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Effects of clozapine and 2,5-dimethoxy-4-methylamphetamine [DOM] on 5-HT_{2A} receptor expression in discrete brain areas

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Abstract

Activation of 5-HT_{2A} receptors has been shown to be an essential component of the discriminative stimulus effects of indoleamine and phenethylamine hallucinogens. The objective of the present study was to determine the neuroanatomical location of the 5HT_{2A} receptors which may be responsible for the stimulus effects of the phenethylamine hallucinogen [−]2,5-dimethoxy-4-methylamphetamine (DOM). It was hypothesized that brain areas containing altered 5-HT_{2A} receptor expression in the context of a similar alteration in DOM-induced stimulus control might be important in mediating the stimulus effects of DOM. Fisher 344 rats were treated with either clozapine (25 mg/kg/day) or DOM (2 mg/kg/day) for 7 days, and the consequences of these drug treatment regimens on DOM-induced stimulus control and on 5-HT_{2A} receptor expression in several brain areas were determined. Chronic administration of clozapine was associated with a widespread decrease in levels of 5-$HT_{2A/2C}$ receptors. Conversely, treatment with DOM had varied effects including a neuroanatomically selective decrease in 5-$HT_{2A/2C}$ receptor levels that was restricted to the olfactory nucleus. Both chronic treatment with DOM and clozapine decreased the stimulus effects of DOM. The present findings suggest a role for the olfactory nucleus in producing the stimulus effects of DOM.

Keywords: 2,5-dimethoxy-4-methylamphetamine (DOM); Clozapine; 5-HT_{2A} receptor; Receptor regulation; Quantitative autoradiography; Drug-induced stimulus control

1. Introduction

Administration of indoleamine (e.g., LSD, psilocybin) and phenethylamine (e.g., DOM, mescaline) hallucinogens exert profound behavioral effects such as distortion of perception, disrupted awareness of time, as well as alterations in mood, affective state and cognition. Many of these same effects are observed in individuals with schizophrenia. For example, schizophrenic patients exhibit positive symptoms such as delusions and hallucinations as well as negative symptoms such as impaired cognition and flattening of affect (Andreasen and Black, 2001; Vollenweider and Geyer, 2001). Furthermore, psilocybin, an indoleamine hallucinogen, produces a psychosis-like syndrome in humans that resembles the first manifestations of schizophrenia (Vollenweider et al., 1998).

In the case of both schizophrenia and the effects of indole and phenethylamine hallucinogens, the 5-HT_{2A} subfamily of serotonin receptors appears to play a critical role (Dean, 2003; Winter et al., 1999). Thus, several studies have reported a lower density of 5-HT_{2A} receptors in schizophrenic patients (Dean et al., 1999; see Dean, 2003). Further, atypical antipsychotics share the common feature of being high-affinity antagonists at the 5-HT_{2A} receptor (Meltzer et al., 2003; Roth et al., 2004), and allelic variations in the 5-HT_{2A} receptor gene influences the clinical response to these compounds (Ellingrod et al., 2002; Lane et al., 2002). Similarly, affinity at 5-HT_2 receptors for a series of phenethylamines correlates both with the potency of these compounds as hallucinogens

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(Titeler et al., 1988; Sadzot et al., 1989) as well as with their potency to substitute for DOM as a discriminative stimuli (Glennon et al., 1984). Using antagonist correlation analysis, Fiorella et al. (1995a,b) concluded that the stimulus effects of LSD involved activation of 5-HT_{2A} receptors rather than 5-HT_{2C} receptors. Similarly, M100907, a selective 5HT_{2A} receptor antagonist, but not the 5HT_{2C} receptor antagonist SB 200,646 blocked the discriminative stimulus effects of phenethylamine hallucinogens (Schreiber et al., 1994; Eckler et al., 2004).

Although 5-HT_{2A} receptors play a prominent role in mediating the stimulus effects of hallucinogenic drugs, the anatomical location of the relevant receptors is unclear. 5-HT_{2A} receptors are distributed throughout the brain, but are found in high density in various cortical regions (especially the frontal cortex), caudate nucleus, nucleus accumbens, olfactory nucleus, and claustrum (Pazos et al., 1985; Appel et al., 1990; López-Giménez et al., 1997). The objective of the present study was to identify the neuroanatomical location of the 5-HT_{2A} receptors that may be responsible for mediating the stimulus effects of DOM, a 5-HT_{2A} receptor agonist with hallucinogenic activity, in the rat. It is hypothesized that brain areas containing altered expression of 5-HT_{2A} receptors in the context of a similar alteration in DOM-induced stimulus control might be important in mediating the discriminative stimulus effects of DOM. Thus in the present study, behavioral results using drug-induced stimulus control after chronic treatment with clozapine or DOM were correlated with changes in 5-HT_{2A} receptor expression in discrete brain areas as measured by quantitative autoradiography. The choice of clozapine and DOM was based on reports that: (i) chronic administration of clozapine, an atypical antipsychotic that blocks several neurotransmitter receptors including 5-HT_{2A} receptors, but not haloperidol, a typical antipsychotic without significant affinity at the 5-HT_{2A} receptor, reduces DOM-induced stimulus control (Doat et al., 2002); (ii) repeated treatment with DOM decreases 5-HT_2 receptor-mediated head-twitch (Leysen et al., 1989); and (iii) chronic exposure to DOM or clozapine alters the density of 5-HT_2 receptors (Leysen et al., 1989; Burnet et al., 1996; Kuoppamäki et al., 1995). In addition, as a clinical relevant drug, any insight obtained on clozapine might be beneficial in elucidating its mechanism of action.

2. Methods

2.1. Subjects

Male Fisher 344 rats obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN) were used in all experiments. Animals were housed in pairs under a 12 h light dark cycle beginning at 6:00 a.m. and allowed free access to water. All training and testing took place during the light cycle. For rats used in behavioral studies caloric intake was controlled to maintain a mean body weight of 250 g. Subjects were fed following experimental sessions. Caloric control has been shown to lengthen the life span and decrease the incidence of a variety of pathologies in Fischer 344 rats (Keenan et al., 1994). Animals used in these studies were maintained in accordance with the 'Guide for Care and Use of Laboratory Animals' of the Institute of Laboratory Animals Resources, National Research Council and the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) were followed. All procedures using animals were approved by the Institutional Animal Care and Use Committee at the University of Buffalo.

2.2. Drugs

The negative isomer of DOM, [−]-DOM, was employed in all experiments and is referred to as DOM throughout the manuscript. DOM was provided by the National Institute on Drug Abuse. Clozapine was purchased from RBI (Natick, MA). DOM was dissolved in water and injected in a volume of 1.0 ml/kg body weight. Clozapine was dissolved in a minimal volume of 8.5% lactic acid, diluted to the appropriate concentration with 0.9% NaCl, and injected in a volume of 2 ml/kg. All injections were intraperitoneal.

2.3. Drug treatments

In the behavioral studies, after stimulus control was established, animals were treated with either vehicle (saline), clozapine (25 mg/kg/day), or DOM (2 mg/kg/day) for seven days. Animals were tested with the training dose 30 h after the last injection in order to minimize the effects of any residual drug on the stimulus properties of DOM.

During treatments, DOM or saline discrimination training was suspended to prevent additional learning. Previously we demonstrated that there is no significant difference in DOM-appropriate responding to the training dose of DOM (0.3 mg/kg) before and after a 21-day suspension of training (Doat et al., 2002). Furthermore, in all experiments the percent DOM-appropriate responding after drug treatment was compared to the percent DOM-appropriate responding after treatment with vehicle. In the autoradiography studies, untrained animals were treated with vehicle, 2 mg/kg/day DOM, or 25 mg/kg/day clozapine for seven days and sacrificed 30 h after the last injection.

3. Drug-induced stimulus control

3.1. Apparatus

Animal test chambers (Coulbourn Instruments Model E10-10) housed in larger lightproof, sound-insulated boxes were used for all experiments. Each box had a house light and exhaust fan. Chambers contained two levers mounted

on opposite ends of one wall. Centered between the levers was a dipper which delivered 0.1 ml of sweetened condensed milk diluted 2:1 with tap water.

3.2. Training

Subjects were trained to discriminate DOM (0.3 mg/kg, 75-min pretreatment time, intraperitoneal injection) from saline as described previously (Fiorella et al., 1995a,b). A fixed ratio 10 schedule of reinforcement was employed. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever. During test sessions, no responses were reinforced and the session was terminated after the emission of 10 responses on either lever. The distribution of responses on the drug-appropriate lever was expressed as a percentage of the total number of responses emitted. Data for any subjects failing to emit 10 responses within the constraints of the 10-min test session were not considered in the calculation of the percent drug-appropriate responding. Response rate was calculated for each session by dividing the total number of responses emitted prior to the emission of 10 responses on either lever by elapsed time.

4. Quantitative autoradiography

4.1. Tissue preparation

Male Fischer 344 rats were sacrificed; the brains were quickly removed and immediately frozen with dry ice onto microtome mounting chucks. Brains were cut into 20 μm thick coronal sections using a cryostat maintained at −14 °C. The sections were thaw-mounted onto gelatin-coated slides and stored with desiccant pellets at −70 °C until use. Sections were cut consecutively and placed on alternating slides (3–4 sections per slide). This sectioning pattern of consecutive sections on alternate slides enabled nearly identical sections to be processed for total and non-specific binding. Prior to the day of the assay the slides were dried under a vacuum overnight at 4 °C and brought to room temperature.

4.2. Autoradiography

Binding of [^{125}I] DOI to the tissue sections was carried out using a modification of a previously described method (Appel et al., 1990). Slides were incubated at room temperature for 10 min in assay buffer [50 mM Tris HCl, 4 mM $MgSO_4$, 0.1% (w/v) BSA, 0.1% (w/v) ascorbate; pH=7.4 at room temperature] and then for 90 min in assay buffer containing 200 pM [^{125}I] DOI (specific activity 2200 Ci/mmol; Perkin Elmer Life and Analytical Sciences, Boston, MA). Following the 90-min incubation, the slides were washed 3 consecutive times for 10 min each in ice-cold assay buffer and dipped in ice-cold deionized H_20. Non-specific binding was determined in the presence of 1 μM unlabeled DOM. The slides were dried overnight under a stream of cool air and then, along with autoradiographic [^{125}I] microscales (Amersham Biosciences, Piscataway, NJ), apposed to Kodak BioMax MR film (Kodak, Rochester, NY) for 7 to 10 days.

In each experiment, slides containing equivalent neuroanatomical areas of interest were processed at the same time. Furthermore, each piece of film was exposed to slides containing neuroanatomically equivalent sections representing both total and non-specific binding from subjects treated with saline, DOM, and clozapine. Thus, in addition to standardizing binding between experiments through the use of autoradiographic microscales, all data within each experiment were identically processed and exposed to film under identical condition.

4.3. Image analysis

Autoradiographic images of the brain sections were digitized using a Bio-Rad 700 scanning densitometer. Image analysis was conducted using a G4 Macintosh computer and the image analysis software Oncore Image. Specific DOM binding was localized by first superimposing images from adjacent sections which represented total and non-specific binding. The gray scales of each image were inverted and the image of non-specific binding was subtracted from the image of total binding to produce an image of specific binding. The gray scale of the resulting image was then re-inverted to produce an image in which darker areas represented greater specific DOM binding. Neuroanatomical areas with DOM binding were identified by comparing the image of specific binding to a rat brain atlas (Paxinos and Watson, 1986). Each region of interest (ROI) was outlined on the image of specific binding using the idraw command. The area of each ROI was determined, the optical density over this area was integrated, and the integrated optical density was normalized by dividing it by the ROI area. For each animal, the normalized integrated optical density was determined bilaterally from 3 to 5 images of specific binding within the ROI and averaged. This mean integrated optical density value for each subject was then averaged among all subjects. Thus, the sample size used for statistical analyses is the number of subjects and not the number of measurements. Integrated optical density values were related to radioactivity using standard curves of intensity versus radioactivity generated by the radiolabeled [^{125}I] microscales exposed to the film along with the sections. Best-fit standard curves were obtained when a log–log function was used to describe the relationship between radioactivity and optical density of the microscales. [^{125}I] DOI binding was quantified in the frontal cortex, olfactory nucleus, striatum, and claustrum. Due to insufficient binding or poor visual detection, optical density measurements were not taken from other areas.

4.4. Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). For the behavioral studies, data were compared using the Mann–Whitney Rank Sum test. For the quantitative autoradiography experiments data were expressed as the percent change from control subjects (i.e. saline treated subjects). Statistical significance of differences in binding was determined using the Differences Method. Differences were considered statistically significant if the probability of their having arisen by chance was less than 0.05. All analyses were conducted using SigmaStat 2.03 for Windows.

5. Results

The effects of chronic treatment with DOM (Expt I) and clozapine (Expt II) on DOM-induced stimulus control and on rates of responding are shown in Table 1. In rats trained to discriminate DOM from saline, suspension of training for 7 days did not alter the stimulus effects of DOM. Thus, following 7 days of treatment with vehicle, the training dose of DOM elicited 100 ± 0% drug-appropriate responding. Chronic treatment with DOM (2 mg/kg/day) produced a statistically significant 39% decrease in DOM-appropriate responding. The rate of responding following treatment with DOM for 7 days was not significantly different than the response rate in the saline treatment control group. Chronic treatment with clozapine [25 mg/kg/day] for 7 days led to a statistically significant decrease to 43% DOM-appropriate responding. However, although all subjects completed the test, it must be noted that there was as well a significant suppression of the rate of responding to 32% of control in these subjects. Thus, the results must be interpreted with caution in that the possible confounding of antagonist effects by rate-suppressing effects in drug discrimination studies is poorly understood (Batman et al., 2005).

Table 1
Effects of chronic treatment with DOM and clozapine on DOM-induced stimulus control

Treatment	% DOM-appropriate responding	*N*/*n*	Rate (responses/min)
Expt I			
Vehicle	100 ± 0	10/10	34 ± 9
DOM	61 ± 10*	9/9	23 ± 4
Expt II			
Vehicle	100 ± 0	10/10	25 ± 4
Clozapine	43 ± 17*	9/9	8 ± 2**

Rats were treated for 7 days with either DOM (2 mg/kg/day) or saline (Expt I) or either clozapine (25 mg/kg/day) or vehicle (Expt II) as described in the Methods. Animals were tested with the training dose of DOM (0.3 mg/kg) 30 h after the last injection of drug or the appropriate vehicle. Data are presented as mean ± S.E.M. *N* = number of subjects completing the test session; *n* = number of subjects tested. *$P<0.05$, **$P<0.01$ (Mann–Whitney Rank Sum test) compared to the appropriate vehicle control.

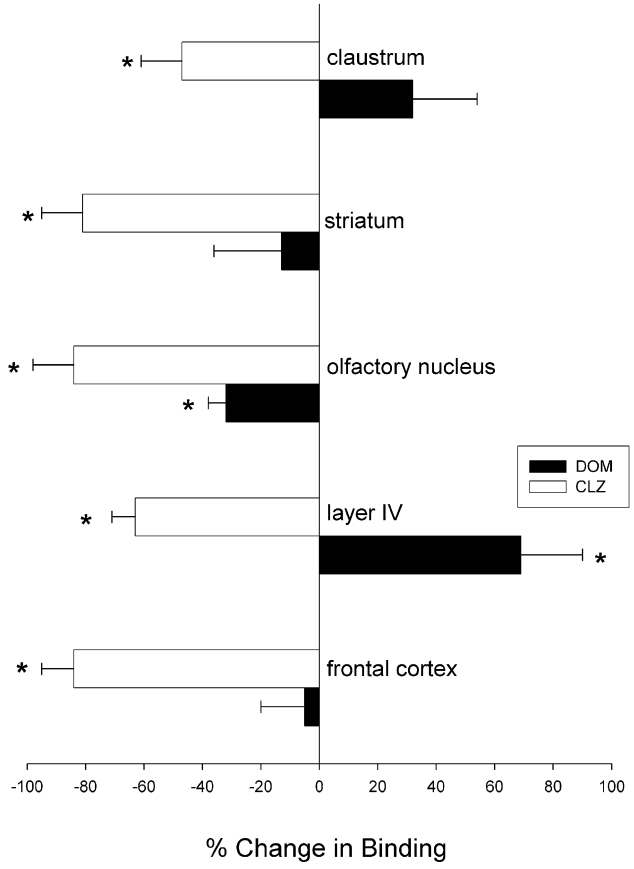

Fig. 1. The regional effects of in vivo treatment with clozapine or [^{125}I] DOI for 7 days on [^{125}I] DOI binding in rat brain. Rats were treated with 25 mg/kg/day clozapine, 2 mg/kg/day DOM, or saline for 7 days and were sacrificed 30 h after the last injection. Data are expressed as the percent change in binding compared to control (binding in saline treated subjects). Ordinate: brain areas where [^{125}I] DOI binding was measured. Abscissa: percent change in [^{125}I] DOI binding. White bars represent the mean percent change in [^{125}I] DOI binding ± SEM from 5 animals treated with clozapine (clz). The black bars represent the mean percent change in [^{125}I] DOI binding ± SEM from 4 animals treated with DOM. Data were compared using the Differences Method. Asterisks denote a statistically significant change in [^{125}I] DOI binding ($P<0.05$).

Fig. 1 shows the effects of 7 days of treatment with either 2 mg/kg/day DOM (black bars) or 25 mg/kg/day clozapine (white bars) on [^{125}I] DOI binding in 5 brain areas. The changes in binding following treatment with clozapine range from a 47% decrease in the claustrum to an 84% decrease in the frontal cortex and olfactory nucleus. Following treatment with DOM there were no statistically significant changes in binding in the claustrum, striatum, or frontal cortex. However, while binding in layer 4 of the cortex was increased by 69%, there was a 32% decrease in the olfactory nucleus.

6. Discussion

Activation of 5-HT$_{2A}$ receptors is a necessary component of the stimulus effects of LSD and phenethylamine

hallucinogens such as DOM (Fiorella et al., 1995a,b; Schreiber et al., 1994). Accordingly, reduction in expression of 5-HT_{2A} receptors should lead to a decrease in DOM-induced stimulus control. Further, brain areas containing a lower density of 5-HT_{2A} receptors in the context of a decrease in stimulus control are likely to be important in mediating the discriminative stimulus effects of DOM. In the present study, 7 day treatment with DOM, a 5-$HT_{2A/2C}$ receptor agonist, or clozapine, a 5-HT_2 antagonist, elicited behavioral tolerance as manifested by a reduction in the stimulus effects of DOM. Similarly, chronic exposure to DOI, the iodinated congener of DOM, also was reported to decreased DOI-induced stimulus control in rats (Smith et al., 1999), while a reduction in 5-HT_2 receptor-mediated head-twitch in mice was found after chronic administration of phenethylamine hallucinogens (Leysen et al., 1989; Darmani et al., 1992). Although development of desensitization after chronic exposure to an antagonist is an unusual response for G protein-coupled receptors, such paradoxical regulation has been shown for 5-HT_{2A} receptors (see Gray and Roth, 2001; Van Oekelen et al., 2003). Furthermore, the observed decrease in DOM-induced stimulus control after chronic clozapine exposure is in agreement with our previous findings with this atypical antipsychotic (Doat et al., 2002). The observed atypical development of tolerance to 5-HT_{2A} receptor-mediated responses in vivo does not appear to be restricted to the CNS as prolonged exposure to DOI and clozapine reduced serotonin-induced contraction of thoracic aorta, a response mediated by $5HT_{2A}$ receptors (Enguix et al., 2003).

Although training was suspended during the drug treatment to prevent additional learning, the reduction in DOM-induced stimulus control was not due to the animals forgetting the cue. After 7 days without training, 100% DOM-appropriate responding was found when animals treated with vehicle were tested with the training dose of DOM. In addition, Doat et al. (2002) showed that in subjects trained to discriminate DOM from saline a 21 day suspension of training does not alter subsequent DOM-induced stimulus control. It is also unlikely that residual drug was responsible for the observed behavioral tolerance. Clozapine is rapidly eliminated in rodents (Baldessarini et al., 1993) such that the 30 h delay after the last injection represents greater than 18 half-lives and should result in complete removal of the drug.

In order to determine whether reduced receptor expression may contribute to the observed decrease in DOM-induced stimulus control, binding of the radiolabeled iodo-analogue of DOM, [^{125}I] DOI, was measured in various neuroanatomical areas in untrained rats exposed to the same drug regimens that produced tolerance to DOM in the behavioral experiments. In rats treated for 7 days with 25 mg/kg/day clozapine, [^{125}I] DOI binding was significantly reduced in all areas examined. A similar down-regulation has been reported following treatment with clozapine (Lee and Tang, 1983; Matsubara and Meltzer, 1989; Wilmot and Szczepanik, 1989; Kuoppamäki et al., 1995; Burnet et al., 1996) as well as following treatment with mianserin, ketanserin and ritanserin (Blackshear and Sanders-Bush, 1982; Gandolfi et al., 1985; Leysen et al., 1986). While the mechanism by which clozapine decreases the density of 5-HT_2 receptors is unclear, it may involve a decrease in transcription. In this context, Buckland et al. (1997) found that treatment with 30 mg/kg/day clozapine for 32 days resulted in a 15% to 40% decrease of 5-HT_{2A} receptor mRNA in various brain areas.

Contrary to the apparent generalized down-regulation of 5-$HT_{2A/2C}$ receptors observed following treatment with clozapine, treatment with DOM (2 mg/kg/day) for 7 days produced varied effects on 5-$HT_{2A/2C}$ receptor expression. In the claustrum, striatum and frontal cortex there were no statistically significant changes in receptor expression. In cortical layer 4, however, there was a 69% increase in [^{125}I] DOI binding, while in the olfactory nucleus there was a 32% decrease. An increase in receptor density following chronic exposure to an agonist might appear to be a curious response for a G protein-coupled receptor. However, several studies have report an increased density of 5-$HT_{2A/2C}$ receptors following exposure to an agonist (Akiyoshi et al., 1993; Grotewiel and Sanders-Bush, 1994; Chen et al., 1995). In addition, chronic treatment with the selective serotonin reuptake inhibitor, fluoxetine, which would be expected to raise synaptic levels of 5-HT and thus increase receptor stimulation, has been reported, in at least some instances, to increase the density of 5-HT_{2A} receptors (Hrdina and Vu, 1993; Li et al., 1997). Thus the present study mirrors the literature where increases, decreases and no change in the density of 5-HT_{2A} receptors have been reported following chronic agonist exposure (Van Oekelen et al., 2003). These varied responses after chronic agonist treatment, however, may reflect localization of 5-HT_{2A} receptors in different cell types as Grotewiel and Sanders-Bush (1994) reported the cellular background was a major determinant in 5-HT_{2A} receptor regulation.

In contrast to the results of the present investigation, Smith et al. (1999) found that tolerance to the discriminative stimulus properties of DOI following an 8 day treatment with DOI was accompanied by a decrease in 5-HT_{2A} receptor expression in the cortex and claustrum. Buckholtz et al. (1990) also reported a decrease in [^{3}H]ketanserin binding in rat cortex after chronic administration of LSD and psilocybin, but did not observe any change in 5-HT_{2A} receptor density after chronic administration of the phenethylamine mescaline. The discrepancy between these studies and the present study may be due to the differences in experimental design. Both Smith et al. (1999) and Buckholtz et al. (1990) used radiolabeled antagonists, and in the present investigation an agonist, [^{125}I] DOI, was used. While the radiolabeled antagonists would be expected to label the entire population of 5-HT_{2A} receptors, [^{125}I] DOI would label the subpopulation of receptors coupled to G proteins as well as any agonist-induced conformational

states or states stabilized by the agonist (Branchek et al., 1990; Teitler et al., 1990; López-Giménez et al., 2001). We may speculate that a reduction in [^{125}I]AMIK binding without any change in [^{125}I] DOI binding might occur if a large receptor reserve existed along with a shortage of G proteins and/or if the receptors had restricted access to the G proteins (Kenakin, 1997; Strange, 1999; Remmers et al., 2000).

As a reduction in 5-HT$_{2A}$ receptor-mediated responses can be associated with down-regulation of the receptors (e.g., Leysen et al., 1989; Grotewiel and Sanders-Bush, 1994;Van Oekelen et al., 2001), the premise of the present study was that brain regions containing altered expression of 5-HT$_{2A}$ receptors in the context of a similar alteration in DOM-induced stimulus control might be important in mediating the discriminative stimulus effects of phenethylamine hallucinogens. Further, the use of two dissimilar compounds to alter the behavior response should lessen the chance of spurious correlations. Treatment both with DOM and with clozapine reduced the stimulus effects of DOM, and the only brain region in which expression of 5-HT$_{2A}$ receptors was decreased by both drug regimens was the olfactory nucleus. Thus, our findings suggest a role for the olfactory nucleus in producing the stimulus effects of DOM. Perhaps because the importance of olfaction in man is overshadowed by other sensory modalities, the role of the olfactory nucleus in the effects of hallucinogenic drugs has largely been overlooked. In rats, however, olfaction is the predominant sensory system. Thus, while the sensory disruptions caused by hallucinogenic drugs are manifest as mainly visual, auditory, and tactile hallucinations in humans, they may have more profound effects on olfaction in the rat. Furthermore, olfactory information is processed by limbic and cortical structures, which are classically associated with hallucinogenesis in humans. Thus, disruptions in the olfactory nucleus could conceivable affect these brain areas to produce more widespread alterations in perception and affect. Interestingly, in schizophrenic patients there is considerable evidence of impaired olfaction (Kopala et al., 1993; Moberg et al., 1999; Malaspina and Coleman, 2003), as well as, reduction in the size of the olfactory bulb (Turetsky et al., 2000).

Because chronic exposure to agonists was reported to decrease 5-HT$_{2A}$ receptor-mediated phosphoinositide hydrolysis with no change (Roth et al., 1995) or an increase in 5-HT$_{2A}$ receptor levels (Akiyoshi et al., 1993; Van Oekelen et al., 2001), the observed behavioral tolerance could involve an impairment in the signaling machinery rather than down-regulation of the receptor. Furthermore, chronic treatment with DOM and clozapine might be acting through entirely different mechanisms to reduce the stimulus effects of DOM. Although these possibilities cannot be ruled out at the present time, the simplest explanation is that both compounds are acting through a single and similar mechanism. If this is the case, than the reduction in density of 5-HT$_{2A}$ receptors in the olfactory nucleus is the most parsimonious explanation for the decreased stimulus effects of DOM.

In conclusion, the results of this study indicate that chronic treatment both with DOM and clozapine produce a functional desensitization of 5-HT$_{2A/2C}$ receptors as evidenced by tolerance to the stimulus effects of DOM. The clozapine-induced functional desensitization is accompanied by a wide-spread decrease in 5-HT$_{2A/2C}$ receptors, while the DOM-induced functional desensitization is associated with region specific changes in the levels of 5-HT$_{2A/2C}$ receptor expression including a decrease in the olfactory nucleus. Because brain areas containing a decrease in 5-HT$_2$ receptor expression might be important in mediating the DOM stimulus, our findings suggest a role for the olfactory nucleus in producing the stimulus effects of DOM.

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