this purpose, regional binding under different experimental conditions is quantified by solubilizing dissected regions of the brain and counting bound radioactivity in a scintillation counter. As discussed earlier, ligands that label 5-HT_2 binding sites would theoretically label these binding sites in living brain and, by extension, identify initial sites of action of hallucinogenic drugs. Drugs tested for this use include derivatives of spiperone (spiroperidol) and ketanserin (Frost 1990; Kuhar et al. 1986). However, these drugs are 5-HT_2 receptor antagonists and do not label 5-HT_2 receptors exclusively (Leysen et al. 1987; Niznik et al. 1985; Stefanini et al. 1980). A tritiated naphthosultam derivative, RP 62203, also is being used as an in vivo probe for 5-HT_2 receptors. This drug is also a 5-HT_2 receptor antagonist but is reported to have greater 5-HT_2 receptor selectivity than spiperone or ketanserin (Fajolles et al. 1992).

Derivatives of LSD also have been used as in vivo probes for 5-HT_2 receptors. They include [^{125}I]-LSD (Hartig et al. 1985*b*), *N*1-methyl-2-[^{125}I]-LSD (Hoffman et al. 1987), and D-(+)-(N1-[^{11}C]methyl)-2-Br-LSD ([^{11}C]MBL) (Lever at al. 1989). [^{11}C]MBL has been used to image 5-HT_2 receptors in living baboon and human brains by PET (Wang et al. 1987). In the baboon brain, specific binding of [^{11}C]MBL to 5-HT_2 receptors in vivo was demonstrated by comparing binding ratios between the frontal cortex and cerebellum in the presence and absence of ketanserin. In a human subject, widespread uniform labeling of brain structures not associated with 5-HT_2 receptors, such as the cerebellum, was seen soon after administration of [^{11}C]MBL. However, this most likely reflected blood flow delivery of the tracer because persistent labeling was seen subsequently in the frontal cortex and temporal poles, sites endowed with 5-HT_2 receptors. No pharmacological effects of drug administration were noted in any subjects receiving [^{11}C]MBL. However, MBL may be an antagonist at 5-HT_2

receptors (Ginzel and Mayer-Gross 1956). As discussed earlier, theoretical arguments support using 5-HT_2 agonists as probes for sites of action of hallucinogens rather than anatagonists (Lyon et al. 1987; Teitler et al. 1990). One such compound under investigation is 1-[2',5'-di-methoxy-4'- (ß-fluoroethyl)phenyl]-2-aminopropane, a phenylalkylamine (Gerdes et al. 1988).

As discussed earlier, presynaptic markers are useful probes to assess structural integrity of neurons. By using this approach, the regional distribution of neurotoxic effects of the hallucinogenic phenylalkylamine MDA and its nonhallucinogenic chemical congener fenfluramine have been reported using radiotracers selective for serotonin uptake sites in vitro (Appel et al. 1990*b*; Battaglia et al. 1991). Radiotracers with in vivo selectivity for serotonin uptake sites also have been reported, including radiolabeled derivatives of paroxetine, quipazine, imipramine, chlorimipramine, cyanoimipramine, citalopram, fluoxetine, and sertralme. However, all these compounds have limitations that compromise their usefulness as radiotracers in a clinical setting (Scheffel et al. 1992).

Recently, radiolabeled congeners of another class of compound have appeared that label cocaine binding sites. For example, RTI-55 is a parasubstituted cocaine analog derived from WIN 35,065-2 that labels DA and serotonin uptake sites (Boja et al., 1992; Madras et al. 1989; Scheffel et al. 1992). In vivo studies in mice using [^{125}I]RTI-55 have yielded satisfactory autoradiographs demonstrating regional binding patterns that reflect the distribution of serotonin and DA uptake sites (Cline et al. 1992). By labeling 5-HT uptake sites in vivo with [^{125}I]RTI-55, investigators have been able to detect regional changes in densities of 5-HT uptake sites following fenfluramine treatment in rats (Scheffel et al. 1992). Fenfluramine effects on 5-HT uptake sites are similar to those seen following MDA treatment (Appel et al. 1990*b* Battaglia et al. 1991). These data suggest that RTI-55 might be useful in a clinical setting to assess potential long-lasting adverse effects on the CNS in individuals who may have been abusers of phenylalkylamine hallucinogens such as MDA.

AUTORADIOGRAPHIC ASSESSMENT OF IN VIVO FATTY ACID INCORPORATION TO STUDY HALLUCINOGENS OR OTHER DRUGS

In addition to its use in receptor or binding site mapping applications, the in vivo autoradiographic approach has been used to study brain metabolism. The best-characterized and most well-known example of this approach is metabolic mapping by assessing incorporation of [^{14}C]deoxyglucose (2-deoxy-D- [1-^{14}C]glucose). 2-Deoxyglucose is transported bidirectionally from blood to brain by the same hexose transport protein as glucose. Glucose is the primary metabolic substrate of the brain in the fed state. The functional activity of the brain in vivo has been correlated with regional cerebral glucose utilization and with regional cerebral blood flow. By assessing incorporation of [^{14}C]deoxyglucose into the brain, regional glucose consumption and, by extension, regional cerebral metabolic activity can be estimated (for a review, see Sokoloff 1985). Using this approach, Freo and colleagues (1991) observed decreased incorporation of [^{14}C]deoxyglucose into rat hippocampus and prosencephalic regions (cingulate; prefrontal; precentral medial; and motor, auditory, and visual cortices) following high (25 milligrams per kilogram [mg/kg]) intraperitoneal (IP) doses of DOI. Another in vivo autoradiographic metabolic mapping approach under development is assessment of local cerebral [^{14}C]leucine incorporation as an index of regional brain protein synthesis (Smith et al. 1984; Sokoloff 1985). However, this model may underestimate actual protein synthesis (Smith et al. 1988).

The Laboratory of Neurosciences of the National Institute on Aging has developed in vivo autoradiographic methods to quantify local cerebral rates of incorporation of radiolabeled long-chain fatty acids into brain phospholipids (DeGeorge et al. 1989; Rimes et al. 1983; Noronha et al. 1990). A partial model underlying this approach has been described (Robinson et al. 1992). The technique is based on infusing radiolabeled fatty acids, such as [^{3}H]palmitic, [^{14}C]arachidonic, or [^{14}C]docosa-hexaenoic acids, into conscious rats. Regional accumulated radioactivity in the brain and integrated plasma fatty acid radioactivity (integrated over the time course of the experiment) are determined. Currently, data are expressed as normalized regional activities (k) having units of milliliters per second times grams (ml/sec x g). However, exact quantification of ‘fatty acid incorporation will not be possible until the kinetics of protein binding of fatty acids and the contributions of different precursor pools

and metabolic pathways have been determined (Robinson et al. 1992). Nevertheless, the technique has proven useful in that regional brain changes in fatty acid incorporation can be correlated with alterations in regional structural integrity and functional activation.

When infused in vivo, [^{3}H]palmitic acid is incorporated into brain membrane phospholipids, with the majority incorporated into phosphatidylcholine. This allows its use as a marker for synthesis and turnover of this phospholipid and, by extension, as a marker for neuronal structural integrity (Gnaedinger et al. 1988; Noronha et al. 1990). Increased [^{14}C]palmitate or [^{3}H]palmitate incorporation has been demonstrated in regions where cell division or proliferation is taking place, such as in active tumors or in areas of glial reaction and phagocytic invasion of the brain having extensive cell death secondary to carotid occlusion (Nariai et al. 1991*a*; Tone et al. 1987). In contrast, regional brain [^{3}H]palmitate incorporation is not altered in response to chemical stimulation of neurotransmitter receptors (DeGeorge et al. 199 1; Freed et al. 1991).

When infused in vivo, [^{14}C]arachidonic acid is incorporated into brain phospholipids primarily as phosphatidylinositol and phosphatidylcholine. Turnover of arachidonate in brain inositol phosphoglycerides, which is catalyzed by phospholipase C, is a metabolic pathway used for signal transduction by several neurotransmitters, including alpha, adrenergic receptors, cholinergic muscarinic receptors, excitatory amino acid receptors, H_1 histaminergic receptors, and 5-HT_2 and 54-HT_{1C} serotonin receptors (Fisher and Agranoff 1987; Sanders-Bush et al. 1990). Thus, incorporation of in vivo administered [^{14}C]arachidonic acid could act as an index of regional functional activation in response to neurotransmitter, drug, or other stimulation whose transduction is coupled to phosphatidylinositol. [14C]Arachidonic acid incorporation is increased in response to muscarinic cholinergic receptor activation with arecoline. In contrast, [^{3}H]palmitate incorporation is unaffected (DeGeorge et al. 1991; Nariai et al. 1991*b*). Similarly, visual stimulation to unilateral enucleated rats resulted in increased [14C]arachidonic acid incorporation in brain structures afferent to the intact orbit when compared with incorporation in the brain structures afferent to the enucleated orbit. No differences between sides were noted for [^{3}H]palmitate incorporation (Wakabayashi et al. 1992). These data support the validity of using fatty acid incorporation as indices of structural integrity and functional activation of neurons.

The fatty acid incorporation technique also has been applied to studying effects on rats treated with hallucinogens. DOI (10 mg/kg IP) resulted in increased [^{14}C]arachidonic acid incorporation in frontal cortex layer IV, claustrum, medial cortex, and motor cortex. In contrast, [^{3}H]palmitic acid incorporation was unaffected in these brain regions assayed (Freed et al. 1991). These brain regions have high densities of 5-HT_2 serotonin receptors (Fishette et al. 1987; Pazos et al. 1985; Slater and Patel 1983) and are strongly labeled by [^{125}I]DOI, which binds both 5-HT_2 and 5-HT_{1C}, serotonin receptors (Appel et al. 1990*a*; McKenna et al. 1989). Activation of 5-HT_2 and 5-HT_{1C} serotonin receptors results in phosphatidylinositol turnover (Sanders-Bush et al. 1990).

Evidence has been presented that hallucinogens are likely acting at 5-HT_2 (Glennon and Rosecrans 1982; Glennon et al. 1984; Heym and Jacobs 1987, 1988; Titeler et al. 1988) and possibly 5-HT_{1C} serotonin receptors (Burris et al. 1991; Sanders-Bush, this volume; Sanders-Bush and Breeding 1991; Titeler et al. 1988). The Freed and colleagues (1991) data demonstrate brain regions that appear to be specifically activated in response to administration of a hallucinogen. Moreover, these data offer further evidence that fatty acid incorporation can be used to study receptors linked to brain lipid metabolism. This approach should be a powerful tool for studying functional aspects of hallucinogen administration.

The fatty acid technique also lends itself to a clinical setting. Fatty acids have been labeled with ^{11}C and detected using PET (Channing et al. 1991). Thus, in the future, it may be possible to study dynamic functional activation of human brain in response to hallucinogen administration to provide a better understanding of how these drugs produce their psychotropic effects.

SUMMARY

Autoradiography provides a powerful tool whereby an investigator can study different aspects of hallucinogens in the laboratory and the clinic. Receptor autoradiography can be performed in vitro to map binding sites of hallucinogens or to assess potential neurotoxic sequelae of hallucinogen treatments. Similarly, such studies can be performed in vivo to the same end. Receptor autoradiography can be performed in a clinical setting using PET to study acute dynamic binding properties of hallucinogens in humans or for long-term followup studies. In vivo

autoradiography of metabolic markers appears useful in the laboratory and potentially in the clinic to help researchers understand not only where, but also the manner in which, the brain responds functionally to hallucinogens.

REFERENCES

Altar, C.A.; Boyar, C.; and Marien, M.R. ^{125}I-LSD autoradiography confirms the prefrontal localization of caudate-putamen S_2 receptors to the caudal (peripallidal) region. *Brain Res* 372:130-136, 1986.

Altar, C.A.; Kim, H.; and Marshall, J.F. Computer imaging and analysis of dopamine (D_2) and serotonin (S_2) binding sites in rat basal ganglia or neocortex labeled by [^{3}H]spiroperidol. *J Pharmacol Exp Ther* 233:527-538, 1985.

Appel, N.M., and De Souza, E.B. Autoradiographic localization of nonreceptor proteins in brain. In: Stumpf, W.E., and Solomon, H.F., eds. *Autoradiography and Correlative Imaging.* New York: Academic Press, in press.

Appel, N.M.; Contrera, J.F.; and De Souza, E.B. Fenfluramine selectively and differentially decreases the density of serotonergic nerve terminals in rat brain: Evidence from immunocytochemical studies. *J Pharmacol Exp Ther* 249:928-943, 1989.

Appel, N.M.; Mathews, S.A.; Storti, G.M.; and Kuhar, M.J. Storage phosphors as an alternative to photographic emulsion for recording and quantifying autoradiograms. *Soc Neurosci Abstr* 17:373, 1991.

Appel, N.M.; Mitchell, W.M.; Garlick, R.K.; Glennon, R.A.; Teitler, M.; and De Souza, E.B. Autoradiographic characterization of [^{125}I]-l-(2,5-dimethoxy-4-iodophenyl)-2aminopropane (^{125}I]DOI) binding to 5-HT_2 and 5-HT_{1C} receptors in rat brain. *J Pharmacol Exp Ther* 255:843-857, 1990*a*.

Appel, N.M.; Mitchell, W.M.; Contrera, J.F.; and De Souza, E.B. Effects of high-dose fenfluramine treatment on monoamine uptake sites in rat brain: Assessment using quantitative autoradiography. *Synapse* 3:33-44, 1990*b*.

Asghar, K., and De Souza, E., eds. *Pharmacology and Toxicology of Amphetamine and Related Designer Drugs.* National Institute on Drug Abuse Research Monograph No. 94. DHHS Pub. No. (ADM)89-1640. Washington, DC: Supt. of Dots., U.S. Govt. Print. Off., 1989.

Audet, M.A.; Descarries, L.; and Doucet, G. Quantified regional and laminar distribution of the serotonin innervation in the anterior half of adult rat cerebral cortex. *J Chem Neuroanat* 2:29-44, 1989.

Battaglia, G.; Shannon, M.; and Titeler, M. Guanyl nucleotide and divalent cation regulation of cortical S_2 serotonin receptors. *J Neurochem* 43:1213-1219, 1984.

Battaglia, G.; Sharkey, J.; Kuhar, M.J.; and De Souza, E.B. Neuroanatomic specificity and time course of alterations in rat brain serotonergic pathways induced by MDMA (3,4-methylene-dioxymethamphetamine): Assessment using quantitative autoradiography. *Synapse* 8:249-260, 1991.

Battaglia, G.; Yeh, S.Y.; O'Hearn, E.; Molliver, M.E.; Kuhar, M.J.; and De Souza, E.B. 3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: Quantification of neurodegeneration by measurement of [^{3}H]paroxetine-labeled serotonin uptake sites. *J Pharmacol Exp Ther* 242:911-916, 1987.

Bleier, R., and Byne, W. Septum and hypothalamus. In: Paxinos, G., ed. *The Rat Nervous System.* Vol. 1. *Forebrain and Midbrain.* Sydney: Academic Press, 1985. pp. 87-118.

Boja, J.W.; Mitchell, W.M.; Patel, A.; Kopajtic, T.; Carroll, F.I.; Lewin, A.H.; and Kuhar, M.J. High affinity binding of [125I]RTI-55 to dopamine and serotonin transporters in rat brain. *Synapse* 12:27-36, 1992.

Burris, K.D.; Breeding, M.; and Sanders-Bush, E. (+)Lysergic acid diethylamide, but not its nonhallucinogenic congeners, is a potent serotonin 5-HT_{1C} receptor agonist. *J Pharmacol Exp Ther* 258:891-896, 1991.

Channing, M.A.; Freed, L.; Wakabayashi, S.; Carson, R.; Simpson, N.; Dunn, B.B.; and Rapoport, S.I. 1-[^{11}C]Labeled polyhomoallylic fatty acids: Phospholipid metabolic tracers for the brain. *Am J Nucl Med* 13:1093, 1991.

Cline, E.J.; Scheffel, U.; Boja, J.W.; Mitchell, W.M.; Carroll, F.I.; Abraham, P.; Lewin, A.H.; and Kuhar, M.J. In vivo binding of [^{125}I]RTI-55 to dopamine transporters: Pharmacology and regional distribution with autoradiography. *Synapse* 12:37-46,1992.

Commins, D.L.; Vosmer, G.; Viris, R.; Woolverton, W.; Schuster, C.R.; and Seiden, L.S. Biochemical and histological evidence that methylenedioxymethamphetamine (MDMA) is toxic to neurons in rat brain. *J Pharmacol Exp Ther* 241:338-345, 1987.

D'Amato, R.J.; Largent, B.L.; Snowman, A.M.; and Snyder, S.H. Selective labeling of serotonin uptake sites in rat brain by [^{3}H]citalopram contrasted to labeling of multiple sites by [^{3}H]imipramine. *J Pharmacol Exp Ther* 242:364-371, 1987.

DeGeorge, J. J.; Nariai, T.; Yamazaki, S.; Williams, W.M.; and Rapoport, S.I. Arecoline-stimulated brain incorporation of intravenously administered fatty acids in unanesthetized rats. *J Neurochem* 56:352-355, 1991.

DeGeorge, J.J.; Noronha, J.G.; Bell, J.; Robinson, P.; and Rapoport, S.I. Intravenous injection of [1-^{14}C]arachidonate to examine regional brain lipid metabolism in unanesthetized rats. *J Neurosci Res* 24:413-423, 1989.

De Souza, E.B. Serotonin and dopamine receptors in the rat pituitary gland: Autoradiographic identification, characterization, and localization. *Endocrinology* 119: 1534-1542, 1986.

De Souza, E.B., and Kuyatt, B.L. Autoradiographic localization of ^{3}H-paroxetine-labeled serotonin uptake sites in rat brain. *Synapse* 1:488-496, 1987.

Engel, G.; Müller-Schweinitzer, E.; and Palacios, J.M. 2-[^{125}Iodo]LSD, a new ligand for the characterization and localisation of 5-HT_2 receptors. *Naunyn-Schmiedebergs Arch Pharmacol* 325:328-336,1984.

Fajolles, C.; Boireau, A.; Ponchant, M.; and Laduron, P.M. [^{3}H]RP 62203, a ligand of choice to label in vivo brain 5-HT_2 receptors. *Eur J Pharmacol* 216:53-72, 1992.

Fisher, S.K., and Agranoff, B.W. Receptor activation and inositol lipid hydrolysis in neural tissues. *J Neurochem* 48:999-1017, 1987.

Fishette, C.T.; Nock, B.; and Renner, K. Effects of 5,7-dihydroxytryptamine on serotonin, and serotonin$_2$ receptors throughout the rat central nervous system using quantitative autoradiography. *Brain Res* 421:263-279, 1987.

Freed, L.M.; Nariai, T.; and Rapoport, S.I. Effect of 5-HT_2 receptor agonist on fatty acid incorporation into brain. *Tr Am Soc Neurochem* 22:189, 1991.

Freo, U.; Soncrant, T.T.; Holloway, H.W.; and Rapoport, S.I. Dose- and time-dependent effects of l-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a serotonergic 5-HT_2 receptor agonist, on local cerebral glucose metabolism in awake rats. *Brain Res* 541:63-69, 1991.

Frost, J.J. Imaging the serotonergic system by positron emission tomography. *Ann NY Acad Sci* 600:272-280, 1990.

Gerdes, J.M.; Mathis, C.A.; and Shulgin, A.T. Synthesis of 1-[2',5'-dimethoxy-4' (ß-fluoroethyl)phenyl]-2aminopropane: Studies related to F-labeled serotonin receptor ligands. *Tetrahed Lett* 29:6537-6540, 1988.

Ginzel, K.H., and Mayer-Gross, W. Prevention of psychological effects of d-lysergic acid diethylamide (LSD 25) by its 2-Brom derivative (BOL-148). *Nature* 178:210, 1956.

Glennon, R.A. Stimulus properties of hallucinogenic phenalkylamines and related designer drugs: Formulation of structure-activity relationships. In: Asghar, K., and De Souza, E., eds. *Pharmacology and Toxicology of Amphetamine and Related Designer Drugs.* National Institute on Drug Abuse Research Monograph No. 94. DHHS Pub. No. (ADM)89-1640. Washington, DC: Supt. of Docs., U.S. Govt. Print. Off., 1989. pp. 43-67.

Glennon, R.A., and Rosecrans, F.A. Indolealkylamine and phenalkylamine hallucinogens: A brief overview. *Neurosci Biobehav Rev* 6489-497, 1982.

Glennon, R.A.; Seggel, M.R.; Soine, W.H.; Herrick-Davis, K.; Lyon, R.A.; and Titeler, M. [^{125}I]-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane: An iodinated radioligand that specifically labels the agonist high-affinity state of 5-HT_2 serotonin receptors. *J Med Chem* 31:5-7, 1988.

Glennon, R.A.; Titeler, M.; and McKenney, J.D. Evidence for 5-HT_2 involvement in the mechanism of action of hallucinogenic agents. *Life Sci* 35:2505-2511, 1984.

Gnaedinger, J.M.; Miller, J.C.; Latker, C.H.; and Rapoport, S.I. Cerebral metabolism of plasma [^{14}C]palmitate in awake, adult rat: Subcellular localization. *Neurochem Res* 13:21-29, 1988.

Hartig, P.R.; Evans, M.J.; Krohn, A.M.; Leder, S.A.; Sze, P.C.; and Stoffers, D.A. [^{125}I]LSD binding to serotonin and dopamine receptors in bovine caudate membranes. *Neurochem Int* 7:699-707, 1985*a*.

Hartig, P.R.; Scheffel, U.; Frost, J.J.; and Wagner, H.N., Jr. In vivo binding of ^{125}I-LSD to serotonin 5-HT_2 receptors in mouse brain. *Life Sci* 37:657-664, 1985*b*.

Heimer, L.; Alheid, G.F.; and Zaborszky, L. Basal ganglia. In: Paxinos, *G.*, ed. *The Rat Nervous System.* Vol. 1. *Forebrain and Midbrain.* Sydney: Academic Press, 1985. pp. 37-86.

Heym, J., and Jacobs, B.L. Serotonergic mechanisms of hallucinogenic drug effects. *Monogr Neural Sci* 13:55-81, 1987.

Heym, J., and Jacobs, B.L. 5-HT_2 agonist'activity as a common action of hallucinogens. In: Rech, R.H., and Gudelsky, G.A., eds. *5-HT Agonists as Psychoactive Drugs.* Ann Arbor, MI: NPP Books, 1988. pp. 95-106.

Hoffman, B.J., and Mezey, E. Distribution of serotonin 5-HT_{1C} receptor mRNA in adult rat brain. *FEBS Lett* 247:453-462, 1989.

Hoffman, B.J.; Scheffel, U.; Lever, J.R.; Karpa, M.D.; and Hartig, P.R. *N*1-methyl-2-^{125}I-lysergic acid diethylamide, a preferred ligand for in vitro and in vivo characterization of serotonin receptors. *J Neurochem* 48:115-124, 1987.

Insel, T.R.; Battaglia, G.; Johannesson, J.; Marra, S.; and De Souza, E.B. 3,4-Methylenedioxymethamphetamine ("Ecstacy") selectively destorys brain serotonin terminals in rhesus monkeys. *J Pharmacol Exp Ther* 249:713-720, 1989.

Javitch, J.A.; Strittmatter, S.M.; and Snyder, S.H. Differential visualization of dopamine and norepinephrine uptake sites in rat brain using [^{3}H]mazindol autoradiography. *J Neurosci* 5: 1513-1521, 1985.

Johnson, M.P.; Hoffman, A.J.; Nichols, D.E.; and Mathis, C.A. Binding to the serotonin 5-HT_2 receptor by the enantiomers of ^{125}I-DOI. *Neuropharmacology* 26:1803-1806, 1987.

Kimes, A.S.; Sweeney, D.; London, E.D.; and Rapoport, S.I. Palmitate incorporation into different brain regions in the awake rat. *Brain Res* 274:291-301, 1983.

Kosofsky, B.E., and Molliver, M.E. The serotoninergic innervation of cerebral cortex: Different classes of axon terminals arise from dorsal and median raphe nuclei. *Synapse* 1:153-168, 1987.

Kovachich, G.B.; Aronson, C.E.; and Brunswick, D.J. Effects of high-dose methamphetamine administration on serotonin uptake sites in rat brain measured using [^{3}H]cyanoimipramine autoradiography. *Brain Res* 505:123-129, 1989.

Kuhar, M.J. Receptor localization with the microscope. In: Yamamura, H.I.; Enna, S.J.; and Kuhar, M.J., eds. *Neurotransmitter Receptor Binding.* New York: Raven Press, 1985. pp. 153-176.

Kuhar, M.J., and Aghajanian, G.K. Selective accumulation of ^{3}H-serotonin by nerve terminals of raphe neurons: An autoradiography study. *Nature New Biol* 241:187-189, 1973.

Kuhar, M.J., and De Souza, E.B. Autoradiographic imaging: Localization of binding sites other than neurotransmitter receptors. In: Ottoson, D., and Rostene, W., eds. *Visualization of Brain Functions.* New York: Stockton Press, 1989. pp. 57-66.

Kuhar, M.J., and Unnerstall, J.R. Receptor autoradiography. In: Yamamura, H.I.; Enna, S.J.; and Kuhar, M.J., eds. *Methods in Neurotransmitter Receptor Binding Analysis.* New York: Raven Press, 1990. pp. 177-218.

Kuhar, M.J.; De Souza, E.B.; and Unnerstall, J.R. Neurotransmitter receptor mapping by autoradiography and other methods. *Annu Rev Neurosci* 9:27-59, 1986.

Kuhar, M.J.; Lloyd, D.G.; Appel, N.; and Kuhar, M.J. Imaging receptors by autoradiography: Computer-assisted approaches. *J Chem Neuroanat* 4:319-327, 1991.

Kung, H.F. Radiopharmaceuticals for CNS receptor imaging with SPECT. *Int J Radiat Appl Instrum [B]* 7:85-92, 1990.

Lever, J.R.; Dannals, R.F.; Wilson, A.A.; Ravert, H.T.; Scheffel, U.; Hoffman, B.J.; Hartig, P.R.; Wong, D.F.; and Wagner, H.F., Jr. Synthesis and in vivo characterization of D-(+)-(N1-[^{11}C]methyl)-2-Br-LSD: A radioligand for positron emission tomographic studies of serotonin 5-HT$_2$ receptors. *Int J Radiat Appl Instrum [B]* 16:697-704, 1989.

Leysen, J.E.; Eens, A.; Gommeren, W.; Van Gompel, P.; Wynants, J.; and Janssen, P.A.J. Non-serotonergic [^{3}H]ketanserin binding sites in striata; membranes are associated with a dopac release system on dopaminergic nerve endings. *Eur J Pharmacol* 134:373-375, 1987.

Lyon, R.A.; Davis, K.H.; and Titeler, M. ^{3}H-DOB (4-bromo-2, 5-dimethoxyphenylisopropyl-amine) labels a guanyl nucleotide-sensitive state of cortical 5-HT$_2$ receptors. *J Pharmacol Exp Ther* 31:194-199, 1987.

MacLean, P.D. Fiber systems of the forebrain, In: Paxinos, G., ed. *The Rat Nervous System.* Vol. 1. *Forebrain and Midbrain. Sydney:* Academic Press, 1985. pp. 417-440.

Madras, B.K.; Spealman, R.D.; Fahey, M.A.; Neumeyer, J.L.; Saba, J.K.; and Millus, R.A. Cocaine receptors labeled by [^{3}H]2ß-carbomethoxy-3-(4-fluorophenyl)tropane. *Mol Pharmacol* 36:518-524, 1989.

McKenna, D.J., and Saavedra, J.M. Autoradiography of LSD and 2,5-dimethoxyphenyl-isopropylamine psychomimetics demonstrates regional, specific cross-displacement in the rat brain. *Eur J Pharmacol* 142:313-315, 1987.

McKenna, D. J.; Nazarali, A. J.; Hoffman, A.J.; Nichols, D.E.; Mathis, C.A.; and Saavedra, J.M. Common receptors for hallucinogens in rat brain: A comparative autoradiographic study using [^{125}I]-LSD and [^{125}I]DOI, a new psychomimetic radioligand. *Brain Res* 476:45-56, 1989.

McTavish, D., and Heel, R.C. Dexfenfluramine: A review of its pharmacological properties and therapeutic potential in obesity. *Drugs* 43:713-733, 1992.

Meibach, R.C.; Maayani, S.; and Green, J.P. Characterization and radioautography of [^{3}H]LSD binding by rat brain slices in vitro: The effect of 5-hydroxytryptamine. *Eur J Pharmacol* 67:371-382, 1980.

Molineaux, S.M.; Jessell, T.M.; Axel, R.; and Julius, D. 5-HT receptor is a prominent serotonin receptor subtype in the central nervous system. *Proc Natl Acad Sci U S A* 86:6793-6797, 1989.

Molliver, D.C., and Molliver, M.E. Anatomic evidence for a neurotoxic effect of (±)-fenfluramine upon serotonergic projections in the rat. *Brain Res* 511:165-168, 1990.

Nakada, M.T.; Wieczorek, C.M.; and Rainbow, T.C. Localization and characterization by quantitative autoradiography of [^{125}I]LSD binding sites in rat brain. *Neurosci Lett* 49:13-18, 1984.

Nariai, T.; DeGeorge, J.J.; Greig, N.H.; and Rapoport, S.I. In vivo incorporation of [9,10-^{3}H]-palmitate into a rat metastatic brain-tumor model. *J Neurosurg* 74:643-649, 1991*a*.

Nariai, T.; DeGeorge, J.J.; Lamour, Y. ; and Rapoport, S.I. In vivo brain incorporation of [1-^{14}C]arachidonate in awake rats, with or without cholinergic stimulation, following unilateral lesioning of nucleus basalis magnocellularis. *Bruin Res* 559:1-9, 1991*b*.

Nichols, D.E., and Oberlender, R. Structure-activity relationships of MDMA-like substances. In: Asghar, K., and De Souza, E., eds. *Pharmucology and Toxicology of Amphetamine and Related Designer Drugs.* National Institute on Drug Abuse Research Monograph No. 94. DHHS Pub. No. (ADM)89-1640. Washington, DC: Supt. of Docs., U.S. Govt. Print. Off., 1989. pp. l-29.

Niznik, H.B.; Grigoriadis, D.E.; Pri-Bar, I.; Buchman, O.; and Seeman, P. Dopamine D_2 receptors selectively labeled by a benzamine neuroleptic: [^{3}H]-TM-09151-2. *Naunyn-Schmiedebergs Arch Pharmacol* 329:333-343, 1985.

Noronha, J.G.; Bell, J.M.; and Rapoport, S.I. Quantitative brain autoradiography of [9,10-^{3}H]palmitic acid incorporation into brain lipids. *J Neurosci Res 26:* 196-208, 1990.

O'Hearn, E.; Battaglia, G.; De Souza, E.B.; Kuhar, M.J.; and Molliver, M.E. Methylenedioxyamphetamine (MDA) and methylenedioxy-methamphetamine (MDMA) cause ablation of serotonergic axon terminals in forebrain: Immunohistochemical evidence. *J Neurosci* 8:2788-2803, 1988.

Palacios, J.M.; Niehoff, D.L.; and Kuhar, M.J. [^{3}H]Spiperone binding sites in brain: Autoradiographic localization of multiple receptors. *Brain Res* 213:277-289, 1981.

Pazos, A.; Cortés, R.; and Palacios, J.M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res* 346:231-249, 1985.

Pearson, R.C.A.; Brodal, P.; Gatter, K.C.; and Powell, T.P.S. The organization of the connections between the cortex and the claustrum in the monkey. *Bruin Res* 234:435-441, 1982.

Peroutka, S.J., and Snyder, S.H. Multiple serotonin receptors: Differential binding of [^{3}H]5-hydroxytryptamine, [^{3}H]lysergic acid diethylamide and [^{3}H]spiroperidol. *Mol Pharmacol* 16:687-699, 1979.

Ricuarte, G.; Bryan, G.; Strauss, L.; Seiden, L.S.; and Schuster, C.R. Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. *Science* 229:153-163, 1985.

Robinson, P.J.; Noronha, J.; DeGeorge, J.J.; Freed, L.M.; Nariai, T.; and Rapoport, S.I. A quantitative method for measuring regional in vivo fatty acid incorporation into and turnover within brain phospholipids: Review and critical analysis. *Brain Res Rev* 17:187-214, 1992.

Rowland, N.E., and Carlton, J. Neurobiology of an anorectic drug: Fenfluramine. *Prog Neurobiol* 27:13-62,1986.

Sanders-Bush, E., and Breeding, M. Choroid plexus epithelial cells in primary culture: A model of 5-HT_{1C} receptor activation by hallucinogenic drugs. *Psychopharmacology* 105:340-346, 1991.

Sanders-Bush, E.; Tsutsumi, M.; and Burris, K.D. Serotonin receptors and phosphatidylinositol turnover. *Ann NY Acad Sci* 600:224-236, 1990.

Scheffel, U.; Dannals, R.F.; Cline, E.J.; Ricuarte, G.A.; Carroll, F.I.; Abraham, P.; Lewin, A.H.; and Kuhar, M.J. [$^{123/125}$I]RTI-55, an in vivo label for the serotonin transporter. *Synapse* 11: 134-139, 1992.

Slater, P., and Patel, S. Autoradiographic distribution of serotonin, receptors in rat brain. *Eur J Pharmacol* 92:297-298, 1983.

Smith, C.B.; Crane, A.M.; Kadekara, M.; Agranoff, B.W.; and Sokoloff, L. Stimulation of protein synthesis and glucose utilization in the hypoglossal nucleus induced by axotomy. *J Neurosci* 4:2489-2496, 1984.

Smith, C.B.; Deibler, G.E.; Eng, N.; Schmidt, K.; and Sokoloff, L. Measurement of local cerebral protein synthesis in vivo: Influence of recycling of amino acids derived from protein degradation. *Proc Natl Acad Sci USA* 85:9341-9345, 1988.

Sokoloff, L. Application of quantitative autoradiography to the measurement of biochemical processes in vivo. In: Reivich, M., and Alavi, A., eds. *Positron Emission Tomography.* New York: Alan R. Liss, 1985. pp. 1-42.

Stefanini, E.; Marchisio, A.M.; Devoto, P.; Vernaleone, F.; Collu, R.; and Spano, P.F. Sodium-dependent interaction of benzamides with dopamine receptors. *Brain Res* 198:229-233, 1980.

Steinbusch, H.W.M. Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6:557-618, 1981.

Teitler, M.; Leonhardt, S.; Weisberg, E.L.; and Hoffman, B.J. 4-[^{125}I]Iodo-(2,5-dimethoxy)phenylisopropylamine and [^{3}H]ketanserin labeling of 5-hydroxytryptamine$_2$ (5-HT$_2$) receptors in mammalian cells transfected with a rat 5-HT$_2$ cDNA: Evidence for multiple states and not multiple 5HT$_2$ receptor subtypes. *Mol Pharmacol* 38:594-598, 1990.

Ter-Pogossian, M.M. Positron emission tomography instrumentation. In: Reivich, M., and Alavi, A., eds. *Positron Emission Tomography. New* York: Alan R. Liss, 1985. pp. 43-61.

Titeler, M.; Lyon, R.A.; and Glennon, R.A. Radioligand binding evidence implicates the brain 5-HT$_2$ receptor subtype as a site of action for LSD and phenylisoproplyamine hallucinogens. *Psychopharmacology* 94:213-216, 1988.

Tone, 0.; Miller, J.C.; Bell, J.M.; and Rapoport, S.I. Regional cerebral palmitate incorporation following transient bilateral carotid artery occlusion in awake gerbils. *Stroke* 18:1120-1127, 1987.

Wakabayashi, S.; Freed, L.M.; Bell, J.M.; and Rapoport, S.I. Fatty acid incorporation into brain lipids after enucleation in the rat. *Tr Am Soc Neurochem* 23:147, 1992.

Wilhite, B.L.; Teyler, T.J.; and Hendricks, C. Functional relations of the rodent claustral-entorhinal-hippocampal system. *Brain Res* 365:54-60, 1986.

Wong, D.F.; Lever, J.R.; Hartig, P.R.; Dannals, R.F.; Villemagne, V.; Hoffman, B.J.; Wilson, A.A.; Ravert, H.T.; Links, J.M.; Scheffel, U.; and Wagner, H.N., Jr. Localization of serotonin 5-HT$_2$ receptors in living human brain by positron emission tomography using N1-([^{11}C]-methyl)-2-Br-LSD. *Synapse* 1:393-398, 1987.

AUTHOR

Nathan M. Appel, Ph.D.
Biologist
Division of Research and Testing
Office of Research Resources
Center for Drug Evaluation and Research
Food and Drug Administration
8301 Muirkirk Road
Laurel, MD 20708