A DOSE-RESPONSE STUDY OF EFFECTS OF 8-OH-DPAT ON LOCOMOTOR SENSITIZATION TO QUINPIROLE

A DOSE-RESPONSE STUDY OF EFFECTS OF 8-OH-DPAT ON LOCOMOTOR SENSITIZATION TO QUINPIROLE

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**Abstract**

Behavioural sensitization models are useful for understanding many disorders, including obsessive-compulsive disorder and drug addiction. Many of these models are produced by sensitization of dopamine neurotransmission, resulting in behaviours which include increased locomotor activity. Alterations to dopamine-mediated locomotor sensitization may be possible via activation of serotonergic neurotransmission, and there is evidence to suggest this may be through repeated activation of serotonin 1A receptors. The current study examines the development of locomotor sensitization in an animal model via repeated exposure of both a dopamine (D2R/D3R) and serotonin 1A (5-HT1A) agonist. To examine this, male Long-Evans rats were exposed to 10 injections of a combination of different doses of quinpirole and 8-OH-DPAT and tested in activity chambers for locomotor stimulation (measured by total distance travelled). Animals were then exposed to challenges of quinpirole, and 8-OH-DPAT and tested again for locomotor activity. Results showed that high doses of quinpirole or 8-OH-DPAT induced locomotor sensitization. However, when the two drugs were co-administered, 8-OH-DPAT displayed some initial disruption of quinpirole-induced sensitization. Animals sensitized to either quinpirole or 8-OH-DPAT did show higher locomotion when challenged with the drug to which they were sensitized. However, simultaneous quinpirole and 8-OH-DPAT sensitization seemed to prevent maximal responding when challenged with quinpirole. In all, our data suggests that sensitization to quinpirole and 8-OH-

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DPAT is occurring via separate neural mechanisms, with 5-HT1A agonism interfering with development of dopaminergic (D2R) sensitization.

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**List of Abbreviations**

5-HTSerotonin

5-HT1A Serotonin 1A receptor

8-OH-DPAT 8-hydroxy-2-(di-n-propylamino)tetralin

ANOVA Analysis of variance

CNS Central Nervous System

D1R Dopamine 1 receptor

D1+ MSN Medium spiny neurons expressing the dopamine 1 receptor

D2R Dopamine 2 receptor

D2+ MSN Medium spiny neurons expressing the dopamine 2 receptor

D3R Dopamine 3 receptor

GABA γ-Aminobutyric acid

GABAA γ-Aminobutyric acid A receptor

Glu Glutamate

GPCR G-protein coupled receptor

L-DOPA L-3,4-dihydroxyphenylalanine

MSN Medium spiny neuron

NAc Nucleus accumbens

OFC Orbito-frontal cortex

SNc Substantia nigra pars compacta

SSRI Selective serotonin reuptake inhibitor

VTA Ventral tegmental area

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WAY 100135 (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-l-yl)-2-phenylpropamine

x

**Introduction**

Repeated exposure to many psychostimulants drugs, including amphetamines and cocaine, leads to a phenomenon termed bevhavioural sensitization, where the drug response becomes more pronounced in subsequent exposures (Hirabayahi, Okada, & Tadokoro, 1991). Behavioural sensitization is believed to be a part of the mechanism underlying a variety of clinical conditions, including addiction (Koob & Le Moal, 1997), and obsessive-compulsive disorder (Ben Pazi, Szechtman, & Eilam, 2001; Szechtman, Sulis, & Eilam, 1998). Locomotor sensitization is a prime example of behavioural sensitization, and can be produced by repeated exposure to psychostimulant drugs, notably, quinpirole or amphetamines (Perrault, Graham, Bisnaire, Simms, Hayton, & Szechtman, 2006; [Przegaliński](http://www.ncbi.nlm.nih.gov.libaccess.lib.mcmaster.ca/pubmed?term=Przegali%C5%84ski%20E%5BAuthor%5D&cauthor=true&cauthor_uid=10724448), Siwanowicz, Baran, & Filip, 2000; Szechtman, Dai, Mustafa, Einat, & Sullivan, 1994). Locomotor sensitization in rodents, particularly rats (Eilam & Szechtman, 2005) and mice (Haleem, 2013), is a well-documented example of behavioural sensitization. Animals sensitized to psychostimulants exhibit a progressive increase of locomotor behaviours. While the locomotor behaviours which become sensitized are variable, they are most often characteristic of the acute drug effects (Perrault et al, 2006).

Psychostimulant drugs exert many of their behavioural effects, including locomotor excitation, by stimulating the various dopamine receptors in the brain (Haleem, 2013). These receptors can be stimulated directly or indirectly by various types of drugs. For example, amphetamines enhance dopaminergic availability at the synapses of all dopamine neurons, whereas agents such as quinpirole stimulate select dopamine receptor subtypes. There are many different subtypes of dopamine receptors located throughout the central nervous system (CNS), all of them differing in structure, mechanism of action, and effect. Of particular interest to the current study is the D2R (dopamine 2 receptor), which is an inhibitory G-protein coupled receptor (GPCR). Upon activation, the D2R activates inhibitory G-proteins that inhibit adenylyl cyclase and decrease intracellular concentrations of cyclic adenosine monophosphate (cAMP) as well as open select K+ channels, leading to local hyperpolarization (Meyer & Quenzer, 2005; Stoof & Kebabian, 1984). This hyperpolarization can occur in the presynaptic cell or post synaptic cell, which will result in two very different effects. In the case of quinpirole (a selective D2R/D3R agonist), presynaptic versus postsynaptic stimulation appears to be dictated by dose, with low doses (less than about 0.05 mg/kg) resulting in preferential presynaptic activation (and locomotor inhibition), and higher doses resulting in preferential postsynaptic activation (and locomotor excitation; Perrault et al, 2006; Szechtman et al, 1994).

Repeated injections of quinpirole (a D2R/D3R agonist), apomorphine (a D1R/D2R agonist), or amphetamine (an indirect dopamine agonist) are all capable of producing locomotor sensitization ([Przegaliński](http://www.ncbi.nlm.nih.gov.libaccess.lib.mcmaster.ca/pubmed?term=Przegali%C5%84ski%20E%5BAuthor%5D&cauthor=true&cauthor_uid=10724448) et al, 2000; Szechtman et al, 1994; [Võikar](http://www.sciencedirect.com.libaccess.lib.mcmaster.ca/science/article/pii/S0924977X99000383) et al, 1999). These dopaminergic drugs are believed to be inducing locomotor sensitization by acting through one of the three major dopamine pathways in the brain: the mesolimbic, nigrostriatal, and mesocortical pathways (Haleem, 2013). At least two of these pathways (mesolimbic and nigrostriatal) may play prominent roles in the genesis of locomotor sensitization. The mesolimbic pathway is characteristically described as being involved in motivated behaviour and addiction, whereas the nigrostriatal pathway is characteristically described as being involved in lower order motor function. An intact nigrostriatal pathway (which connects the substantia nigra pars compacta (SNc) to the striatum; Andén et al, 1966) appears to be necessary for generation of cocaine-induced locomotor sensitization, as mutant mice that lack a nigrostriatal pathway do not show evidence of locomotor sensitization in the open field when repeatedly exposed to the psychostimulant, cocaine (Beeler, Cao, Kheirbek, & Zhuang, 2008). The mesocortical pathway (originiating in the ventral tegmental area, or VTA, and terminating in the frontal cortex; Andén et al, 1966) appears to be more important in sensitization of other movement-related behaviours. This suggestion is based on the findings in a lesion study where neurons in either the nucleus accumbens (NAc) or orbito-frontal cortex (OFC) in rats were destroyed via intracerebral infusion of excitotoxin (Dvorkin, Silva, McMurran, Bisnaire, Foster, & Szechtman, 2010). Consistent with a prominent role of the mesolimbic pathway (which connects the VTA to the NAc; Andén et al, 1966), the study demonstrated that animals which had NAc lesions displayed an increase in distance travelled in the open field which was independent of any drug.

Dvorkin et al’s (2010) finding that NAc lesions produced spontaneous locomotor excitation is illuminating becausethat the NAc lesion produced locomotor output levels which were on par with quinpirole-sensitized sham controls. The neural composition of the NAc is primarily (95%) inhibitory γ-Aminobutyric acid (GABA) medium spiny neurons (MSNs) (Lalchandani, van der Goes, Partridge, & Vicini, 2013; Maguire et al, 2014). Given this information, it would appear that progressive inhibition of the NAc MSNs is necessary in order to achieve locomotor sensitization. However, it is important to note that there are two sub-types of GABAergic MSN neurons within the NAc: those that are regulated by D1Rs (dopamine 1 receptors), and those that are regulated by D2Rs (Jung & Shim, 2011; Lobo & Nestler, 2011). The neurons in the NAc expressing these dopamine receptor subtypes are thought to be differentially responsible for sensitization. This finding coincides with the hypothesis that D1+ MSNs (neurons that express the D1R) modulate the direct pathway of movement, while D2+ MSNs (neurons that express the D2R) modulate the indirect pathway of movement, although this is a bit of a simplification since there may be some overlap in the projections of the two types of MSNs (Smith, Lobo, Spencer, & Kalivas, 2013). Both D1R and D2R agonists have been found to increase locomotor activity in rodents when administered systemically (Jung & Shim, 2011; Szechtman et al, 1994; Võikar et al, 1999). Since the current study examines the effects of the D2R agonist quinpirole on locomotion, we will be focusing on D2R modulated pathways (for an illustration of direct and indirect pathways, see Figure 1 below).

*Figure 1.* A simplified schematic representation of the direct (left) and indirect (right) pathways of the striatum. In the direct pathway (left), excitatory glutamatergic (Glu) signalling from the cortex will increase inhibition of the internal palladium which will in turn decrease inhibition of the thalamus. This will enable the thalamus to increase Glu (stimulatory) signalling to the cortex. Indirect dopamine agonists or D1R agonists will stimulate the striatum, further driving the pathway. In the indirect pathway (right), excitatory Glu signalling from the cortex to striatum inhibits the external palladium. This inhibition will disinhibit (decrease inhibition) GABA signalling to the subthalamic nucleus which will decrease Glu transmission to the internal palladium. The internal palladium will increase inhibition of the thalamus which will decrease Glu (excitatory) signalling to the cortex. Indirect dopamine agonists or D2R agonists will inhibit the striatum further driving the pathway.

The exact mechanism that causes inhibition of D2+ MSNs during sensitization is not fully understood, but may be a result of increased VTA-D2+ MSN connections. A study by Lalchandani, van der Goes, Partridge, & Vicini (2013) supports this hypothesis, as those authors showed that chronic quinpirole administration *in vitro* to mouse corticostriatal brain cultures increased striatal MSN susceptibilityto D2R and GABAA inhibition. These authors also demonstrated that repeated quinpirole exposure led to an increase in MSN dendritic complexity, which may have allowed for greater synapse formation with presynaptic terminals. The authors were able to demonstrate that the increased MSN dendritic complexity occurred in conjunction with stronger response to quinpirole. All together, these results would seem to suggest that D2R stimulation may increases VTA-MSN synapse formation, allowing for greater GABAergic and dopaminergic influence, both ultimately increasing inhibition of D2+ MSNs.

Although progressive inhibition of D2+ MSNs of the NAc may be a key feature for quinpirole-mediated locomotor sensitization, there are many other agents which appear to be capable of producing or altering sensitization at sites which may be independent of dopaminergic sensitization. Serotonin 1A (5-HT1A) agents appear to be capable of inducing locomotor sensitization at high doses in a neural site which is independent from quinpirole. One study demonstrated that exposure to a high dose of a 5-HT1A agonist (8-OH-DPAT), as well as the D2R agonist quinpirole, are both capable of inducing locomotor sensitization in the open field (Alkhatib, Dvorkin-Gheva, & Szechtman, 2013). However, animals in the study did not display any evidence of cross-sensitization, meaning that sensitization to quinpirole did not bestow an increased sensitivity to 8-OH-DPAT, and vice versa.

Serotonergic receptors are somewhat ubiquitous throughout the CNS, and there are seemingly endless ways in which serotonergic activity could alter locomotion. Many 5-HT fibres project from the dorsal raphe (DR) nucleus, innervating a wide array of brain structures, including the VTA, NAc, and SNc (Hornung, 2003; Meyer & Quenzer, 2005). Neurons located in these areas will express many of the serotonergic receptor subtypes, including the 5-HT1A subtype (Azmitia & Segal, 1978; Köhler & Steinbusch, 1982). Many other structures both upstream and downstream from these structures in the movement pathway may also express serotonergic receptors, suggesting that 8-OH-DPAT-induced locomotor sensitization is likely influenced by serotonergic action in a wide array of neural structures.

The 5-HT1A receptor is one of the many serotonin receptor subtypes expressed throughout the body. It is of particular interest because of its possible involvement in the mechanism of action of selective serotonin reuptake inhibitors (SSRIs), as well as their ability to alter the course of psychostimulant-based locomotor sensitization (Carey, DePalma, Damianopoulos, Shanahan, Müller, & Huston, 2005; Hervás, Vilaró, Romero, Scorza, Mengod, & Artigas, 2000; Przegaliñski & Filip, 1997). Like the D2R, the 5-HT1A receptor is an inhibitory GPCR which upon activation will lead to decreased cAMP concentrations and hyperpolarization of the cell (Innis & Aghajanian, 1987). This inhibition will result in markedly different neurological effects depending on whether presynaptic (autoreceptors) or postysnaptic receptors are primarily stimulated. Stimulation of autoreceptors may be occurring at low doses of 8-OH-DPAT (lower than approximately 0.05 mg/kg, as suggested by Carey et al (2004a; Carey, Damianopoulous, & Shanahan, 2009). The autoreceptor activation bias occurring from low-doses of 8-OH-DPAT may be due to increased autoreceptor versus postsynaptic receptor sensitivity(Casanovas, Lesourd, & Artigas, 1997).

The effects of the 5-HT1A system on development of psychostimulant-induced sensitization remain somewhat of a mystery. High doses (1 mg/kg) of 8-OH-DPAT are capable of producing locomotor sensitization on their own (Alkhatib et al, 2013). Previous studies exploring the effects of high (post-synaptic) doses of 8-OH-DPAT on development of dopaminergic sensitization have varied considerably in their methods and results which has generated some debate into the relationship between the two systems. Studies conducted by Przegaliński et al (1997; 2000) have suggested that treatments of 8-OH-DPAT have arrested the development of locomotor sensitization in amphetamine treated rats. The studies have demonstrated that a wide array of 8-OH-DPAT doses inhibit the progression of amphetamine sensitization, an effect which is blocked by co-treatment of (S)-N-tert-butyl-3-(4-(2-methoxyphenyl)piperazin-l-yl)-2-phenylpropamine (WAY 100135), a 5-HT1A antagonist. The doses used in the study were all likely to be activating postsynaptic receptors as they ranged from 0.125 mg/kg to 0.5 mg/kg (Carey et al, 2005; Przegaliński et al, 2000; Przegaliñski & Filip, 1997). There are other studies which support this finding, a research team consisting of Tomiyama, Kimura, Maeda, Kannari, Mastsunaga, & Baba (2005) demonstrated that behavioural sensitization to L-DOPA (L-3,4-dihydroxyphenylalanine), a dopamine precursor, was arrested by 8-OH-DPAT, a result also supported by an earlier finding (Kannari, Yamato, Shen, Tomiyama, Suda, & Matsunaga, 2001). Others, however, have suggested something a little different. For example, a study by Carey, DePalma & Damianopoulous (2002), found that doses of 0.2 and 0.4 mg/kg of 8-OH-DPAT co-administered with cocaine enhanced maximal locomotor output of rats following several treatments compared to animals just receiving cocaine. Like the other studies previously mentioned, the authors did note that there was an initial inhibition of sensitization of cocaine-induced locomotion, but this effect dissipated and then reversed over repeated injections. This finding has also been supported in follow-up studies by the same group of researchers using the same doses of cocaine (10 mg/kg) and 8-OH-DPAT (0.2 mg/kg) (Müller, Carey, Salloum, & Huston, 2003).

While the effects of higher (postsynaptic) doses of 8-OH-DPAT doses on psychostimulant-induced sensitization remains somewhat unclear, the effect of low (presynaptic) doses seems to slow the rate of dopaminergic locomotor sensitization. A study by Carey et al (2005) demonstrated that low (presynaptic) doses of 8-OH-DPAT (0.05 mg/kg) slowed the rate of locomotor sensitization to cocaine. The researchers also showed that the presynaptic dose of 8-OH-DPAT suppressed spontaneous locomotor activity compared to controls. Suppression of locomotor activity from low doses (equal to or less than 0.05 mg/kg) has also been found in other studies by the same researchers (Carey et al, 2004a, Carey et al, 2004b). The neural mechanisms underlying the effects described are, at the present moment, very unclear. Unlike the dopaminergic system, serotonergic innervation is much more extensive, and 5HT1A receptors are relatively ubiquitous throughout the CNS (Haleem, 2013, Meyer & Quenzer, 2005).

Although the potential action sites for 5-HT1A drugs to exert their locomotor altering effects seem almost limitless, the evidence provided by Alkhatib et al (2013), suggests that 8-OH-DPAT must be inducing sensitization by acting on areas that are either independent from quinpirole, or further downstream in the neural pathways responsible for quinpirole-induced locomotor sensitization. The study by Alkhatib et al (2013), however, did have two important caveats. First, the study only examined the effects of sensitization to either quinpirole or 8-OH-DPAT; they did not explore how the dynamics of sensitization was altered by repeated co-administration of the two drugs. Also, the study did not establish a dose-response relationship for the two drugs, only one dose of each of the drugs was used for the study.

The current study seeks to explore the two caveats from the Alkhatib et al (2013) study and will further probe the relationship between the dopaminergic and serotonergic systems responsible for sensitization. The current study will establish a dose-response relationship for sensitization to both quinpirole and 8-OH-DPAT through repeatedly co-administering various doses (including both presynaptic, and postsynaptic doses) of the two drugs to Long Evans rats and testing for locomotor activity. This will be in contrast to many studies which have used general dopaminergic agonists in conjunction with 5-HT1A agonists. Much like the Alkhatib et al study, drug challenges for each of the drugs will be employed to examine how sensitization alters locomotor response to acute challenges of each of the two drugs. Our hypothesis is that if animals are co-administered 8-OH-DPAT and quinpirole, the 8-OH-DPAT should attenuate sensitization to quinpirole. It is predicted that locomotor sensitization to quinpirole and 8-OH-DPAT should occur at high doses, and the doses of 8-OH-DPAT should slow the rate of quinpirole sensitization. Rats sensitized to quinpirole and/or 8-OH-DPAT should present higher locomotor activity when they are acutely challenged to the drug(s) in which they were sensitized to. Both quinpirole and 8-OH-DPAT should exert their effects by acting through separate neural mechanisms, and there should be no evidence of cross-sensitization.

**Method**

**Subjects**

Subjects were 215 male Long-Evans rats (Charles River, St Constant, Quebec, Canada), weighing approximately 250–300 g at the start of the experiments. Rats were housed individually in cages measuring 35 x 30 x 16 cm. The colony room was climate-controlled (temperature was maintained at 22 oC), with a 12 h light/dark cycle (lights on at 0700). All experiments were conducted during light hours. Upon arrival, rats were allowed to acclimatize to the colony room for 1 week, and then handled for 2-3 minutes daily for 5 days prior to the start of the study. Animals were provided with *ad libitum* access to food and water and were all housed and tested in compliance with guidelines described in the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

**Drugs**

Quinpirole hydrochloride and 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) were dissolved in separate 0.9% (physiological) saline (NaCl) solutions and injected subcutaneously under the nape of the neck at a volume of 1.0 mL/kg. Rats were injected and tested twice weekly until termination of the experiments, and received 10 injections of both quinpirole (administered first) and 8-OH-DPAT. Animals were then subjected to three drug challenges (vehicle, 8-OH-DPAT, and quinpirole).

A previous study has demonstrated that following subcutaneous injection of quinpirole (0.125 mg/kg), there is a steady rise in locomotor activity, followed by plateau at approximately 40 minutes. Decline in locomotion occurs approximately 90 minutes post injection. In contrast, low doses of quinpirole (0.03 mg/kg) produce maximal inhibition lasting from 5 to 25 minutes post-injection (Eilam & Szechtman, 1989). For subcutaneous injections of 0.25 mg/kg 8-OH-DPAT in rats, maximal behavioural responses occur approximately 5 minutes post injection, and disappear after 30 minutes. The half-life in the striatum is approximately 31 minutes, with half-lives in the brain stem slightly higher (41 minutes) and plasma slightly lower (27 minutes) (Yu & Lewander, 1997). Given this information, we feel that a 1 hour testing period will be sufficient to capture the majority of the response profiles of both drugs.

**Testing Apparatus**

 The testing room was located down the hall from the colony room and was climate controlled to the same temperature (22 oC) as the colony room. The room consisted of 10 empty Plexiglas activity chambers (measuring 40 x 40 x 35 cm). These chambers were interfaced to a Digiscan 16 monitor and a computer that provided automated recording of locomotor activity using VersaMax software (AccuScan Instruments, Columbus, OH). Infrared photobeams surrounded the perimeters of the chambers, and broken beams generated horizontal activity measures which included total distance travelled. Chambers were covered with Plexiglas ventilated lids, which were taped down during testing with masking tape to prevent animals from escaping.

**Design**

Due to technical limitations which prevented running all rats concurrently, rats were tested in three successive replicates. The entire study consisted of two portions: a sensitization phase and a challenge phase. For the sensitization phase, assignment to treatment groups was quasi-random (based on weight) such that rats in every treatment group had approximately equal weights prior to the start of the study.

The sensitization phase was constructed as a 4 x 4 factorial design, repeated over 10 testing periods to examine the effects of 8-OH-DPAT on quinpirole sensitization. *Between-factors* variables were “QNP Dose” (0 vs 0.03125 vs 0.0625 vs 0.125 mg/kg of quinpirole hydrochloride), and “DPAT Dose” (0 vs 0.03125 vs 0.0625 vs 0.125 mg/kg of 8-OH-DPAT hydrobromide). The *between-factors* variables were fully crossed to create 16 treatment groups (N ranging from 11-14 in experimental groups, and 23 for saline-saline controls). The doses of quinpirole were selected to ensure that there was one inhibitory dose, a weakly excitatory dose, and an excitatory dose of quinpirole, as demonstrated in previous studies (Perrault et al, 2006; Tucci et al, 2013; Tucci et al, 2014). The doses of 8-OH-DPAT were selected to ensure that there was a range of doses which included at least one dose (0.0625 mg/kg) capable of altering locomotor properties (Tucci et al, 2014). *Within-factor* variable was “Injection” (amount of treatments the animal has received). We elected to use 10 repeated injections administered bi-weekly because the behavioural effects of chronic quinpirole treatment plateau after 8–10 drug injections administered 2–8 days apart (Szechtman et al, 1994). The behavioural effects of 8-OH-DPAT may plateau much sooner, possibly around injection 4 or 5 (Alkhatib et al, 2013; Tucci et al, 2014).

For the challenge phase of the study all rats were reassigned to challenge groups for Injections 11-13. During this phase of the study, the injection and testing regimen remained the same (one hour testing period, bi-weekly injections), however rats received each of the three challenges (0.9 % saline, 0.1 mg/ kg of quinpirole hydrochloride, and 0.1 mg/kg of 8-OH-DPAT hydrobromide). Saline challenges were all conducted during Injection 11 (all rats received saline), but quinpirole and 8-OH-DPAT challenges took place during Injections 12, and 13, with all rats receiving both challenges. Treatment assignment for Injections 12, and 13 was quasi-random, based on weight, and such that sensitization phase treatment groups were balanced across the two injections, with half receiving a quinpirole challenge first, and half receiving an 8-OH-DPAT challenge first.

The challenge phase was constructed as a 4 x 4 factorial design, conducted over 3 challenge injections (vehicle, 8-OH-DPAT, and quinpirole) to test for drug cross-sensitization. *Between-factors* variables were “QNP Dose” from the sensitization phase (0 vs 0.03125 vs 0.0625 vs 0.125 mg/kg of quinpirole hydrochloride), and “DPAT Dose” from the sensitization phase (0 vs 0.03125 vs 0.0625 vs 0.125 mg/kg of 8-OH-DPAT hydrobromide). *Between-factors* variables were fully crossed to create 16 treatment groups. *Within-factor* variable was “Drug Challenge” (which challenge the animal received: 0.9 % saline, 0.1 mg/ kg of quinpirole hydrochloride, or 0.1 mg/kg of 8-OH-DPAT hydrobromide).

**Procedure**

On the day of testing, all animals were weighed at the beginning of the day to determine the appropriate drug solution volume for injection. 8 or 9 animals were brought from the colony room in their home cages and transported to testing room. Animals were then removed from their cages, injected with the appropriate doses of drug, and immediately placed in the testing chambers, and their locomotor activity was measured for 1 hour. Each animal was tested in the same chamber, at the same time throughout the study. Testing chambers were thoroughly cleaned with a 50:50 solution of Windex in water following each use.

**Data Analysis**

The first research question for the current study is whether the drug 8-OH-DPAT, a 5-HT1A agonist alters the locomotor properties characteristic of quinpirole sensitization, a D2R/D3R agonist. The experiment tests the hypothesis that repeated injections of high doses (0.125 mg/kg) of each of the two drugs should produce locomotor sensitization, and 8-OH-DPAT should arrest the rate of quinpirole sensitization. To test this hypothesis, data was analyzed by a 4 x 4 ANOVA with repeated measures (a split-plot ANOVA) to test effects of the independent variable “Injection” (*within subjects factors*), “QNP Dose,” and “DPAT Dose” (*between subjects factors*) on dependent variable “Total Distance Travelled.” *Post-hoc* Duncan’s multiple range (DMR) test was used to evaluate group differences following significant *main or interaction effects* (*p* < 0.05).

The second research question examined if animals responded differently to acute drug challenges following sensitization. A third question was whether the drugs quinpirole and 8-OH-DPAT are acting on different neural sites. To answer these questions, animals were subjected to 3 drug challenges. The experiment tested the hypothesis that animals sensitized to quinpirole and/or 8-OH-DPAT should respond at a higher level (and display greater locomotor activity) when challenged with the drug(s) they were sensitized to compared to saline controls that received an acute injection of the challenge drug. The other hypothesis is that the two drugs are exerting their effects by acting on independent neural sites. To test this hypothesis, data was analyzed by a 4 x 4 ANOVA with repeated measures (a split-plot ANOVA) to test the effects of independent variable “Drug Challenge” (*within subjects factor*), “QNP Dose,” and “DPAT Dose” (*between subjects factors*) on dependent variable “Total Distance Travelled.” Significant results from the ANOVA (*p* < 0.05) were followed-up with *post-hoc* DMR tests.

**Results**

**Sensitization**

A split-plot ANOVA was conducted for dependent variable “Total Distance Travelled” with *within subjects factor* “Injection,” and *between subjects factors* “QNP Dose” and “DPAT Dose” to examine how repeated doses of quinpirole and 8-OH-DPAT affected total locomotor activity. Mauchly’s Test of Sphericity was significant: χ2(35) = 1187, *p* < 0.001, so Greenhouse-Geisser values were used to correct degrees of freedom (ε = 0.31) where appropriate. A test of the standardized skewness indicated that the data were normally distributed.

*Total Locomotor Activity*

The split-plot ANOVA revealed a significant main effect for *between subjects factor* QNP dose (*F*(3, 199) = 79.7, *p* < 0.001, ηp2 = 0.53). Results from a *post-hoc* DMR test demonstrated that the low quinpirole dose (0.03125 mg/kg) produced significantly lower locomotor activity (and thus was inhibitory) compared to controls receiving only saline. In contrast, the high dose of quinpirole (0.125 mg/kg) produced significantly higher locomotor activity (and thus was stimulatory) compared to controls receiving no quinpirole (see Table 1).

*Table 1.* High doses of quinpirole and 8-OH-DPAT produced excitation over the 10 injections. Data are presented as mean distance travelled (m) ± SEM.

|  |  |  |
| --- | --- | --- |
| Drug | Dose (mg/kg) | Mean distance travelled (m) ± SEM |
| Quinpirole  | 0 | 72.8 ± 6.42 |
|  | 0.03125 | 30.8 ± 6.91^ |
|  | 0.0625  | 66.1 ± 6.77 |
|  | 0.125  | 170 ± 6.99\* |
| 8-OH-DPAT | 0  | 76.5 ± 6.27  |
|  | 0.03125 | 74.1 ± 6.91 |
|  | 0.0625 | 81.9 ± 6.84 |
|  | 0.125 | 107 ± 7.05\* |

For quinpirole, the low dose was significantly lower than the control dose (0 mg/kg) which is indicated by ^. The high dose of quinpirole was significantly greater than the control dose which is indicated by \*. For 8-OH-DPAT, the high dose was significantly greater than the control dose (0 mg/kg) which is indicated by \*.

The split-plot ANOVA also revealed a significant main effect for *between subjects factor* DPAT Dose (*F*(3, 199) = 4.83, *p* = 0.003, ηp2 = 0.07). The *post-hoc* DMR test showed that the high dose of DPAT (0.125 mg/kg) produced significantly higher locomotor activity compared to controls receiving no DPAT. In other words, the high DPAT dose was stimulatory. In contrast to quinpirole, there was no apparent inhibition of locomotion with any of the doses of 8-OH-DPAT (see Table 1).

There was a significant interaction between QNP dose and DPAT dose: *F*(9, 199) = 2.09, *p* = 0.03, ηp2 = 0.09. This result shows that there was an interaction between the two drugs with doses of 8-OH-DPAT, despite being stimulatory on rat locomotion in the absence of quinpirole, having attenuated the stimulatory effects of quinpirole when the two drugs were co-administered (see Table 2).

*Table 2.* 8-OH-DPAT attenuated quinpirole sensitization across the 10 injections. Data are presented as mean total distance travelled (m) ± SEM.

|  |  |  |
| --- | --- | --- |
| Quinpirole Dose (mg/kg) | 8-OH-DPAT dose (mg/kg) | Mean distance travelled (m) ± SEM  |
| 0 | 0 | 40.7 ± 8.68 |
|  | 0.03125 | 59.7 ± 13.9 |
|  | 0.0625  | 78.1 ± 13.9 |
|  | 0.125  | 112 ± 13.9 |
| 0.03125 | 0  | 20.8 ± 13.9  |
|  | 0.03125 | 25.4 ± 13.9 |
|  | 0.0625 | 29.6 ± 13.4 |
|  | 0.125 | 47.5 ± 13.9 |
| 0.0625 | 0  | 47.1 ± 13.4  |
|  | 0.03125 | 65.5 ± 13.4 |
|  | 0.0625 | 67.6 ± 13.4 |
|  | 0.125 | 84.1 ± 13.9 |
| 0.125 | 0  | 198 ± 13.4 |
|  | 0.03125 | 146 ± 13.9^ |
|  | 0.0625 | 152 ± 13.9^ |
|  | 0.125 | 185 ± 14.6 |

For the high dose of quinpirole crossed with 8-OH-DPAT, the low dose and medium dose of 8-OH-DPAT produced significant attenuationof the quinpirole response (denoted by ^).

The split-plot ANOVA also revealed a significant main effect for *within subjects* variable “Injection” on dependent variable Total Distance Travelled: *F*(2.51, 499) = 233, *p* < 0.001, ηp2 = 0.54, meaning that rat locomotion changed over the course of the 10 testing periods. There was a significant interaction between Injection and QNP dose (*F*(7.52, 499) = 92.2, *p* > 0.001, ηp2 = 0.58), and between Injection and DPAT dose (*F*(7.52, 499) = 1.98, *p* = 0.05, ηp2 = 0.03). There was a three-way interaction that approached significance between Injection, QNP dose, and DPAT dose: *F*(22.6, 499) = 1.41, *p* = 0.09, ηp2 = 0.06, such that doses of 8-OH-DPAT may have affected the rate of quinpirole sensitization (see Figure 2).

In conclusion, the total distance travelled in the chambers changed as a factor of QNP dose, and DPAT dose, meaning that the drugs altered locomotor activity (see Figure 2) at the high (0.125 mg/kg) doses of both drugs (confirmed by a DMR test). A possible three-way interaction suggests that the relationship between co-administration of the high doses of quinpirole and 8-OH-DPAT may change over subsequent injections (see Figure 3), such that initially, 8-OH-DPAT is slowing the acquisition of locomotor sensitization to quinpirole. This effect is perhaps short-lived, as there appears to be no discernable differences in the plateau levels of locomotor activity following sensitization (see Figure 3).

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*Figure 2.* The effect of 8-OH-DPAT on quinpirole locomotor sensitization for the drug sensitization phase (Injections 1 through 10). QNP dose and DPAT dose each had four different treatment levels (0, 0.03125, 0.0625, and 0.125 mg/kg) which were fully crossed to create 16 treatment groups. Low doses of quinpriole were inhibitory. High doses of both 8-OH-DPAT and quinpirole produced sensitization. Doses of 8-OH-DPAT appeared to slow the rate of quinpirole sensitization initially, but this effect dissipated by the 10th injection (see Figure 3). Locomotor activity (*y*-axis) is presented as mean total distance travelled in activity chambers over 60 minutes ± SEM. Quinpirole doses are displayed in the columns (Quinpirole Vehicle = 0 mg/kg, Quinpirole Low Dose = 0.03125 mg/kg, Quinpirole Medium Dose = 0.0625 mg/kg, Quinpirole High Dose = 0.125 mg/kg). The 8-OH-DPAT doses are represented by the symbols indicated in the figure (DPAT Vehicle = 0 mg/kg, DPAT Low Dose = 0.03125 mg/kg, DPAT Medium Dose = 0.0625 mg/kg, DPAT High Dose = 0.125 mg/kg). Individual lines represent data for the quinpirole doses crossed with the 8-OH-DPAT doses. N ranges from 11 to 24 per treatment group.



*Figure 3.* Possible three-way interaction between Injection, DPAT dose, and quinpirole dose showing that a high 8-OH-DPAT dose slows the rate of locomotor sensitization to quinpirole initially (left), but the effect dissipates after repeated injections (right). \* Indicates for the given injection, locomotor activity is significantly lower compared to animals receiving the high dose of quinpirole (0.125 mg/kg) plus vehicle. Data presented here is for Injection 4 (left) and 10 (right) for the high dose (0.125 mg/kg) of quinpirole crossed with all levels of 8-OH-DPAT (0, 0.1325, 0.0625, and 0.125 mg/kg). Locomotor activity (*y*-axis) is presented as mean total distance travelled in activity chambers over 60 minutes ± SEM. HQV = 0.125 mg/kg quinpirole plus vehicle (N = 13), HQLD = 0.125 mg/kg quinpirole plus 0.03125 mg/kg 8-OH-DPAT (N = 12), HQMD = 0.125 mg/kg quinpirole plus 0.0625 mg/kg 8-OH-DPAT (N = 12), HQHD = 0.125 mg/kg quinpirole plus 0.125 mg/kg 8-OH-DPAT (N = 11).

**Drug Challenges**

A split-plot ANOVA was conducted for dependent variable “Total Distance Travelled” with *within subjects factor* “Drug Challenge,” and *between subjects factors* “QNP dose” and “DPAT dose” to examine how repeated doses of quinpirole and 8-OH-DPAT affected total locomotor activity when challenged with vehicle, quinpirole, and 8-OH-DPAT. Mauchly’s Test of Sphericity was significant: χ2(2) = 140, *p* < 0.001, so Greenhouse-Geisser values were used to correct degrees of freedom (ε = 0.66) where appropriate. A test of the standardized skewness indicated that the data were normally distributed.

*Total Locomotor Activity*

The split-plot ANOVA revealed a significant main effect for *between subjects factor* DPAT Dose (*F*(3, 192) = 3.69, *p* < 0.05, ηp2 = 0.04), meaning that prior exposure to DPAT had an effect on locomotor response. The *post-hoc* DMR test showed that the medium (0.0625 mg/kg) and high dose of DPAT (0.125 mg/kg) produced significantly higher locomotor activity than animals receiving the 0 dose of DPAT.

There was an interaction between QNP dose and DPAT dose: *F*(9, 192) = 1.80, *p* = 0.08, ηp2 = 0.08, which approached significance, suggesting that sensitization to quinpirole and 8-OH-DPAT may affected the locomotor performance across the challenges which was different from the main effects of the two drugs.

The ANOVA also revealed a significant main effect for *within subjects* variable “Drug Challenge” on dependent variable Total Distance Travelled: *F*(1.32, 253) = 253.0, *p* < 0.001, ηp2 = 0.57.

There was a significant interaction between, Drug Challenge and QNP dose (*F*(3.95, 253) = 59.5, *p* > 0.001, ηp2 = 0.48) and Drug Challenge and DPAT dose (*F*(3.95, 253) = 3.51, *p* = 0.009, ηp2 = 0.05). Prior exposure to quinpirole affected animals such that they displayed greater locomotor activity across the drug challenges, as did prior exposure to 8-OH-DPAT for the 8-OH-DPAT challenge. There was a significant three-way interaction between Drug Challenge, QNP dose, and DPAT dose: *F*(11.9, 253) = 2.67, *p* = 0.002, ηp2 = 0.11. The presence of this three-way interaction demonstrates that 8-OH-DPAT altered baseline responding rates (see Table 3). In other words, rats sensitized to 8-OH-DPAT had baseline locomotor levels altered such that when they were challenged with saline, they displayed greater activity than 8-OH-DPAT naïve rats. The three-way interaction also demonstrates that 8-OH-DPAT sensitization has quelled the acute quinpirole response in rats sensitized to quinpirole. Said differently, this means that when rats are co-sensitized to quinpirole and 8-OH-DPAT, they display a blunted locomotor response when acutely challenged with quinpirole (see Figure 4).

*Table 3.* The effects of 8-OH-DPAT and quinpirole sensitization on basal locomotion. Animals were injected repeatedly with various doses of 8-OH-DPAT and quinpirole over 10 injections, and then challenged with saline to test for basal locomotion. Data are presented as mean total distance travelled (m) ± SEM

|  |  |  |
| --- | --- | --- |
| Sensitized Drug | Dose (mg/kg) | Mean distance travelled (m) ± SEM  |
| 8-OH-DPAT | 0 | 48.1 ± 2.92 |
|  | 0.03125 | 54.8 ± 3.16 |
|  | 0.0625  | 63.8 ± 3.13\* |
|  | 0.125  | 71.8 ± 3.23\* |
| Quinpirole | 0 | 52.9 ± 2.99 |
|  | 0.03125 | 49.3 ± 3.26 |
|  | 0.0625 | 63.8 ± 3.10^ |
|  | 0.125 | 72.4 ± 3.20^ |

Higher 8-OH-DPAT doses produced increases in basal locomotion (denoted by \*) when challenged with saline. The highest two dose of quinpirole produced increases in basal locomotion (denoted by ^) when challenged with saline



*Figure 4.* The effect of sensitization to regimes of 8-OH-DPAT and quinpirole on locomotor response following a challenge of 0.1 mg/kg of quinpirole (right), and 0.1 mg/kg of 8-OH-DPAT (left) occurring at Injections 12 and 13. QNP dose and DPAT dose each had four different treatment levels (0, 0.03125, 0.0625, and 0.125 mg/kg) which were fully crossed to create 16 treatment groups animals were repeatedly injected with drug for Injections 1-10, then challenged with 8-OH-DPAT and quinpirole (shown here). Animals sensitized to quinpirole displayed higher locomotion than did controls sensitized to vehicle when challenged with quinpirole (denoted by #, shown on right of graph). Animals sensitized to 8-OH-DPAT displayed higher locomotion than did controls sensitized to saline when challenged with 8-OH-DPAT (denoted by \*, shown on left of graph). Animals co-sensitized to 8-OH-DPAT and quinpirole had lower locomotor activity than those sensitized to only quinpirole (denoted by ^, shown on right of graph). Cross-sensitization is not apparent, as animals sensitized to quinpirole only did not respond different than saline controls when challenged with 8-OH-DPAT. Similarly, animals sensitized to 8-OH-DPAT did not respond different than saline controls when challenged with quinpirole. Locomotor activity (*y*-axis) is presented as mean total distance travelled in activity chambers over 60 minutes ± SEM. VV = vehicle plus vehicle (N = 24), VLD = vehicle plus 0.03125 mg/kg 8-OH-DPAT (N = 12), VMD = vehicle plus 0.0625 mg/kg 8-OH-DPAT (N = 12), VHD = vehicle plus 0.125 mg/kg 8-OH-DPAT (N = 12), HQV = 0.125 mg/kg quinpirole plus vehicle (N = 13), HQLD = 0.125 mg/kg quinpirole plus 0.03125 mg/kg 8-OH-DPAT (N = 12), HQMD = 0.125 mg/kg quinpirole plus 0.0625 mg/kg 8-OH-DPAT (N = 12), HQHD = 0.125 mg/kg quinpirole plus 0.125 mg/kg 8-OH-DPAT (N = 11).

**Discussion**

 The purpose of the current study was to examine the relationship between the dopaminergic (D2R/D3R) and serotonergic (5-HT1A) systems and how they interact to produce behavioural (locomotor) sensitization. The first portion of the study probed the generation of locomotor sensitization by repeatedly co-administering the dopaminergic agonist quinpirole and the serotonergic agonist 8-OH-DPAT over 10 injections, testing for locomotor activity following each subcutaneous injection. The second portion of the study examined how animals responded to acute drug challenges following sensitization. For this phase, animals were exposed to acute challenges of saline, 8-OH-DPAT (0.1 mg/kg), and quinpirole (0.1 mg/kg). Saline challenges occurred at Injection 11, whereas quinpirole and 8-OH-DPAT challenges were randomized over the 12th and 13th injections. All animals received all injections. We hypothesized that 8-OH-DPAT should attenuate sensitization to quinpirole. Our first prediction was that 8-OH-DPAT would slow the rate of quinpirole sensitization. This prediction was confirmed, as repeated 8-OH-DPAT injections slowed the rate of quinpirole sensitization (see Figure 3). Our second prediction was that animals sensitized to quinpirole and/or 8-OH-DPAT should present higher locomotor activity when they are acutely challenged to the drug(s) in which they were sensitized to. This prediction was confirmed as animals who were sensitized to quinpirole displayed higher locomotor activity than controls when they were acutely challenged with quinpirole. Animals sensitized to 8-OH-DPAT also displayed higher locomotor activity than controls when they were acutely challenged with 8-OH-DPAT (see Figure 4). Our prediction was that 8-OH-DPAT and quinpirole should be exerting their effects on separate neural areas and thus there should be no evidence of cross-sensitization. This prediction was also confirmed as animals sensitized to quinpirole did not sensitize to 8-OH-DPAT and vice-versa (the high quinpirole + vehicle group did not display a different locomotion level compared to the vehicle + vehicle treatment group when challenged with 8-OH-DPAT; see Figure 4).

There is a large body of evidence suggesting that 5-HT1A receptors are capable of altering locomotion (Alkhatib et al, 2013; Carey, DePalma, Damianopoulous, Müller, & Huston, 2004; Carey et al, 2004), as well as altering the course of dopaminergic sensitization. Although the effects of the 5-HT1A system on psychostimulant-induced sensitization remain controversial, there is reasonable evidence to suggest that 8-OH-DPAT is capable of arresting the rate of locomotor sensitization to dopamine agonists (Carey et al, 2005; Przegaliński et al, 2000; Przegaliñski & Filip, 1997). Our results confirmed this prediction, as all doses of 8-OH-DPAT used in the study slowed the rate of quinpirole sensitization (see Figure 2, right graph). What was perhaps more curious, is the fact that all of the doses of 8-OH-DPAT were able to produce this effect, albeit with varying degrees of efficacy. This finding challenges the notion that low doses (less than 0.05 mg/kg) of 8-OH-DPAT may be preferentially activating presynaptic receptors, whereas higher doses may be preferentially activating postsynaptic receptors (Carey et al, 2004a; Carey et al, 2009; Casanovas, Lesourd, & Artigas, 1997).

 Further challenging the idea that low doses of 8-OH-DPAT activate presynaptic receptors and high doses activate postsynaptic receptors, is the absence of a biphasic dose-response relationship for locomotor output. The locomotor response for 8-OH-DPAT was entirely dose dependent (there was a significant main effect for 8-OH-DPAT for the sensitization phase), with no doses producing locomotor inhibition (see Figure 2, left graph). It appears unlikely that even higher doses of 8-OH-DPAT than the ones used in this study would warrant a different conclusion, as the study by Alkhatib et al (2013) used an extremely high dose of the drug (1 mg/kg), finding that locomotor sensitization was rapid and profound when animals were repeatedly injected and tested in the open field on the same schedule as the one used in the current study. Although there may be small variations in preferential activation of the two types of receptors based on drug dose, they are not detectable from the data we obtained in this study. Based on the results found in the current study, it seems likely that there is no substantial preferential activation of presynaptic versus postsynaptic 5-HT1A receptors; instead it seems plausible that all the doses of 8-OH-DPAT are producing a compound effect, activating both types of receptors relatively equally. We must then conclude that an overall serotonergic tone generated by 8-OH-DPAT is responsible for the interference with the acquisition of quinpirole sensitization.

 Our results are also supported by the work of Przegaliñski & Filip (1997) who found that animals repeatedly co-exposed to amphetamine and 0.125 mg/kg of 8-OH-DPAT were delayed in their acquisition in locomotor sensitization compared to animals only sensitized to amphetamine. Amphetamine functions as an indirect dopamine agonist (it release dopamine from pre-synaptic terminals), activating all dopaminergic receptors (unlike the current study which employs a selective dopaminergic agonist), suggesting that the ability of 5-HT1A agonists to arrest dopaminergic locomotor sensitization may be more general and not only limited to the D2Rs and/or D3Rs. This finding also may extend to the lower doses of 8-OH-DPAT used in the current study by Carey et al (2005) who found the same thing as Przegaliñski and Filip (1997) using a lower dose of 8-OH-DPAT (0.05 mg/kg) and cocaine rather than amphetamine. Like our study, these doses were repeatedly administered, with locomotor activity tested after each injection.

 The possible mechanisms underlying 8-OH-DPAT’s slowing of quinpirole sensitization are quite numerous, and unfortunately, based on our results we are not able to make any concrete claims surrounding a neural mechanism. We did, however, gain some valuable insight from the challenge phase of the study, for example, we found that sensitization to 8-OH-DPAT blunted the acute quinpirole response in quinpirole-sensitized animals. It appears from the figure that this may be occurring in a dose-dependent manner (see Figure 4, right). In other words, animals sensitized to quinpirole (high dose) along with 8-OH-DPAT had lower locomotor activity when they were challenged with quinpirole, and this effect was stronger with higher doses of 8-OH-DPAT used in the sensitization phase. Along with further challenging the dichotomy of presynaptic vs postsynaptic 5-HT1A preference based on 8-OH-DPAT dose, this illustrates that 8-OH-DPAT has initiated some long lasting neural changes to the dopaminergic system which is not enabling it to sensitize to maximal levels. This finding raises an important question relating to the three-way interaction found between our three variables in the sensitization phase (Injection-DPAT-quinpirole interaction), namely, why does the DPAT-quinpirole interaction fade with repeated injections? It seems that while repeated doses of 8-OH-DPAT prevented complete quinpirole sensitization, this effect may have been masked during the plateau of the sensitization phase of the study. The evidence for this comes from the challenge phase of the study in which we found that quinpirole sensitization did not interfere with the acute 8-OH-DPAT response, and animals exposed to repeated injections of 8-OH-DPAT displayed greater locomotor activity when challenged with 8-OH-DPAT compared to controls. This effect also appears to be dose-dependent, as higher doses of repeated 8-OH-DPAT injections led to a higher locomotor response to the 8-OH-DPAT challenge. Based on all this, it appears plausible that the plateau phase of the animals sensitized to the high dose of quinpirole with the various doses of 8-OH-DPAT is made up of two components: the quinpirole response, and the DPAT response, which appear to be independent. In other words, what an animal lacks in a quinpirole response may be made-up by an enhanced 8-OH-DPAT response (see Figure 4).

 Our third prediction was that quinpirole and 8-OH-DPAT should be producing their effects by acting through separate neural mechanisms, and thus there should be no evidence of cross-sensitization. This is important to establish because it would establish that the drugs are not simply interfering with each other by competing for cellular resources (both drugs are inhibitory GPCRs, sharing many of the same downstream proteins) in order to exert their effects. The results we have obtained in our study appear to support our prediction, as there does not appear to be any evidence of cross-sensitization. This conclusion comes on the basis of multiple findings, but perhaps most importantly, sensitization to quinpirole did not increase the acute 8-OH-DPAT response and vice-versa (see Figure 4). Said differently, animals sensitized to one of the two drugs did not become sensitized to the other without previously being exposed to it. More support for independent neural mechanisms comes from the finding that quinpirole did not interfere with 8-OH-DPAT sensitization (as animals co-sensitized to the two drugs still displayed evidence of sensitization when challenged with 8-OH-DPAT).

 If the two drugs are exerting their effects by acting independently, this gives us some information about the mechanism of action of 8-OH-DPAT. Based on our findings, 8-OH-DPAT is producing sensitization through neural mechanisms which are distinct from that of quinpirole. Quinpirole appears to be inducing sensitization by operating through the mesolimbic and/or the nigrostriatal pathways. This means that were 8-OH-DPAT to be acting on any of these sites in the same manner as quinpirole, animals sensitized to 8-OH-DPAT should have had increased locomotor activity when challenged to quinpirole, which was not the case. One could still make the argument that although 8-OH-DPAT is inducing sensitization in a manner which is distinct from quinpirole, it is interfering with quinpirole sensitization by depleting cellular resources at the sites of quinpirole action. This conclusion seems unlikely, however, given the data we have obtained here. A shared cellular mechanism would enable 8-OH-DPAT to act on modifications made by quinpirole sensitization. Since we already have demonstrated that 8-OH-DPAT does not cross-sensitize to animals sensitized to quinpirole, and 8-OH-DPAT does not abolish quinpirole sensitization, it makes the prospect of shared neural mechanisms highly unlikely. Although the sensitization mechanisms appear to be separate, however, it does not discount the possibility that acute doses of 8-OH-DPAT may be interfering with acquisition of sensitization to quinpirole.

 The conclusion that quinpirole and 8-OH-DPAT are operating independently is supported by the study by Alkhatib et al (2013) who found no evidence of cross-sensitization in their study. In contrast to the current study, the study by Alkhatib et al (2013) did not co-administer both quinpirole and 8-OH-DPAT; rather, animals were sensitized to one drug or the other. Despite using a high dose of quinpirole, or a high dose of 8-OH-DPAT (1 mg/kg) to induce sensitization, animals in that study did not display any evidence of enhanced locomotion when they were acutely challenged with the opposite drug to which they were sensitized. With such a wide range of doses of 8-OH-DPAT and quinpirole employed across the Alkhatib et al (2013) study and the current study, we can be relatively confident in concluding that quinpirole and 8-OH-DPAT are inducing sensitization through independent mechanisms.

 As previously discussed, another finding in the Alkhatib et al (2013) study was that locomotor sensitization was possible not only with quinpirole, but 8-OH-DPAT as well. Our results support this finding, as animals that were repeatedly injected with the high dose of 8-OH-DPAT (0.125 mg/kg) displayed progressive increases in locomotor activity (see Figure 2). In contrast to other studies, we did not observe a decrease in locomotion following injections of low doses of 8-OH-DPAT (Carey et al, 2004a; Carey et al, 2004b; Carey et al, 2005). Instead, locomotion increased in a dose dependent manner; with the highest dose showing signs of sensitization (see Figure 2). The evidence of sensitization was not present only in the first phase of the study, it extended into the second (challenge phase of the study). Our third prediction stated that animals should respond at a higher level to controls when challenged with the drug(s) in which they were sensitized. In other words, an animal sensitized to quinpirole should display elevated locomotion when challenged with quinpirole compared to controls, and an animal sensitized to 8-OH-DPAT should display elevated locomotion compared to controls when challenged with 8-OH-DPAT. This finding was confirmed and further supports that both 8-OH-DPAT and quinpirole are capable of producing locomotor sensitization.

 The mechanism surrounding quinpirole sensitization has been well studied, and may be the result of a progressive increase in amount of post-synaptic receptors, and increased post-synaptic dendritic projections (Lalchandani et al, 2013). Our results add an important dimension to the body of research with the finding that basal locomotor levels are raised following sensitization (animals that were sensitized to quinpirole had significantly higher locomotion when challenged with saline compared to controls sensitized to saline). This finding does support the findings by Lalchandani et al (2013), as increased connections between the pre-synaptic dopaminergic neuron and post-synaptic neuron would enable more sites for neurotransmitter binding and thus a stronger post-synaptic effect.

 Less is known about the changes to the serotonergic network following sensitization, so our finding of increased basal locomotion following repeated 8-OH-DPAT injections lends some much needed information to the field. Rats repeatedly injected with 8-OH-DPAT had higher locomotion than controls following a saline challenge (see Figure 4). This increase in basal locomotion appears to be dose-dependent, with higher doses further increasing locomotion. Unfortunately, the results obtained in our study do not shed much light on the cellular mechanisms surrounding the change in basal locomotion following repeated 8-OH-DPAT. The effect could be due to a similar mechanism found by Lalchandani et al (2013) underlying the changes in dopaminergic connections following sensitization. However, the effect is likely not to be as strong because it would enable sensitization (which we only see in our highest 8-OH-DPAT dose). Another possible mechanism may be that there is a change in the relative levels of pre-synaptic to post-synaptic 5-HT1A receptors following repeated exposure.

The question of the underlying mechanism leading to increased basal locomotion would be an interesting one to probe in future studies. One component which the current study lacked was a binding assay to examine whether results were due to increased drug-receptor binding at specific sites. Possible sites of interest would include the NAc, SNc, and striatum. Such an addition would enable us to further assess the independence of the serotonergic and dopaminergic systems in inducing sensitization, as well as give more information to underlying cellular mechanisms following long term changes in locomotion and locomotor response to 5-HT1A and D2R agents. For a future study, it would be interesting to add this component to an open field study to examine other movement behaviours. This could be accomplished by fully crossing doses of 0 (control), and 0.125 mg/kg of 8-OH-DPAT and quinpirole and testing animals in the open-field in a repeated measures design. Such an experiment would examine how the dopamine sensitization altering properties of 8-OH-DPAT translate in the open field. The open field design would enable the dissection of behaviours related to obsessive compulsive disorder, as previously done in numerous studies (Dvorkin, Perreault, & Szechtman, 2006; Szechtman et al, 1998; Tucci et al, 2013). Based on our findings in the current study, the prediction would be that co-administration of 8-OH-DPAT along with quinpirole would arrest the rate of sensitization of some behaviours characteristic of obsessive compulsive disorder in an animal model. This test could be followed up with a binding assay as previously described. However, the current study offers a valuable contribution to the scientific community, as it presents a comprehensive analysis of the relationship of the dopaminergic and serotonergic systems and how they contribute to behavioural sensitization. The study employed a wide range of doses of both drugs to gain a full understanding of the relationship between the two systems. To further understand the cellular mechanisms surrounding the two systems and how they interact, binding assays will be necessary in future studies.

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