QUINPIROLE AND 8-OH-DPAT INDUCE COMPULSIVE CHECKING BEHAVIOR IN MALE RATS BY ACTING ON DIFFERENT FUNCTIONAL PARTS OF AN OCD NEUROCIRCUIT

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Running Title: Dopamine-Serotonin Interaction in OCD
Figures: 3; Tables: 1; Abstract: 213 words

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ABSTRACT

The study investigated whether the serotonin 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) can induce compulsive checking in a large open field, as does the dopamine D2/D3 receptor agonist quinpirole. To induce compulsive checking, male rats were exposed to 8 injections of either 8-OH-DPAT (1 mg/kg), quinpirole (0.2 mg/kg) or saline. Subsequently, to assess cross sensitization, rats received an acute challenge of 8-OH-DPAT or quinpirole. Results showed that treatment with 8-OH-DPAT induces compulsive checking and may have a stronger effect on this behavior compared to quinpirole. However, there was no cross sensitization between 8-OH-DPAT and quinpirole on measures of compulsive checking and locomotion. Moreover, the spatial distribution of locomotor paths in 8-OH-DPAT animals was more confined and invariant than in quinpirole rats; their rate of locomotor sensitization was also faster than in quinpirole animals. Thus, while 8-OH-DPAT and quinpirole can induce compulsive checking in a large open field, results suggest they do so differently. It is suggested that 8-OH-DPAT and quinpirole probably produce compulsive behavior by acting on different parts of a security motivation circuit underlying OCD. Quinpirole may induce compulsive checking behavior by directly driving dopaminergic activity mediating the motivational drive to check. Conversely, 8-OH-DPAT may perpetuate the activated motivational state by inhibiting the serotonergic negative feedback signals that normally deactivates the OCD circuit.

KEYWORDS

Compulsive checking behavior; dopamine-serotonin interaction; OCD; security motivation

INTRODUCTION

In the spontaneous alternation paradigm, the serotonin 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) (Yadin *et al*, 1991) and the dopamine D2/D3 receptor agonist quinpirole (Einat and Szechtman, 1995) induce perseverative behavior in rats. Specifically, rats treated with either 8-OH-DPAT or quinpirole show an increased tendency to repeat a choice of the same goal arm in a T-maze. This perseverative tendency is suggested to be analogous to the repetitive motor patterns seen in patients with obsessive-compulsive disorder (OCD) (Yadin *et al*, 1991).

In the quinpirole sensitization model of OCD, chronic administration of quinpirole to rats in a large open field induces compulsive checking behavior (Szechtman *et al*, 1998; Eilam and Szechtman, 2005; Szechtman and Eilam, 2005). Since both 8-OH-DPAT and quinpirole induce perseverative tendencies in the spontaneous alternation

paradigm, the question arises whether 8-OH-DPAT can also produce compulsive checking in a large open field, as quinpirole does. If so, do both drugs produce their effects through a common mechanism? To address these questions the present study compared the effects of 8-OH-DPAT and quinpirole on the induction of compulsive checking, followed by a test of cross sensitization.

Compulsive checking behavior in the rat is characterized by exaggerated preoccupation with one location in the environment, to which the animal returns repeatedly. Four lines of evidence suggest that this rat behavior constitutes a reasonable model of human OCD checking compulsions (reviewed in Man *et al*, 2004; Eilam *et al*, 2005; Joel, 2006; Korff and Harvey, 2006; Westenberg *et al*, 2007; Hoffman, 2011). First, the spatial-temporal structure of compulsive checking in the rat matches the salient performance features of an OCD compulsion (Szechtman *et al*, 1998), namely, an exaggerated preoccupation with the item(s) of concern, a ritual-like quality in motor performance and environmental dependence for display of the behavior. Second, the motivational basis of quinpirole-induced and OCD checking appear similar (Szechtman *et al*, 1998; Szechtman and Woody, 2004; Woody and Szechtman, 2005; Boyer and Lienard, 2006; Feygin *et al*, 2006; Whishaw *et al*, 2006), in that both represent an exaggerated form of normal checking of stimuli related to safety and security (the 'home base' in the case of the rat model). Third, compulsive checking in the rat is subject to similar modulation as OCD compulsions in that the performance of each is modified by external stimuli and can be temporarily suppressed (Ben Pazi *et al*, 2001; Szechtman *et al*, 2001; Zadicario *et al*, 2007). Finally, treatments that are therapeutically useful for OCD are also effective in attenuating quinpirole-induced compulsive checking, e.g. clomipramine (Szechtman *et al*, 1998; Foa *et al*, 2005), nicotine (Tizabi *et al*, 2002; Salin-Pascual and Basanez-Villa, 2003; Lundberg *et al*, 2004) and deep brain stimulation (Greenberg *et al*, 2006; Winter *et al*, 2008; Mundt *et al*, 2009; Djodari-Irani *et al*, 2011).

MATERIALS AND METHODS

SUBJECTS

A total of 36 experimentally naive Long-Evans male rats (Charles River, St Constant, Quebec, Canada), weighing 250–300 g at the start of the experiment, entered the study. Rats were housed individually in polyethylene cages (35 cm x 30 cm x 16 cm) lined with Tek-Fresh Laboratory bedding made from 100% reclaimed virgin wood pulp (Harlan Teklad, Madison, WI) in a temperature controlled (22 °C) colony room, maintained on a 12-hr light-dark cycle (lights on at 0700), with free access to food and water. Rats were allowed to acclimatize to the colony room for 1 week following arrival and were handled for 2–3 min daily for 5 days before the start of the

experiment. All treatment and testing were conducted during the light phase of the day-night cycle. Animals were 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-OH-DPAT hydrobromide (Sigma Aldrich) were dissolved in physiological saline and injected subcutaneously under the nape of the neck at a dose of 0.2 mg⁄kg and 1 mg⁄kg, respectively. The particular dose of quinpirole was selected because the drug reaches a maximum effect at a dose of about 0.2–0.5 mg/kg (Szechtman *et al*, 1994a; Szechtman *et al*, 1994b; Szumlinski *et al*, 1997; Perreault *et al*, 2005; Dvorkin *et al*, 2006). The selected dose of 8-OH-DPAT was similar to that used by Yadin et al. (Yadin *et al*, 1991) in the proposed spontaneous alternation model of OCD. Saline was administered at a volume of 1 mL/kg.

APPARATUS

Animals were tested after each injection in a large open field consisting of a solid surface table top (160 x 160 cm and 60 cm high); the distance from table edge to nearest wall was at least 0.64 m. The table top was constructed of material used in making kitchen counter-tops – it was smooth, non-porous, composed of unsaturated polyester and acrylic resin blends (Acryflek Industries), and had a custom blue color to facilitate the video detection of dark and white objects. Four small Plexiglas ⁄ glass boxes (approximately 8 x 8 x 7.5 cm) were present at the same fixed location of the open field throughout the study—two at the corners and two at locations near the center of the open field. The open field table surface was subdivided virtually into 25 rectangular places (locales) used to define the location of the animal in the field. The open field and objects were wiped clean after use by each rat with a diluted solution of an antibacterial cleaner (Lysol).

A camera affixed to the ceiling, providing a stationary top view of the entire open field and the rat, videotaped behavior continuously. Videotapes were converted to MPEG files (Canopus MPEGPro EMR realtime MPEG-1 MPEG-2 encoder) and these digitized videos were used to automatically track the trajectories of locomotion using EthoVision 3.1 (Noldus Information Technology bv, Netherlands) (Noldus *et al*, 2001; Spink *et al*, 2001). The spatial sensitivity of the tracking system was 8 x 8 mm ⁄ pixel, with a temporal resolution of 30 frames⁄s.

EXPERIMENTAL DESIGN AND PROCEDURE

The first part of the experiment (injections 1 to 8) evaluated the development of compulsive checking behavior induced by 8-OH-DPAT and quinpirole. 8-OH-DPAT was administered according to the treatment regimen of quinpirole (Szechtman *et al*, 1998; Dvorkin *et al*, 2006). The behavioral effects of chronic treatment with quinpirole reach a plateau at approximately 8 injections administered 2–8 days apart (Szechtman *et al*, 1994a; Szechtman *et al*, 1994b; Perreault *et al*, 2005; Dvorkin *et al*, 2006). The second part of the experiment (injections 9 to 10) assessed whether 8-OH-DPAT and quinpirole exhibit cross-sensitization. Each group received both 8-OH-DPAT and quinpirole challenges across injections 9 to 10, administered in random order.

At the start of the experiment, subjects were allocated at random into three treatment groups (N=12/group): 8-OH-DPAT (1 mg/kg), quinpirole (0.2 mg/kg) and saline (1 mL/kg). Each group was treated with 8 injections to monitor the development of compulsive checking. Rats were injected and tested on a twice-weekly schedule (e.g., every Monday and Thursday, or every Tuesday and Friday). On injection 9, half of the subjects in each treatment group received a quinpirole challenge, and the other half received an 8-OH-DPAT challenge. On injection 10, subjects that were administered quinpirole on injection 9 received 8-OH-DPAT, while those that received 8-OH-DPAT were administered quinpirole. Animals receiving each drug across injections 9-10 were grouped for analysis to give an N=12/group for each respective challenge.

On the day of testing, animals were weighed, transported in their home cage to an adjoining non-colony testing room, and administered the appropriate injection. Immediately afterwards, rats were placed onto the open field for 55 min and their behavior videotaped for offline data analysis. Each rat was tested throughout the study at its assigned time of day. After each use, the open fields were thoroughly cleaned with Lysol diluted with water.

BEHAVIORAL ANALYSIS

EthoVision 3.1 software was used to extract from the digitized video recordings of the rat's path plots the time series of *x*, *y* coordinates as described previously (Dvorkin *et al*, 2006; Dvorkin *et al*, 2010). Digitized tracking data were pre-processed to remove noise (by applying appropriate filters to smooth the *x*, *y* coordinates; (Hen *et al*, 2004)), and the obtained coordinates were divided into episodes of forward locomotion (called progression) and episodes of small movements or immobility (called lingering), as described previously (Golani *et al*, 1993; Drai *et al*, 2000; Drai and Golani, 2001). These values were used to calculate the compulsive checking measures and amount and spatial distribution of locomotion for each treatment group.

COMPULSIVE CHECKING BEHAVIOR

Using a virtual implementation of the coordinate system of 25 open field locales (places) (Szechtman *et al*, 1998; Dvorkin *et al*, 2010), the frequency of visits and duration of stops in each locale were computed (the terms 'visit' and 'stop' are equivalent and are used interchangeably). The obtained values were used to identify the locale with the highest cumulative frequency of visits as well as the place with the maximal cumulative duration of stops. Checking behavior was defined with reference to the most visited locale (labeled 'key place' or 'key locale'; these terms are equivalent), which was in almost all instances also the locale with the longest total duration of stops (Eilam and Golani, 1989; Szechtman *et al*, 1998). If several locales had an equal number of visits then the locale with the higher cumulative duration of stops was used as the key locale. A visit to the key place is referred to as a 'check' or 'checking'.

The spatial-temporal structure of compulsive checking in OCD patients is characterized by an exaggerated preoccupation with the performance of the behavior and a reluctance to leave the place/object on which the behavior is focused (Eilam *et al*, 2005; Szechtman *et al*, 2005). In the animal model, these characteristics are indexed by the following measures, defined during earlier studies of checking behavior (Szechtman *et al*, 1998; Szechtman *et al*, 2001; Tizabi *et al*, 2002; Dvorkin *et al*, 2006).

I. Frequency of checking: total number of visits to the key locale.
II. Recurrence time of checking: mean duration of return times to the key place ('return time' is the interval from departure to next arrival at the locale).
III. Stops before returning to the key locale: mean number of places visited between returns to the key locale.
IV. Length of check: total duration of stay at the key locale divided by the frequency of visits there.

Compulsive checking behavior in the animal model is identified by the presence of a significant difference between drug treated and saline-treated rats for all of the above criteria (Szechtman *et al*, 1998; Dvorkin *et al*, 2006). Hence, the group of these four dependent variables is termed 'criteria measures' for compulsive checking. These criteria measures were used to assess and compare the behavioral effects of 8-OH-DPAT and quinpirole and are reported here for injection 8 and the cross-sensitization test (injections 9-10).

AMOUNT AND SPATIAL DISTRIBUTION OF LOCOMOTION

Two dimensions of locomotor activity in the open field were quantified in the present study: distance travelled and the spatial distribution of locomotor

trajectories. The distance travelled was computed as the sum of the distances during progression and lingering. To describe the spatial distribution of locomotor trajectories, two indices were used: *path stereotypy ratio* and *area of two standard deviational ellipse*, computed according to the method detailed elsewhere (Dvorkin *et al*, 2006; Dvorkin *et al*, 2010). The first index, path stereotypy ratio, reflects the relative frequency of repetitions of travel along the same paths, while the second index is a measure of the extent of the area covered by the trajectories of locomotion. The area of two standard deviational ellipse (2SDE) is one of the basic types of descriptors of spatial distribution used in centrographic statistics; it represents the area of an ellipse encompassing data points within 2 standard deviations of the mean along the long and short axes of the ellipse (Ebdon, 1985). 2SDE is computed by using data from 1x1cm grids that were visited by the rat during the session; therefore, if the rat traversed through many places, visiting each grid similar number of times, the resulting 2SDE will be high, reflecting a lack of spatial preference; however, if the rat focuses on visiting a limited number of grids, the 2SDE will be lower, reflecting preference to certain places on the arena. The total distance travelled per 55-minute interval, the path stereotypy ratio and the 2SDE were computed across injections 1-8 to describe and compare the behavioral effects of 8-OH-DPAT and quinpirole. The total distance travelled per 55-minute interval and the time course for locomotor activation were computed for the cross-sensitization test (injections 9-10) to compare the mechanisms of action of quinpirole and 8-OH-DPAT.

STATISTICS

To assess presence of compulsive checking at end of chronic treatment (injection 8), a one-way analysis of variance (ANOVA) was computed for each dependent variable of the criteria measures, followed by between Group comparisons using Duncan's Multiple Range Test. For the cross-sensitization test, a 3x2 ANOVA was performed with two between group factors: Chronic Drug Treatment Group (Saline *vs.* Quinpirole *vs.* 8-OH-DPAT) and Challenge Drug (Quinpirole *vs.* 8-OH-DPAT); for *post-hoc* tests, simple effects were evaluated by comparing the relevant marginal means, and non-overlapping 95% confidence intervals were considered to be statistically significant.

Development of locomotor activity from injection 1 to 8 was evaluated using a 3x8 ANOVA with Chronic Drug Treatment Group (Saline *vs.* Quinpirole *vs.* 8-OH-DPAT) as a between group factor and Injection (1 to 8) as a within group factor; where appropriate, simple effects were evaluated by comparing the relevant marginal means and 95% confidence intervals. An asymmetric sigmoid equation was fitted to distance travelled data (Figure 2b) using a nonlinear curve-fitting algorithm (Fig.P Version 2.98, Fig.P Software Corporation, Hamilton, Ontario, Canada), as described

previously (Szechtman *et al*, 1994b; Beerepoot *et al*, 2008). Chosen level of significance was $p < 0.05$. Calculations were performed using SPSS 20 for Windows. Graphs show marginal means and standard error of the mean (SEM).

RESULTS

INDUCTION OF COMPULSIVE CHECKING

Figure 1 shows that at the end of chronic treatment (injection 8), both 8-OH-DPAT and quinpirole treated animals differed significantly from saline controls on all four criteria measures for compulsive checking. Accordingly, in addition to quinpirole, 8-OH-DPAT can also induce compulsive checking behavior.

Figure 1 suggests that at the doses of the two drugs tested 8-OH-DPAT may be more powerful than quinpirole in inducing compulsive checking. This is suggested by the finding that 8-OH-DPAT had a significantly stronger effect than quinpirole on two of the four criteria measures of compulsive checking, and was similar to quinpirole on the remaining measures (*length of check* and *recurrence time of checking*).

TEST FOR CROSS-SENSITIZATION BETWEEN 8-OH-DPAT AND QUINPIROLE ON COMPULSIVE CHECKING

Table 1. Test for cross-sensitization between 8-OH-DPAT and quinpirole on criteria measures for compulsive checking.

Compulsive Checking Measure	Challenge	Chronic Drug Treatment Group[1]			ANOVA		
		Saline	*Quinpirole*	*8-OH-DPAT*	*Group Effect*	*Challenge Effect*	*GroupxChallenge Interaction*
Frequency of Checking	Quinpirole	40.9 ± 7.8	83.2 ± 7.8[a]	23.3 ± 7.8[b]	F(2,33)=4.29, p=0.022	F(1,33)= 27.16, p<0.001	F(2,33)= 16.41, p<0.001
	8-OH-DPAT	69.1 ± 10.5	76.5 ± 10.5	124.6 ± 10.5[2ab]			
Length of Check (s)	Quinpirole	89.1 ± 26.5	15.8 ± 26.5	151.6 ± 26.5[ab]	F(2,33)=3.26, p=0.051	F(1,33)= 1.49, n.s.	F(2,33)= 3.86, p=0.031
	8-OH-DPAT	100.8 ± 33.2	43.3 ± 33.2	14.9 ± 33.2[2]			
Recurrence Time of Checking (s)	Quinpirole	46.9 ± 4.4	27.4 ± 4.4[a]	35.8 ± 4.4	F(2,33)=4.55, p=0.018	F(1,33)=69.03, p<0.001	F(2,33)= 4.73, p=0.016
	8-OH-DPAT	18.3 ± 1.5[2]	16.9 ± 1.5	14.7 ± 1.5[2]			
# of Stops Before Returning To Check	Quinpirole	3.5 ± 0.2	2.5 ± 0.2[a]	2.8 ± 0.2[a]	F(2,33)=8.07, p=0.001	F(1,33)=99.01, p<0.001	F(2,33)=2.96, *p=0.066*
	8-OH-DPAT	1.7 ± 0.1[2]	1.6 ± 0.1[2]	1.5 ± 0.1[2]			

[1] Values are the adjusted marginal means and 1 S.E. from Chronic Drug Treatment Group by Challenge ANOVA.
[2] Numerals indicate significant difference compared to the challenge with quinpirole in the same chronic treatment group
[a] Significant difference compared to the acute effects of the same challenge drug in the chronic saline group
[b] Significant difference compared to the same challenge drug in the chronic quinpirole group

Table 1 shows, for each chronic treatment group, how the display of compulsive checking behavior responded to a challenge injection of 8-OH-DPAT and quinpirole. In the chronic 8-OH-DPAT group, compulsive checking was significantly attenuated after a challenge injection of quinpirole, compared to checking performance after an injection of 8-OH-DPAT. Moreover, after a challenge injection of quinpirole, checking performance in the chronic 8-OH-DPAT group was significantly reduced even when compared to performance under quinpirole in the chronic quinpirole

group. Together, these results show that chronic 8-OH-DPAT effects on compulsive checking are not reproduced by quinpirole substitution and hence that the effects of 8-OH-DPAT do not cross-sensitize to quinpirole.

As shown in Table 1, the display of compulsive checking in the chronic quinpirole group showed a seemingly different pattern of results after challenge injections than observed in the chronic 8-OH-DPAT group. In the chronic quinpirole group, performance of compulsive checking after challenge with 8-OH-DPAT was not attenuated and instead it was at the same (or higher) level of performance as after a challenge injection of quinpirole. This shows that 8-OH-DPAT can substitute for quinpirole in producing compulsive checking. On the surface, such findings suggest cross-sensitization between 8-OH-DPAT and quinpirole. However, further analysis presented below indicates a more complex interpretation.

Rather than reproducing the effects of chronic quinpirole, it is plausible that the 8-OH-DPAT challenge had its own unique effects on compulsive checking. This possibility is raised by the comparison of the effects of 8-OH-DPAT in the chronic quinpirole group to those in the chronic saline animals (Table 1). As is evident, the effects of 8-OH-DPAT in the chronic quinpirole group were no different from the acute effects of 8-OH-DPAT. This indicates that 8-OH-DPAT can induce compulsive checking even in rats not sensitized to quinpirole. Hence, the substitution of 8-OH-DPAT for quinpirole may be only apparent and as such, the evidence that the effects of chronic quinpirole cross-sensitize to an injection of 8-OH-DPAT is not compelling.

Considering that the effects of 8-OH-DPAT on compulsive checking do not cross-sensitize to quinpirole, and that a similar lack of cross-sensitization may hold for the effects of chronic quinpirole to 8-OH-DPAT, this raises the suggestion that 8-OH-DPAT and quinpirole induce compulsive checking by two separate and independent mechanisms. This hypothesis is supported by findings presented below showing that each drug had its own unique profile of effects on the amount and spatial distribution of locomotion.

AMOUNT AND SPATIAL DISTRIBUTION OF LOCOMOTION

The path plots in Figure 2a are of a representative rat from each chronic treatment group on the last test day (injection 8), selected on the basis of its 2SDE value being closest to group mean. Inspection of Figure 2a suggests that: (1) both 8-OH-DPAT and quinpirole increased the amount of locomotion compared to the saline control; (2) the routes of travel were more stereotyped under 8-OH-DPAT than quinpirole; and, (3) the area over which the rat travelled was more spread out under quinpirole than 8-OH-DPAT. A quantitative measure of each of the above suggestions is captured by, respectively: (1) distance travelled (Figure 2b); (2) path stereotypy (Figure 2c); and, (3) 2 standard deviational ellipse (2SDE; Figure 2d). These graphs

show not only performance on the final test (injection 8) but also changes in performance across injections. Indeed, the three suggestions are supported by the data depicted in Figures 2b-d as well as statistical analyses described below.

AMOUNT OF LOCOMOTION (FIGURE 2B)

As shown in Figure 2b, on injection 8, the 8-OH-DPAT and quinpirole groups travelled four times as much as saline controls. However, the profiles by which these two groups reached this level of performance were different. Specifically, the 8-OH-DPAT group showed significantly more locomotion than did the quinpirole group on the first injection (151.8±14.2 m *vs.* 77.0±14.2 m, $p < 0.05$). Furthermore, while both 8-OH-DPAT and quinpirole groups showed locomotor sensitization, the rate of sensitization was faster in the 8-OH-DPAT animals than the quinpirole group: 8-OH-DPAT reached the half-maximum level of sensitized locomotion on 1.9±0.2 injections compared to 3.2±0.3 injections for the quinpirole group.

PATH STEREOTYPY (FIGURE 2C)

Figure 2c provides a measure of path stereotypy, the frequency of travel along the same path. As shown, on injection 8, both drug treated groups had a more stereotyped route of travel than the saline group. Moreover, 8-OH-DPAT animals were more stereotyped than the quinpirole group (ratio of 6.2±0.3 *vs.* 4.1±0.3, $p < 0.05$). Rats injected with 8-OH-DPAT exhibited this level of path stereotypy from injection 1 onwards, whereas path stereotypy in the quinpirole group increased across injections, reaching its peak level at about injection 4 (Figure 2c).

2 STANDARD DEVIATIONAL ELLIPSE (2SDE) (FIGURE 2D)

Figure 2d shows 2SDE, a measure of the area over which the trajectories of locomotion are distributed. As is evident, across all injections, both drug treated groups travelled in a more constricted space than the saline animals (for Group, $F(2,32) = 83.1$, $p < 0.001$). Moreover, in 8-OH-DPAT animals this area was even more confined compared to the quinpirole group (1.7±0.2 m^2 *vs.* 3.2±0.2 m^2, $p < 0.05$). In both drug groups there was no marked sensitization of 2SDE (for Injection, $F(6,192) = 1.303$, $p = 0.258$; for Injection x Group, $F(12, 192) = 1.458$, $p = 0.143$).

TEST FOR LOCOMOTOR CROSS-SENSITIZATION (FIGURE 3)

Figure 3 shows how the locomotor-activating effects for each chronic treatment group responded to a challenge injection of 8-OH-DPAT and quinpirole. As was the case for compulsive checking (Table 1), Figure 3a shows that there was no cross-

sensitization for locomotor distance, because substituting quinpirole for 8-OH-DPAT produced significantly less locomotion in the chronic 8-OH-DPAT group and similarly, for the 8-OH-DPAT challenge in the chronic quinpirole group. Moreover, as shown in Figure 3b, the sensitized state of the animals did not alter the locomotor-activating effects of the challenge drugs. In particular, the time profile of the quinpirole challenge in the chronic 8-OH-DPAT group did not differ from the acute effects of quinpirole in the chronic saline group. And similarly, the time profile of the 8-OH-DPAT challenge in the chronic quinpirole group did not differ from the acute effects of 8-OH-DPAT in the chronic saline group. Thus, there is no evidence for cross-sensitization between quinpirole and 8-OH-DPAT on their effects on locomotion.

As shown in Figure 3b, the change from acute to sensitized locomotion under 8-OH-DPAT was strikingly different than the transformation to sensitized locomotion under quinpirole. In particular, the sensitized locomotor response to 8-OH-DPAT retains the time profile of the acute response—only the entire time course profile is displaced upwards by a constant amount. In contrast, with quinpirole, the biphasic acute response (Figure 3b, left panel) is transformed to a monotonic form (Figure 3b, centre panel), with the amount of locomotion increasing as a function of time after injection of the drug. These differences are consistent with the notion that the similar behavioral effects on measures of compulsive checking of 8-OH-DPAT and quinpirole are produced by separate mechanisms.

DISCUSSION

Results showed that 8-OH-DPAT induced compulsive checking behavior in a large open field, as did quinpirole. Hence, the two drugs have similar compulsive behavior-inducing effects in the spontaneous alternation paradigm and also in the open field. However, 8-OH-DPAT and quinpirole did not exhibit cross-sensitization for any measure of compulsive checking and locomotion. Moreover, the two drugs, at the dose used, had some differences in their effects on both these measures. 8-OH-DPAT produced a stronger effect than quinpirole on two of the four measures of compulsive checking, and was similar to quinpirole on the remaining two measures. Additionally, each drug had a distinct profile of effects on the amount and the spatial distribution of locomotion. This set of findings, together with the fact that one drug is an agonist of D2/D3 receptors while the other drug stimulates $5HT_{1A}$ receptors, suggests that the pathology of quinpirole- and 8-OH-DPAT-induced compulsive checking behavior—despite their similarity in the manifestation of “compulsive” behavior—must stem from dysfunctions of different parts of a specialized neural circuit underlying OCD.

The circuit underlying OCD is considered to be composed of cortico-striatal-thalamic-cortical loops (Modell *et al*, 1989; Wise and Rapoport, 1989; Baxter, 1992; Insel,

1992; Graybiel and Rauch, 2000; Saxena *et al*, 2001; Stein, 2002; Aouizerate *et al*, 2004; Szechtman *et al*, 2004; Huey *et al*, 2008; Vermeire *et al*, 2012). It has not been established what normal function this neurocircuit performs. One recent theory proposes that it mediates a special motivation for handling potential threats. This motivation system is called the Security Motivation System (Szechtman *et al*, 2004; Woody and Szechtman, 2011) or the Hazard-Precaution System (Boyer and Liénard, 2007). According to the authors (Szechtman *et al*, 2004; Woody *et al*, 2011), the motivational drive is induced by potential threat and mediated by dopaminergic inputs from the ventral tegmental area and the substantia nigra, while the negative feedback signals that de-activate the motivation upon goal-attainment are mediated by serotonergic pathways from the brainstem to the limbic striatum and the medial and orbital frontal cortex. Within this framework, OCD is a pathology of the security motivation circuit that can arise from either excessive dopaminergic motivational drive, insufficient serotonergic negative feedback or both.

The security motivation schema can be used to explain how quinpirole and 8-OH-DPAT can induce compulsive checking by acting on different mechanisms. The open field environment presents a potential threat that activates the dopaminergic motivational drive of the security motivation circuit. Quinpirole induces compulsive checking behavior by driving continually the dopamine receptors of the circuit and over-powering any negative feedback to terminate the behavior. In contrast, 8-OH-DPAT yields compulsive checking behavior not by stimulating motivational drive. Rather, by virtue of its inhibitory effects on serotonin activity (Barnes and Sharp, 1999; Alex and Pehek, 2007; Albert and Le François, 2010), 8-OH-DPAT prevents the activation of a serotonergic negative feedback that would normally de-activate the dopaminergic motivational state induced by the potential danger of the open field. Indeed, 8-OH-DPAT can perpetuate an excitatory effect on dopaminergic activity via its combined action on 5-HT_{1A} autoreceptors and heteroreceptors (Barnes *et al*, 1999; Fink and Göthert, 2007; Hayes and Greenshaw, 2011). Interestingly, a recent study provides evidence for a dysfunctional negative feedback in OCD patients (Hinds *et al*, 2012).

In all, both 8-OH-DPAT and quinpirole can induce compulsive checking in a large open field. Yet, 8-OH-DPAT and quinpirole probably produce this effect by acting on different parts of a security motivation circuit underlying OCD. Quinpirole may induce compulsive checking behavior by directly driving dopaminergic activity mediating the motivational drive to check. Conversely, 8-OH-DPAT may perpetuate the activated motivational state by inhibiting the serotonergic negative feedback signals that normally deactivate the OCD circuit.

COMPETING INTERESTS

The authors declare no competing interests.

ACKNOWLEDGEMENTS

We thank Dawn Graham, John Peel, Leena Taji, Paul Cheon and Renee Sharma for their contribution and help in the implementation of the experiment and collection of data. This study was supported by an operating grant (MOP-64424) from the Canadian Institutes of Health Research (CIHR) to HS.

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FIGURES

FIGURE 1 - EFFECTS OF 8-OH-DPAT AND QUINPIROLE ON A TEST FOR COMPULSIVE CHECKING ON INJECTION 8

Both drug-treated groups differed significantly from saline controls on all four criteria measures for compulsive checking behavior, and thus are said to show compulsive checking. * $p < 0.05$ *vs* chronic saline (Sal) group, ** $p < 0.05$ *vs* chronic saline and chronic quinpirole (QNP) groups, Duncan multiple range test.

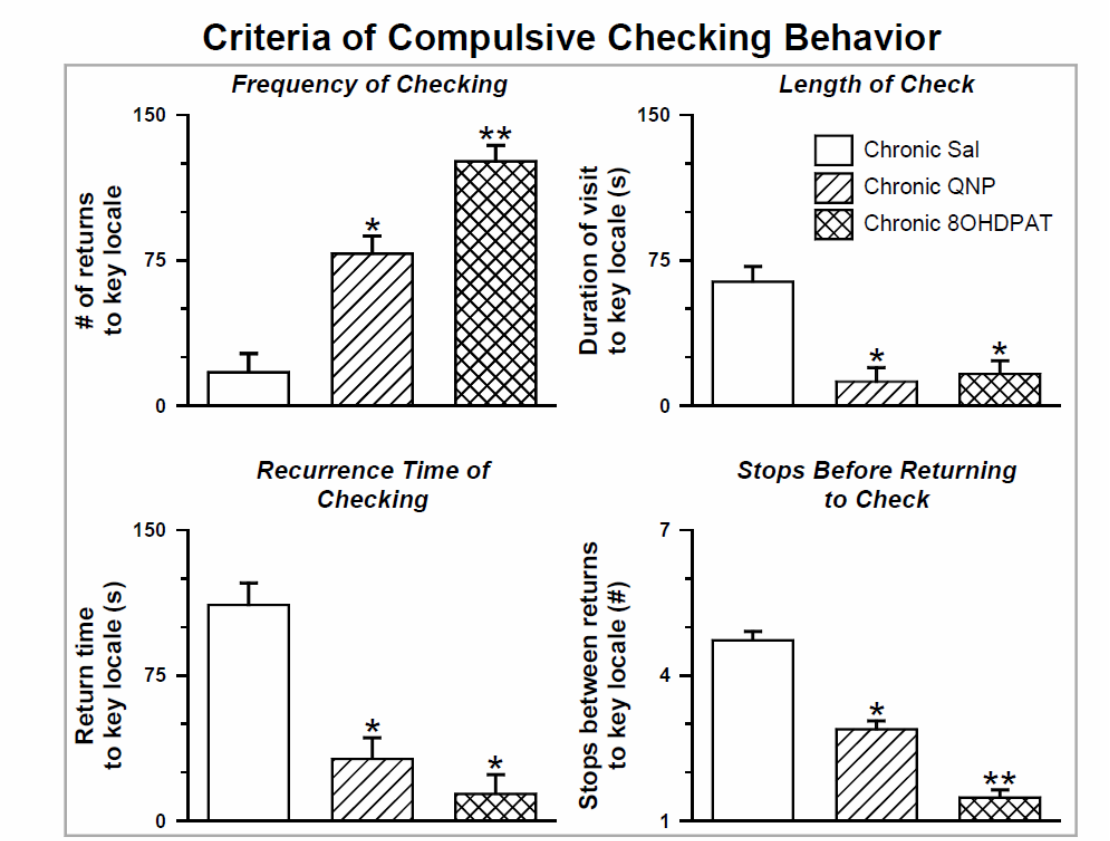


FIGURE 2 - EFFECTS OF 8-OH-DPAT AND QUINPIROLE ON THE AMOUNT AND THE SPATIAL DISTRIBUTION OF LOCOMOTION

a. Path plots on injection 8 for a representative rat treated chronically with saline (*left*), quinpirole (*middle*) and 8-OH-DPAT (*right*); selected rat has 2SDE value closest to group mean. Locomotor trajectories during the entire 55 min session are shown, and each line represents a trajectory of locomotion; density of trajectory lines corresponds to amount of locomotion. Gray squares indicate locations of the four objects in the open field. **b**. Total distance (m) travelled in 55 min across injections. Fitted parameters of the sigmoid curves are: for quinpirole, number of drug injections required to reach the half-maximal response (I_{50}) = 3.2±0.3, maximal response (R_{max}) = 338.7±26.3 m, sigmoidicity of the curve (n) = 3.1±0.9, lowest

response serving as a fixed parameter in the equation (R_{min}) = 77.0 m; for 8-OH-DPAT, I_{50} = 1.9±0.2, R_{max} = 302.9±9.3 m, n = 4.1±1.7, R_{min} = 151.8 m. **c.** Changes in path stereotypy across injections. **d.** Changes in 2 standard deviational ellipse (2SDE) across injections.

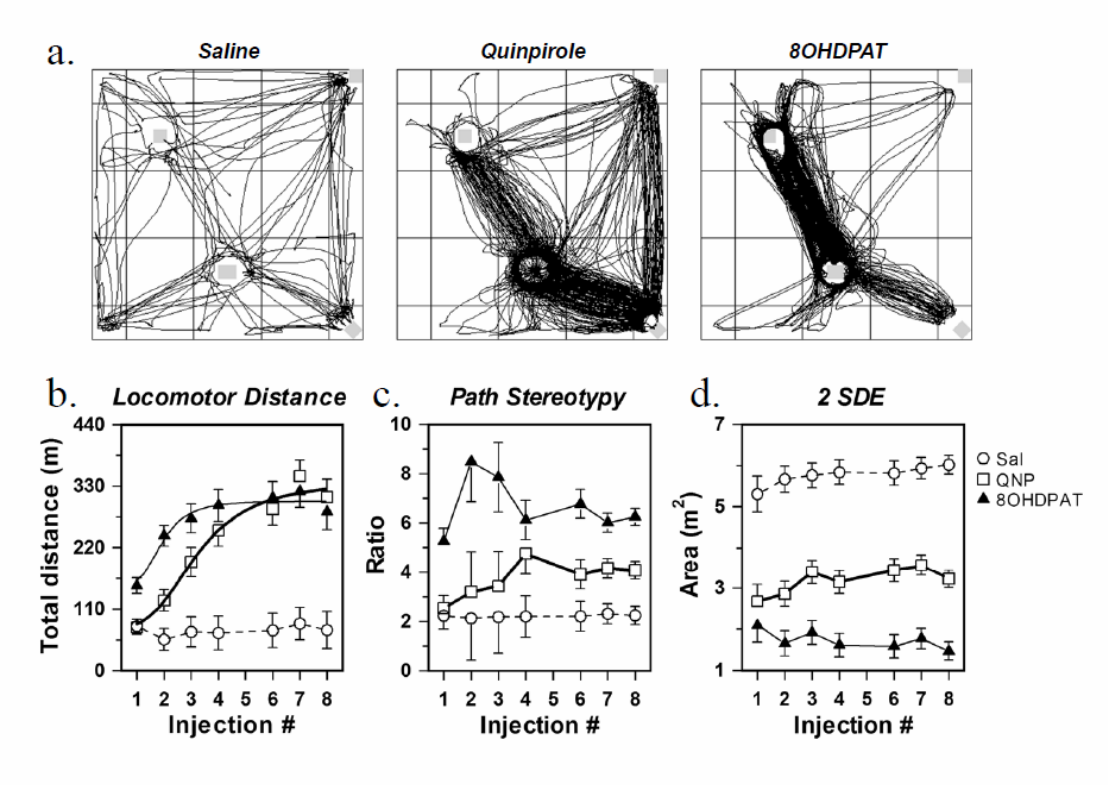
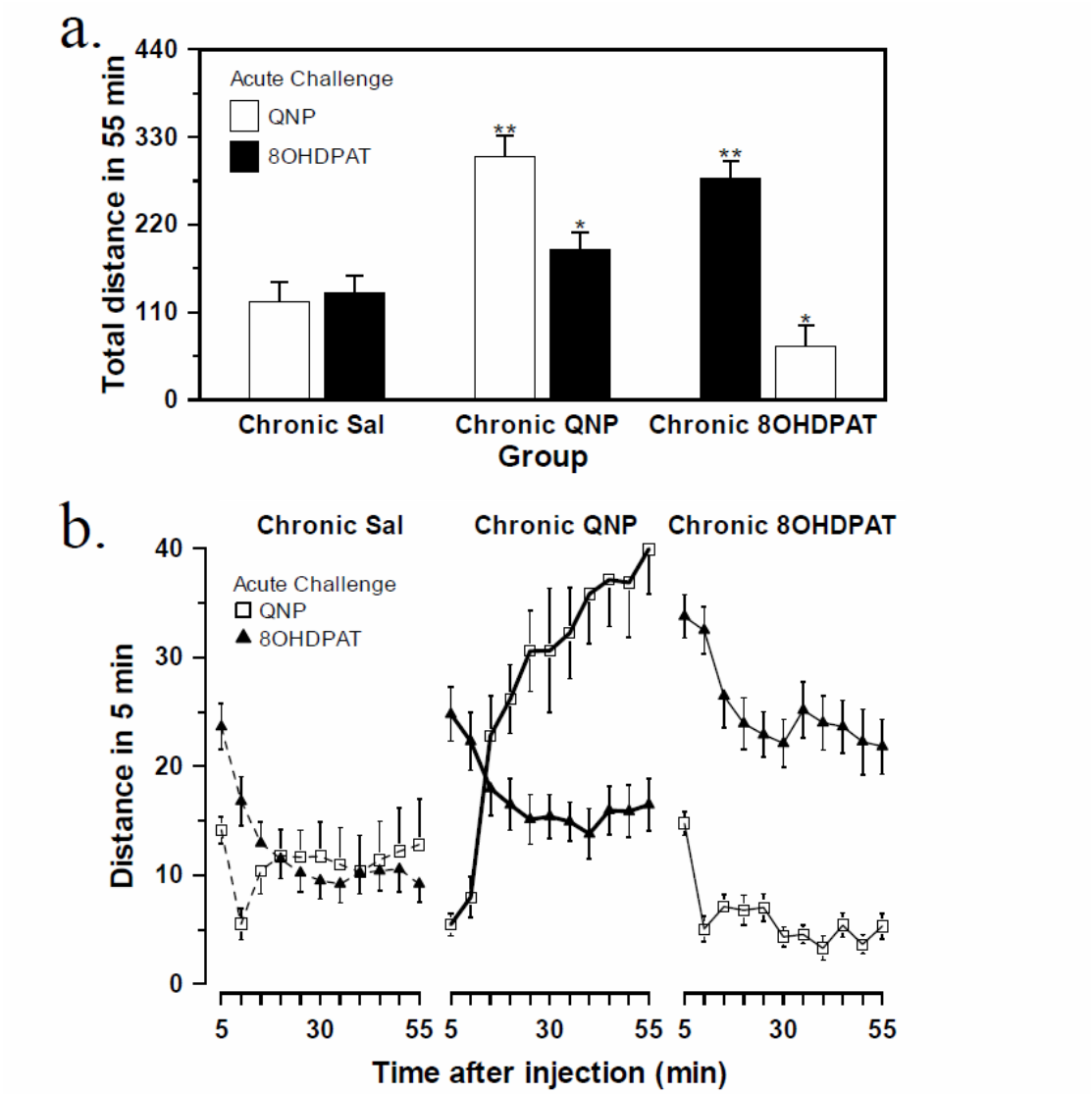


FIGURE 3 - TEST FOR CROSS-SENSITIZATION BETWEEN THE LOCOMOTOR-ACTIVATING EFFECTS OF 8-OH-DPAT AND QUINPIROLE

a. Total distance (m) travelled in 55 min after a challenge dose of 8-OH-DPAT or quinpirole administered to rats pretreated chronically with saline (Sal), quinpirole (QNP) or 8-OH-DPAT (8OHDPAT). **b**. Time profiles of distance travelled after drug injection. *p<.05 *vs.* other challenge drug the group received, **p<.05 *vs.* saline group injected with same challenge drug.


a.
Total distance in 55 min
440
330
220
110
0
Acute Challenge
QNP
8OHDPAT
**
*
**
*
Chronic Sal
Chronic QNP
Chronic 8OHDPAT
Group
b.
Chronic Sal
Chronic QNP
Chronic 8OHDPAT
Distance in 5 min
40
30
20
10
0
Acute Challenge
QNP
8OHDPAT
5
30
55
5
30
55
5
30
55
Time after injection (min)