Hallucinogenic drugs, such as 5-lysergic acid diethylamide (LSD), produce profound changes in consciousness and perception in humans; these effects are primarily responsible for their abuse potential. Understanding the neuronal substrates that mediate the effects of a drug of abuse is important for developing strategies for treatment and intervention. It is well established that the hallucinogenic effects of LSD and other hallucinogens are mediated via actions at 5-hydroxytryptamine (serotonin) (5-HT)2A receptors. Evidence for this comes primarily from studies in animals using the drug-discriminative paradigm. 5-HT2A receptor agonists substitute for hallucinogens (Glennon et al., 1984; Callahan and Appel, 1988; Winter and Rabin, 1988), a significant correlation exists between the binding of hallucinogens at cortical 5-HT2 receptors and their potency in drug discrimination (Glennon et al., 1984), and 5-HT2 receptor antagonists block the discriminative-stimulus effects of hallucinogenic drugs (Colpaert et al., 1985; Glennon and Hauck, 1985; Cunningham and Appel, 1987; Meert et al., 1989). More recent behavioral studies using selective 5-HT2A antagonists support a pivotal role for the 5-HT2A receptor in hallucinogen drug discrimination (Ismaiel et al., 1993; Schreiber et al., 1994; Fiorella et al., 1995b). Furthermore, studies in humans suggest that most of the subjective hallucinogenic properties of psilocybin, an indoleamine hallucinogen, are blocked by the 5-HT2A receptor antagonist ketanserin (Vollenweider et al., 1997, 1998). Thus, 5-HT2A receptor activation is proposed to

**ABSTRACT**

d-Lysergic acid diethylamide (LSD), an indoleamine hallucinogen, produces profound alterations in mood, thought, and perception in humans. The brain site(s) that mediates the effects of LSD is currently unknown. In this study, we combine the drug discrimination paradigm with intracerebral microinjections to investigate the anatomical localization of the discriminative stimulus properties of LSD in rats. Based on our previous findings, we targeted the anterior cingulate cortex (ACC) to test its involvement in mediating the discriminative stimulus properties of LSD. Rats were trained to discriminate systemically administered LSD (0.085 mg/kg s.c.) from saline. Following acquisition of the discrimination, bilateral cannulae were implanted into the ACC (AP, +1.2 mm; ML, ±1.0 mm; DV, −2.0 mm relative to bregma). Rats were tested for their ability to discriminate varying doses of locally infused LSD (0.1875, 0.375, and 0.75 μg/side) or artificial cerebrospinal fluid (n = 3–7). LSD locally infused into ACC dose-dependently substituted for systemically administered LSD, with 0.75 μg/side LSD substituting completely (89% correct). Systemic administration of the selective 5-hydroxytryptamine (serotonin) (5-HT)2A receptor antagonist R-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)-ethyl]-4-piperidine-methanol (M100907; 0.4 mg/kg) blocked the discriminative cue of LSD (0.375 μg/side) infused into ACC (from 68 to 16% drug lever responding). Furthermore, M100907 (0.5 μg/μl/side) locally infused into ACC completely blocked the stimulus effects of systemic LSD (0.04 mg/kg; from 80 to 12% on the LSD lever). Taken together, these data indicate that 5-HT2A receptors in the ACC are a primary target mediating the discriminative stimulus properties of LSD.
mediate the behavioral and subjective effects of LSD and other hallucinogens.

However, the precise neuronal circuitry involved in the hallucinogenic drug action is less certain. Earlier studies using microinjections to identify brain regions that may be involved in the actions of LSD have suggested a role for the nucleus accumbens and dorsal raphe nucleus (Minnema et al., 1980; Nielsen and Scheel-Kruger, 1986). However, these studies used a relatively high dose of LSD, and none applied antagonists to identify the serotonin receptor that mediated the effect of LSD. Our group, using c-fos as a biomarker for neuronal activation, reported an increase in c-fos protein expression in anterior cingulate cortex (ACC), medial prefrontal cortex, and amygdala after an acute dose of LSD (Gresch et al., 2002). In addition, we observed that the behavioral tolerance to repeated LSD administration, as measured by an attenuation of the discriminative stimulus, is associated with decreased 5-HT \textsubscript{2A} receptor signaling and receptor density in the medial prefrontal cortex and ACC (Gresch et al., 2005). Based on these findings and studies indicating the ACC influences many functions, including integration of sensory input to modulate complex behaviors, the current study targeted the ACC to test the involvement of this brain region in mediating the discriminative-stimulus properties of LSD. Experiments were designed to evaluate the ability of LSD administered directly into the ACC to produce a discriminative stimulus that substitutes for the systemic LSD discriminative stimulus and to determine whether the discriminative stimulus produced by systemic LSD is mediated by 5-HT \textsubscript{2A} receptors within the ACC.

**Materials and Methods**

**Animals.** Adult male Sprague-Dawley rats (225–249 g; Harlan, Indianapolis, IN) were individually housed and food-deprived to 85% of their free-feeding weight 1 week before the beginning of the drug discrimination experiments. All animals had continuous access to water except during training, and they were given enough food immediately following training and on weekends to maintain their weights at 85% of their expected nondeprived weights. Rats were maintained in a colony room (ambient temperature 22–23°C, 12:12-h light/dark cycle). All animal use procedures were in strict accordance with the National Institutes of Health Guide to the Care and Use of Laboratory Animals and were approved by Vanderbilt University Animal Care Committee.

**Materials.** M100907 was a gift from Merrill Dow (Cincinnati, OH). LSD was obtained from the National Institute on Drug Abuse (Bethesda, MD). WAY-100635 was purchased from Tocris Cookson (Bristol, UK). LSD was obtained from the National Institute on Drug Abuse (Bethesda, MD). WAY-100635 was purchased from Tocris Cookson (Bristol, UK). M100907 was a gift from Merrill Dow (Cincinnati, OH). WAY-100635 was purchased from Tocris Cookson (Bristol, UK). M100907 was a gift from Merrill Dow (Cincinnati, OH).

**Apparatus.** Six commercially available operant conditioning chambers (BRS/LVE model RTC-024; BRS/LVE, Beltsville, MD), each housed in a sound-attenuated chamber, were used. The operant chambers were equipped with two response levers, a liquid dipper centered between the two levers, and a house light. The equipment and experimental parameters were programmed using MED Associates software and controlled by MED Associates interface (MED Associates, St. Albans, VT) and MS-DOS-compatible computers.

**Drug Discrimination Training.** Rats \((n = 60)\) were shaped to lever-press for food reinforcement (Borden's condensed milk diluted 1:1 with tap water) on an fixed ratio (FR) \(1\) schedule of reinforcement during daily 20-min sessions given Monday through Friday. After shaping to lever-press, the reinforcement contingency was changed to a variable interval (VI) \(15\)-s schedule of reinforcement with a 15-s time-out for incorrect responses. The time-out contingency, a 15-s period following incorrect responses during which no responses were reinforced, served to punish incorrect responses. At this point, discrimination training began. The VI schedule was changed to a VI 30-s schedule of reinforcement at the end of the first week of training, where it remained for the duration of the experiment. On alternate days, the rats were injected subcutaneously 30 min before training with either 0.085 mg/kg LSD or saline. For half of the rats, responding on the right lever was LSD-correct and responding on the left lever was saline-correct; for the remainder of the rats, the reverse was true. Discrimination learning was monitored twice weekly by calculating the percentage of correct lever responses (number of correct responses/total number of responses) during 2.5-min extinction test sessions given at the beginning of the training session. During the remaining 17.5 min of the training session, the VI 30-s schedule was in effect. Training continued until the rats were averaging 85% correct or greater during the 2.5-min extinction sessions for both LSD and saline.

For the animal’s choice behavior to be included in the results, the rat was required to make a minimum of five responses. Thus, the length of the test session for all the experiments described below was 5 min in contrast to the 2.5-min extinction session used to monitor acquisition of the discrimination. The additional time is particularly important when the treatment conditions produce response suppression.

**Dose-Response Curve.** Following acquisition of the LSD-saline discrimination, a dose-response function was determined for LSD during 5-min extinction tests. For this experiment, 60 rats were assigned to one of five groups \((n = 12)\). Rats were injected with LSD \((0.01, 0.02, 0.043, and 0.085\ mg/kg s.c.) or saline 30 min before testing. At the end of the 5-min extinction test, rats were returned to their home cages.

**Antagonist Studies.** To determine the role of the 5-HT \textsubscript{2A}, 5-HT \textsubscript{2C}, and 5-HT \textsubscript{1A} receptors in mediating the discriminative-stimulus effects of LSD, animals were tested for their ability to discriminate LSD following pretreatment with M100907, a selective 5-HT \textsubscript{2A} receptor antagonist; ketanserin and mianserin, selective 5-HT \textsubscript{1A} antagonists; SB260553, a selective 5-HT \textsubscript{2C} antagonist; and WAY-100635, a selective 5-HT \textsubscript{1A} receptor antagonist. For the M100907 experiment, animals were divided into four groups \((n = 12)\), and they were injected with one of three doses of M100907 \((0.025, 0.1, and 0.4\ mg/kg s.c.) or saline 30 min before testing. Thirty minutes before testing, all rats were injected with 0.06 mg/kg LSD s.c. The test dose of LSD \((0.06\ mg/kg) was a submaximal dose, thus ensuring sensitivity to even subtle effects produced by the various antagonists. At the end of the pretreatment interval, animals were placed in the operant conditioning chamber and given 5-min extinction tests. Following testing, all animals were returned to their home cages.

After a week of retraining, mianserin was tested for its ability to block the discriminative stimulus effects of LSD. Rats were assigned to one of four groups \((n = 11)\) and pretreated with mianserin \((0.125, 0.25, and 0.5\ mg/kg s.c.) or saline 30 min before testing. Fifty minutes subsequent to mianserin administration, animals received 0.06 mg/kg LSD s.c. Thirty minutes after LSD injections, the rats were tested during 5-min extinctions sessions. At the end of the test session, all animals were returned to their home cages.

The same procedure was used to test ketanserin, SB260553, and WAY-100635. For each experiment, animals were assigned to one of four groups \((n = 11 or 12)\), and they were pretreated with three doses of drug or saline. Ketanserin \((0.1, 0.3, and 1.0\ mg/kg s.c.) was administered 60 min before testing, SB260553 \((1.2, and 8\ mg/kg s.c.) was administered 60 min before testing, and WAY-100635 \((0.1, 0.3, and 1.0\ mg/kg s.c.) was administered 60 min before testing. LSD \((0.06\ mg/kg s.c.) was always administered 30 min before testing.

**Stereoxic Surgery.** Animals were anesthetized with ketamine and xylazine, and they were placed in a stereotaxic apparatus. Bilateral 26-gauge guide cannulae (Plastics One, Roanoke, VA) were surgically implanted for the injection cannulae to target the ACC.
(AP, +1.2 mm and ML, (1.0 mm relative to bregma and DV, −2.0 mm relative to dura; Paxinos and Watson, 1986). After 5 to 7 days of recovery, discrimination training and behavioral testing resumed.

Drug Infusions and Testing Sessions. After recovery from surgery, the animals were retrained and tested on the LSD-saline discrimination to confirm that the discrimination was still intact. Once retraining was complete (1–2 weeks), microinjections of LSD, M100907, or aCSF in a volume of 1 μl was administered through a 33-gauge internal cannula extending 1 mm below the tip of the guide cannula using a microsyringe and hand-driven micromanipulator. Drugs were simultaneously injected bilaterally into the ACC over a 1-min period, and the cannula was left in place for an additional 2 min. Immediately after removal of the cannula, the animals were placed in the operant chamber and given 5-min extinction tests. Animals were habituated to the injection procedure before the onset of the experiment.

To determine whether intra-ACC infusions of LSD would substitute for systemic LSD, LSD was locally infused into the ACC once per week with systemic training being given on the remaining 4 days. All tests were conducted on a Friday, which allowed the animals the weekend to recover before resuming training on Monday. Tests were run 2 min following local infusions and consisted of 5-min extinction sessions at the end of which the animals were returned to their home cages. During their weekly training, the rats were given 2.5-min extinction tests to confirm that they were still discriminating both cues at 85% correct or greater. The order for local infusion of LSD test doses was aCSF, 0.75, 0.375, and 0.1875 μg/μl/side LSD.

To determine whether systemically administered M100907 would block intra-ACC infused LSD discrimination, rats were tested for their ability to discriminate locally infused LSD following s.c. pretreatment with M100907. This experiment was conducted over 4 weeks. On week 1, rats received saline (1 ml/kg s.c.) 20 min before local infusion of 1 μl of aCSF. For the second week, rats received saline (1 ml/kg s.c.) 20 min before local infusion with 0.375 μg/μl/side LSD. The third week, the 5-HT2A receptor antagonist M100907 (0.4 mg/kg s.c.) was injected 20 min before local infusion with 0.375 μg/μl/side LSD. On the fourth week, rats were retested for their ability to discriminate local infusion of 0.375 μg/μl/side LSD. The fourth experiment was to confirm that the reduction in drug lever selection observed in week 3 was due to direct antagonism of the 5-HT2A receptors and not the result of a loss of tissue or cannula viability. Each week during the experiment, rats received 4 days of training with testing being given on Friday. All tests were 5-min extinction sessions.

To determine whether intra-ACC infusions of M100907 would block discrimination of systemically administered LSD, animals were tested for their ability to discriminate s.c. LSD in the presence and absence of locally infused M100907. These experiments were conducted over 3 weeks. During week 1, rats received intra-ACC infusion of aCSF 20 min before 0.04 mg/kg LSD. During week 2, animals were given local infusions of M100907 (0.5 μg/μl/side) 20 min before s.c. injections of 0.04 mg/kg LSD. During week 3, the week 1 experiment was repeated (intra-ACC infusion of aCSF 20 min before 0.04 mg/kg LSD s.c.) to evaluate possible changes in tissue and cannula viability. Rats were tested on Friday during 5-min extinction sessions and received training from Monday to Thursday.

Histological Analysis. After completion of the study, rats were sacrificed. Their brains were removed, sectioned, mounted on microscope slides, and stained. The placement of the cannulae was verified with reference to the coordinates from the atlas of Paxinos and Watson (1986). A schematic diagram of the extent of bilateral cannulation placement for ACC infusions is shown in Fig. 1A.

Statistical Analysis. The data were analyzed using a one-way analysis of variance. Level of significance for the analysis of variance was set at p < 0.05. Contribution of individual group means to the overall significant F value was determined by Tukey’s least significant difference post hoc test (p < 0.05). Statistical analyses were preformed with SPSS 7.0 software (SPSS Inc., Chicago, IL).

Results

Discrimination Training and Dose-Response Curve. The initial training dose of LSD was gradually incremented over the first 26 days of training from 0.06 to 0.085 mg/kg LSD. The rats were averaging 85% or greater correct lever pressing during the 2.5-min extinction sessions for both training cues by day 31 of training. After acquisition of the discrimination, a dose-response curve was determined by testing several doses of LSD (0.085, 0.043, 0.02, and 0.01 mg/kg s.c.) and saline (Fig. 2). As can be seen, percent of choice of LSD lever was dose-dependent [F(4,50) = 47.4; p < 0.0001]. All doses except for the lowest dose of LSD tested were significantly different from saline (p < 0.05; Tukey’s test). Response rates varied significantly as a function of different doses of LSD [F(4,50) = 4.6; p < 0.0037]. Rate of responding was significantly higher following 0.01 and 0.02 mg/kg LSD than at the other doses of LSD tested or than saline (p < 0.05; Tukey’s test).

Systemic Pretreatment with 5-HT2A Receptor Antagonists Blocks the LSD-Mediated Discriminative Stimulus. To determine the role of various 5-HT receptors in mediating the discriminative stimulus effects of LSD, rats were pretreated with increasing doses of M100907, a selective 5-HT2A receptor antagonist; SB206553, a 5-HT2A,2C receptor antagonist; WAY-100635, a 5HT1A receptor antagonist; and mianserin and ketanserin, mixed 5-HT1 receptor antagonists followed by 0.06 mg/kg LSD s.c. M100907
Fig. 2. LSD dose-response curve. The data represent mean percent of responding ± S.E.M. (n = 12) on the LSD lever (top) and mean number of responses ± S.E.M. made during 5-min test session (bottom) as a function of various doses of LSD. *, top, indicates LSD-treated groups whose choice behavior was significantly different from vehicle controls (p < 0.05; Tukey’s test); **, bottom, indicates rate of responding was significantly different from other doses of LSD tested or from saline (p < 0.05; Tukey’s test).

\[ F(3,40) = 31.7; p < 0.0001 \], ketanserin \[ F(3,40) = 21.5; p < 0.0001 \], and mianserin \[ F(3,40) = 12.2; p < 0.0001 \] significantly reduced LSD lever selection compared with saline, whereas SB206553 and WAY-100635 did not significantly alter LSD lever selection, confirming a major role for the 5-HT\(_{2A}\) receptor.

**Intra-ACC Injection of LSD Substitution for Systemically Administered LSD Is Dose-Dependent.** Intra-ACC infusion of 0.1875, 0.375, and 0.75 \(\mu g/\)side of LSD produced 41.0 ± 15.5, 73.5 ± 6.0, and 88.7 ± 4.6% responding on the LSD lever \[ F(3,17) = 36.1; p < 0.0001 \] (Fig. 4). The dose of 0.75 \(\mu g/\)side of LSD injected into the ACC substituted fully for systemically administered LSD, whereas aCSF injection elicited only 14% responding on the LSD lever. A dose of 0.29 \(\mu g/\)side produces 50% LSD lever selection (based on linear regression analysis). Response rates did not differ significantly over the course of the dose-response curve \[ F(3,17) = 0.347; p = 0.792 \] (Fig. 4, bottom). LSD (0.75 \(\mu g/\)side; \( n = 4 \)) injected into brain sites outside of ACC was ineffective in substituting for systemically administered LSD (Fig. 1B), eliciting a mean of 24.3 ± 6.7% responding on the LSD lever.

Systemic M100907 Pretreatment Blocks the Substitution Produced by Intra-ACC LSD Injection. Administration of the 5-HT\(_{2A}\) receptor antagonist M100907 (0.4 mg/kg s.c.) 20 min before LSD infusion into the ACC blocked the ability of LSD (0.375 \(\mu g/\)side) locally infused into the ACC to substitute for systemic LSD \[ F(3,26) = 38.7; p < 0.0001 \] (Fig. 5). Systemic M100907 reduced LSD lever responding by 52% (from 68 to 16%; \( p < 0.001 \)). A week later, the animals were pretreated with saline and retested with the same dose of LSD (0.375 \(\mu g/\)side). The percent responding on the LSD lever was similar to that observed previously (74 compared with 68%), indicating that the discrimination was still intact and confirming that the effects of M100907 were specific and not due to cannula malfunction or loss of ACC tissue viability. Rate of responding did not differ significantly between the test conditions \[ F(3,26) = 0.772; p < 0.520 \] (Fig. 5, bottom).

**Intra-ACC Injection of M100907 Blocks Systemic LSD Substitution.** Local infusion of the 5-HT\(_{2A}\) receptor antagonist M100907 (0.5 \(\mu g/\)side) 20 min before systemic administration of LSD (0.04 mg/kg s.c.) blocked the discriminative stimulus effects of systemically administered LSD \[ F(2,11) = 37.3; p < 0.0001 \] (Fig. 6). Local infusion of M100907 reduced LSD lever responding from 80 to 12% (\( p < 0.001 \); Tukey’s test). When the animals were retested with 0.04 mg/kg s.c. LSD, percent of LSD lever selection was similar to that which occurred before local infusions of LSD.
and the 5-HT$_2$ receptor antagonists ketanserin and mianserin completely blocked the LSD-induced discriminative stimulus, whereas the 5-HT$_{1A}$ antagonist WAY-100635 and 5-HT$_{2B/2C}$ receptor antagonist SB206553 had no effect on choice behavior. This finding is in agreement with reports demonstrating the blockade of the discriminative cue by 2,3-dimethoxy-4-iodoamphetamine (DOI) or LSD in rats with M100907 pretreatment (Schreiber et al., 1994; Winter et al., 2004), supporting the general conclusion that 5-HT$_{1A}$ receptors are key mediators of the discriminative stimulus effects of hallucinogens in rats (for review, see Nichols, 2004). However, because LSD also has high affinity for the 5-HT$_{1A}$ receptor, many drug discrimination studies have investigated a possible role for the 5-HT$_{1A}$ receptor in the stimulus effects of LSD. The results have been variable, but in general they suggest that there is not a large 5-HT$_{1A}$ component to the LSD stimulus in the rat. For example, the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)-tetralin has been found to either partially substitute (Reissig et al., 2005) or not substitute at all for LSD (Cunningham and Appel, 1987) in rats. Interestingly, the involvement of 5-HT$_{1A}$ receptor to the LSD discriminative stimulus may be species-dependent; the LSD discriminative stimulus in the monkey, pigeon, and mouse is reported to have a 5-HT$_{1A}$ receptor component (Nielsen, 1985; Walker et al., 1991; Benneyworth et al., 2005). The possible role for the 5-HT$_{2C}$ receptor is less certain due to the lack of specific agonists available for testing (Nichols, 2004); however, Fiorella et al. (1995a) demonstrated that 5-HT$_{2C}$ receptors mediate an enhanced sensitivity to LSD discriminative stimulus following serotonin depletions.

In addition, we showed that direct bilateral injections of LSD into the ACC of rats substituted completely for systemically administered LSD in drug discrimination. This substitution was dose-dependent with a dose of approximately 0.29 μg of LSD per side producing 50% LSD lever selection. The discriminative stimulus produced by LSD infused directly into the ACC was completely blocked by systemic administration of the selective 5-HT$_{2A}$ receptor antagonist M100907. Injections of LSD into the orbital or frontal cortex did not substitute for systemic LSD, suggesting that not all cortical sites that express 5-HT$_{2A}$ receptors (Cornea-Hebert et al., 1999) are involved in producing the discriminative cue LSD. The major finding of the present study was that direct bilateral injections of M100907 into the ACC completely blocked the discriminative stimulus produced by systemic LSD. Taken together, these results suggest that activation of 5-HT$_{2A}$ receptors located within the ACC is necessary to produce the discriminative stimulus of LSD.

The hypothesis that the hallucinogenic drug LSD is acting centrally on the 5-HT system was proposed almost immediately after the discovery of 5-HT as a central nervous system neurotransmitter. Based primarily on the similarities in chemical structure between LSD and 5-HT, Gaddum and Hameed (1954) proposed that the effects of LSD might result from actions within the central nervous system acting on the 5-HT system. Furthermore, Freedman (1961) demonstrated that administration of LSD elevated brain 5-HT levels. More recently, it was demonstrated that direct injections of 7.5 or 25 μg of LSD into the lateral ventricle of rats trained to

**Fig. 5.** Systemic M100907 blocks intra-ACC-injected LSD. Pretreatment with the specific 5-HT$_{2A}$ antagonist M100907 (M) (0.4 mg/kg s.c.) administered 20 min before LSD blocked the ability of bilateral, intra-ACC injection of LSD (0.375 μg/μl/side) to substitute for systemic LSD (top). **+ +, p < 0.001 compared with aCSF; ††, p < 0.001 compared with intracerebral (i.c.) LSD. §§, p < 0.001 compared with retest i.c. LSD (n = 6–8). Bottom, mean ± S.E.M. total responses the rats made during the 5-min test period.

**Fig. 6.** Intra-ACC injection of M100907 blocks systemic LSD. The 5-HT$_{2A}$ receptor antagonist M100907 (M) (0.5 μg/μl/side) was injected bilaterally into the ACC 20 min before systemically administered LSD (0.04 mg/kg s.c.). Top, intra-ACC injection of M100907 significantly reduced the ability of rats to discriminate LSD, given systemically. **+ +, p < 0.001 compared with aCSF-a.s. LSD. ††, p < 0.001 compared with retest i.c. aCSF-s.c. LSD (n = 4–5). The mean ± S.E.M. total responses the rats made during the 5-min test period are shown in the bottom panel.**

M100907 (85 compared with 80%), confirming tissue and cannula viability. Response rates were not altered significantly by the test conditions (F(2,11) = 0.996; p = 0.40) (Fig. 6, bottom).

**Discussion**

In the present study, we demonstrate that systemic administration of the specific 5-HT$_{2A}$ receptor antagonist M100907
discriminate systemically administered 0.1 mg/kg LSD from saline produced a full generalization with a similar time course as systemic LSD (Doat et al., 2003). Indeed, we replicated this finding in rats trained to discriminate the hallucinogen DOI, a mixed 5-HT2A and 5-HT2C agonist. We observed that LSD injected into the lateral ventricle in rats trained to discriminate 0.75 mg/kg DOI substitutes completely for systemic DOI with a dose of 0.45 μg producing 50% DOI lever selection, confirming that LSD acts centrally (unpublished data).

The first evidence that a discrete brain site might be mediating the effects of LSD was from the early studies of Aghajanian and colleagues. They reported that LSD administered either systemically or directly by iontophoresis to the serotoninergic raphe cell bodies suppressed the firing of raphe neurons (Aghajanian et al., 1968, 1970, 1972). This lead to the hypothesis that hallucinogens exert their profound effects by altering raphe function, thus having extensive influence on many brain regions because of the widespread projections of raphe neurons. However, not all hallucinogenic drugs used by humans have this property; for example, the phenethylamines such as mescaline do not suppress raphe neuron firing (Aghajanian et al., 1970; Haigler and Aghajanian, 1973); therefore, other brain areas were hypothesized to be involved in the actions of hallucinogenic drugs.

The strategy of intracranial drug injection has been successfully used to identify brain areas in which drugs of abuse initiate their actions. For example, one of the most intensely studied brain systems is the dopamine system that is involved in the actions of cocaine. Through various methodologies, including direct injections, it has been demonstrated that the nucleus accumbens and frontal cortex are involved in the rewarding and reinforcing properties of the actions of cocaine (Wise and Hoffman, 1992). This method of direct injection has also been used to explore the brain regions that mediate the actions of LSD. Mokler and Rech (1984) demonstrated that LSD administered intracerebrally disrupted responding on an FR40 operant task with an ED50 of 15 μg. Other investigators have used the drug discrimination paradigm to determine the involvement of brain regions in mediating the discriminative stimulus of LSD. The drug discrimination paradigm is a powerful and sensitive method that has been used in both animals and humans to evaluate the stimulus properties of drugs. Results from most (Schuster and Johanson, 1988; Kamien et al., 1993; Johanson et al., 2006), but not all (Brauer et al., 1997), drug discrimination studies in humans show that laboratory animals and humans place drugs into similar drug classifications. A dose of LSD (1 μg) injected bilaterally into nucleus accumbens substituted completely for systemic LSD in rats trained to discriminate 0.16 mg/kg LSD in a FR32 schedule (Nielsen and Scheel-Kruger, 1986). The raphe nucleus was targeted in the study by Minnema et al. (1980) using a two-lever VI15 operant procedure with rats trained to discriminate 96 μg/kg LSD. Direct injection of LSD into the dorsal raphe produced a stimulus that generalized to systemically administered LSD with a similar time course.

The doses of LSD used in most of these studies were relatively high, clouding interpretation. For example, the study of Nielsen and Scheel-Kruger (1986) used a training dose of 160 μg/kg compared with our training dose of 60 μg/kg, and Minnema et al. (1980) injected 60 μg/kg into the raphe compared with our approximate doses of 1.0 to 5.0 μg/kg injected into the ACC. At high doses, LSD interacts with multiple receptors including 5-HT1A and dopamine D2 receptors (Nichols, 2004). Furthermore, none of these studies used selective serotonin receptor antagonists to identify which receptors were mediating the actions of the centrally administered LSD. Thus, the results presented here are the first to demonstrate that 5-HT2A receptors within the ACC are essential for the stimulus produced by LSD. The current results do not rule out a role for 5-HT2A receptors in other brain sites.

Major methodological issues inherent in direct brain microinjections include tissue damage due to cannula implantation and region of influence of a lipid-soluble, diffusible drug. Although tissue damage to the cortex could alter behavior in the drug discrimination paradigm, we observed a similar ~80% correct lever pressing with 0.043 mg/kg LSD in noncannula-implanted animals (Fig. 2) and with 0.04 mg/kg LSD in bilateral-cannulated animals (Fig. 6), indicating that surgery and subsequent tissue damage did not alter the ability of systemic LSD to produce discrimination. This conclusion was reinforced in our experimental design in which the stimulus properties of LSD were compared the week before and week after microinjections and found to be identical. We injected dye in a subset of rats to help localize the injection site; qualitative analysis indicated that diffusion of the dye was limited to the ACC. However, the diffusion coefficient of the dye differs compared with the drugs used in this study and post mortem tissue manipulation confounds the use of dye diffusion to conclude the precise anatomical localization of the drugs infused. Placement of the injection cannula extended into border regions of the ACC and frontal cortex (Fig. 1); thus, we cannot exclude the possible involvement of this region of the frontal cortex in the LSD discriminative stimulus. However, direct injection of LSD bilaterally into the frontal cortex 0.5 to 1 mm anteriorly was ineffective, suggesting that the diffusion of LSD is relatively anatomically discrete.

The region of the rat ACC targeted in our experiments corresponds to areas 24a and 24b of the rat cingulate cortex (Vogt and Peters, 1981; Vogt et al., 2003). Areas 24a and 24b contain most neuronal types found in neocortical areas; in addition to pyramidal cells, there are a variety of multipolar, bitufted, and bipolar cells (Vogt and Peters, 1981; Devinsky et al., 1995; Gabbott et al., 1997; Vogt et al., 2003). In the ACC, 5-HT2A receptors are located throughout the cortical layers, with the densest expression on layer V pyramidal cells (Cornea-Hebert et al., 1999). This region of the ACC receives projections from the anteromedial and laterodorsal thalamus and amygdala and projects to motor and visual cortices, periaqueductal gray, striatum, nucleus accumbens, raphe nucleus, nucleus of the solitary tract, and dorsal nucleus of the vagus (Vogt and Peters, 1981; Vogt and Miller, 1983; Devinsky et al., 1995; Vogt et al., 2003). The ACC is unique in that its role is to integrate inputs from multiple thalamic nuclei for the initiation of goal-directed behaviors. The unifying theory of ACC function is to integrate the individual’s affect into cognitive and motor behaviors (Devinsky et al., 1995). Because the ACC influences many functions, including modulating autonomic activity, endocrine function, nociceptive processing, visuomotor integration, and decision-making behaviors (Devinsky et al., 1995; Vogt et al., 2003), our finding that LSD produced a discriminative cue by acting
on 5-HT2A receptors in the ACC may reflect modulation of these systems to produce its unique intrathecal stimulus. Psilocybin, an indoleamine hallucinogen that shares similar psychological effects in humans as LSD, in normal human subjects increases cerebral glucose utilization in cortical regions, including the ACC as measured by positron emission tomography (Vollenweider et al., 1997). They hypothesized that the association between the subject’s drug-induced de-realization and ego pathologies and the increase in glucose metabolism in the ACC may be a result of disruption of ACC network circuits leading to a mismatch of internal and external reality, change of sense of time and space, and impairment of ego functioning. Interestingly, neuromaging studies have reported that both auditory and visual hallucinations in schizophrenics are associated with activation of the ACC (Silbersweig et al., 1995; Weiss and Heckers, 1999; Shergill et al., 2000). Moreover, the ACC has been implicated in the neuropathology of schizophrenia because of structural and morphological differences observed from post mortem analyses of schizophrenic brains, including a reduction of gray matter volume, reduced laminar thickness, and reduction in number and size of pyramidal neurons (Benes et al., 2001; Bouras et al., 2001; Chana et al., 2003). These clinical observations point to a role of a dysfunctional ACC in schizophrenia.

In conclusion, we confirmed previous studies demonstrating that 5-HT2A receptors mediate the discriminative stimulus of systemic LSD in rats, whereas 5-HT2C receptors and 5-HT1A receptors do not seem to be involved in the LSD discriminative stimulus in the rat. In addition, we observed that direct bilateral injection of LSD into the ACC of rats substituted completely for systemically administered LSD. Furthermore, we showed that local antagonism of 5-HT2A receptors in the ACC completely blocked the effects of systemic LSD, suggesting that activation of 5-HT2A receptor in the ACC is necessary for the production of the unique effects of LSD.

Acknowledgments
We thank Kathleen Patterson for technical assistance.

References


Address correspondence to: Dr. Randy L Smith, Department of Psychiatry, 8148 Medical Research Bldg. 3, Vanderbilt University-School of Medicine, Nashville, TN 37232. E-mail: randy.s.barrett@vanderbilt.edu