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A DOSE-RESPONSE STUDY OF SEPARATE AND COMBINED EFFECTS OF SEROTONIN AGONIST 8-OH-DPAT AND DOPAMINE AGONIST QUINPIROLE ON LOCOMOTOR SENSITIZATION, CROSS-SENSITIZATION AND CONDITIONED ACTIVITY

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Corresponding Author:	H Szechtman McMaster University Hamilton, FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	McMaster University
Corresponding Author's Secondary Institution:	
First Author:	Eric F Johnson
First Author Secondary Information:	
Order of Authors:	Eric F Johnson
	H Szechtman
Order of Authors Secondary Information:	
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ERIC F. JOHNSON AND HENRY SZECHTMAN[§]

Department of Psychiatry and Behavioral Neurosciences, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada

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§Correspondence:
Dr. H Szechtman
Department of Psychiatry and Behavioral Neurosciences
McMaster University
1280 Main Street West
Health Science Centre, Room 4N82
Hamilton, Ontario
CANADA L8S 4K1
Tel: (905) 525-9140 ext 22201
FAX: (905) 522-8804
Email: szechtma@mcmaster.ca

ABSTRACT

Chronic treatment with the dopamine D2/D3 agonist quinpirole or the 5-HT_{1A} agonist 8-OH-DPAT induces behavioral sensitization. It is not known whether both drugs produce sensitization through a shared mechanism. Here we examine whether quinpirole and 8-OH-DPAT show cross-sensitization and impact sensitization, as would be expected from shared mechanisms. Male rats (N=208) were assigned randomly into 16 groups formed by crossing 4 doses of quinpirole (0, 0.03125, 0.0625, or 0.125 mg/kg) with 4 doses of 8-OH-DPAT (0, 0.03125, 0.625, or 0.125 mg/kg). After a course of 10 drug treatments administered twice per week in locomotor activity chambers, all groups were challenged on separate tests with quinpirole (0.1 mg/kg), 8-OH-DPAT (0.1 mg/kg) or saline, and locomotor activity was evaluated. Challenge tests with quinpirole and 8-OHDPAT showed no cross-sensitization between the drugs. Chronic quinpirole (0.125 mg/kg) induced a sensitized quinpirole response that was attenuated dose-dependently by chronic 8-OH-DPAT co-treatment. Quinpirole (0.0625 mg/kg) co-treatment with 8-OH-DPAT (all doses) induced quinpirole sensitization. Chronic 8-OH-DPAT (0.125 mg/kg) induced a sensitized 8-OHDPAT response that was prevented by chronic co-treatment with the lowest but not the highest dose of quinpirole. 8-OHDPAT (0.0625) co-treatment with quinpirole (0.125 mg/kg) induced sensitization to 8-OH-DPAT. Saline challenge test showed elevated locomotor activity in chronic quinpirole (0.125 mg/kg) and 8-OHDPAT (0.0625, 0.125 mg/kg) alone groups, and 7 of 9 co-treated groups. Absence of cross-sensitization suggests separate mechanisms of sensitization to quinpirole and 8-OH-DPAT. Co-treatment effects suggest induction of sensitization can be modulated by 5-HT_{1A} and D2/D3 activity.

KEYWORDS

Dopamine-serotonin interaction; 8-OH-DPAT; quinpirole; locomotor sensitization

INTRODUCTION

Behavioral sensitization refers to the phenomenon of augmented responding to a drug as a result of repeated exposure to the drug (Robinson and Becker, 1986). There is a long-standing and extensive research on this phenomenon because of considerations that understanding sensitization may reveal the mechanisms underlying development of various psychopathologies, including schizophrenia (Ellinwood, 1968; Ellison, 1979; Angrist, 1983; Segal and Schuckit, 1983; Robinson *et al*, 1986), mania (Post and Contel, 1981), drug abuse and addiction (Piazza *et al*, 1989; Robinson and Berridge, 1993), post-traumatic stress and panic disorders (Antelman, 1988; Post and Weiss, 1988), as well as obsessive-compulsive disorder (OCD; Szechtman *et al*, 1998; Szechtman *et al*, 1999; Eilam and Szechtman, 2005; Szechtman and Eilam, 2005). However, most of this research has focused on the mechanisms underlying sensitization produced by drugs of abuse, such as cocaine and amphetamine. These psychostimulants are indirect dopamine agonists and hence much research using such compounds is focused on mechanisms for enhanced pre-synaptic dopamine neurotransmission as a key to understand sensitization. Yet, direct dopamine agonists that suppress pre-synaptic dopamine release also induce sensitization (Hoffman and Wise, 1993; Szechtman *et al*, 1994b; Delius *et al*, 2015). This suggests that the relevance of sensitization for psychopathology is not necessarily confined to mechanisms of enhanced pre-synaptic dopamine levels. Indeed, the sensitization induced by quinpirole had been proposed to underlie OCD (Eilam *et al*, 2005), opening the possibility of different mechanisms of sensitization for different types of psychopathology.

In the present study we compared the sensitization induced by two direct agonists, quinpirole and 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT). Quinpirole is a direct agonist of D2/D3 dopamine receptors and 8-OH-DPAT is an agonist of serotonin 1A (5-HT_{1A}) receptors (Levant *et al*, 1992; Müller *et al*, 2007). However, chronic treatment of rats with either agonist can induce compulsive checking proposed to model OCD psychopathology (Alkhatib *et al*, 2013), raising the question whether the two drugs induce sensitization by acting on a common mechanism. The present results indicate that each drug induces sensitization by a different pathway but nevertheless stimulation of 5-HT_{1A} receptors can modulate the sensitization to quinpirole, and conversely D2/D3 receptor stimulation can affect the sensitization induced by 8-OH-DPAT.

MATERIALS AND METHODS

ANIMALS

Subjects were 208 experimentally naïve male Long-Evans rats (Charles River, Quebec) that weighed a mean ± SEM of 384 ±2 g at the time of first drug treatment (range 306-441 g). Animals were housed individually in a climate controlled colony room, and exposed to a 12 hour light/dark cycle (7 AM lights on, 7 PM lights off). Food and water were freely available. Upon arrival, rats were allowed to habituate to the animal facility for 7 days and were then handled for approximately 2-5 minutes each day for 5 days before start of experiment. Testing occurred during the light phase. Animals were housed and tested as approved by the Animal Research Ethics Board, McMaster University in compliance with the Canadian Council on Animal Care guidelines.

DRUGS

(-)-Quinpirole hydrochloride (QNP; Q102) and (+/-)-8-hydroxy-2-(di-*n*-propylamino)-tetralin hydrobromide (8-OH-DPAT; H8520) were obtained from Sigma-Aldrich. Each drug was dissolved in 0.9% saline solution and injected subcutaneously under the nape of the neck at a volume of 1.0 mL/kg. For tests where two drugs were co-administered, quinpirole solution was administered first, immediately followed by the 8-OH-DPAT injection; for non-drug injections an equivalent volume of saline was used. Throughout the study, rats received two injections before each trial and were tested twice weekly until termination of the experiment.

APPARATUS

Locomotor activity was measured using an automated apparatus equipped with the VersaMax Animal Activity Monitoring System (AccuScan Instruments, Columbus, OH). It consisted of 10 empty Plexiglas activity chambers (40x40x35 cm) located in a non-colony room. The activity chambers were interfaced to a Digiscan 16 analyzer that monitored the state of 30 infra-red beams forming a horizontal X-Y grid over the bottom of the activity chamber. A computer with VersaMax software captured the beam breaks and derived from their sequence and timing “distance traveled” that served as the dependent variable of the present study.

DESIGN

The research questions addressed by the present experiment are whether sensitization induced by quinpirole and by 8-OH-DPAT shows cross-sensitization and whether the induction of such sensitization by one drug is modulated by the other drug. To address these questions, a regimen of chronic treatment to induce sensitization was employed with each drug separately and together. All groups were then challenged, on separate tests, with quinpirole (0.1 mg/kg), 8-OH-DPAT (0.1 mg/kg) and saline, and evaluated for

the presence of sensitization to each drug and for changes in baseline activity due to drug treatment history. Thus, the design of the study consisted of two between-groups factors related to doses of quinpirole and 8-OHDPAT employed in the treatment regimen to induce sensitization and a third within-groups factor related to the drug used on challenge tests. In particular, one factor was Chronic Pretreatment Quinpirole Dose with 4 levels (0, 0.03125, 0.625, 0.125 mg/kg) and another one was Chronic Pretreatment 8-OH-DPAT Dose also with 4 levels (0, 0.03125, 0.625, 0.125 mg/kg). The third factor, which was a repeated measures factor, was Challenge Drug with 3 levels (quinpirole, 8-OH-DPAT, saline). The two between-groups factors were fully crossed and formed 16 independent groups that were tested. The number of subjects per group was 12-13, except for the quinpirole (0.125 mg/kg) + 8-OH-DPAT (0.125 mg/kg) group (N=11) and the saline + saline group (N=24). Due to technical limitations in running all rats at once, the experiment was conducted by testing three separate batches of rats but not as fully random replicates.

PROCEDURE

The regimen of chronic drug treatment used in the present study followed our established protocol for induction of sensitization of twice weekly injections for a total of 10 injections (Szechtman *et al*, 1994a; Szumlinski *et al*, 1997; Coscina *et al*, 1998; Culver *et al*, 2000; Lomanowska *et al*, 2004; Perreault *et al*, 2005; Beerepoot *et al*, 2008; Alkhatib *et al*, 2013). Following the 10^{th} injection, the same schedule was continued without interruption for injections 11 to 13 except that on injection 11 all groups were administered saline and for injections 12 and 13 they were injected with a challenge dose of quinpirole (0.1 mg/kg) and 8-OH-DPAT (0.1 mg/kg). Half the rats in each group received quinpirole and then 8-OH-DPAT and the other half received these challenge injections in reverse order.

Rats were allocated into groups at random, with the proviso of approximately equal body weight across treatments prior to start of study. For all trials throughout the study the same procedure was followed: Animals were weighed, transported in their home cage to the non-colony experimental testing room, and administered the appropriate injections. Immediately afterwards, the rat was placed into the activity chamber for 60 min and locomotor activity recorded. Each animal was tested in the same activity chamber, at approximately the same time throughout the study. Each rat had 2 trials per week, and was run on the same day of the week (Mon/Thu or Tue/Fri or Wed/Sun). Testing chambers were cleaned with a 50:50 solution of Windex in water following each use.

The schedule of twice weekly injections was used because a previous study showed that the induction of sensitization to quinpirole is equally effective with inter-injection intervals from 2 to 8 days apart (Szechtman *et al*, 1994a) and because this schedule maximizes the number of animals that can be run concurrently. The doses of quinpirole for chronic treatment (0.03125, 0.0625, and 0.125 mg/kg) were selected to include the range from pre-synaptic to a post-synaptic doses (Szechtman *et al*, 1994a; Perreault *et al*, 2006) and did not include higher doses of quinpirole to avoid ceiling effects and maximize the possibility of modulation by 8-OH-DPAT co-treatment. The doses of 8-OH-DPAT for chronic treatment (0.03125, 0.0625, and 0.125 mg/kg) were selected based on the literature to approximate the considerations guiding the choice of quinpirole doses (De La Garza and Cunningham, 2000; Przegaliński *et al*, 2000; Carey *et al*, 2004; Müller *et al*, 2007; Haleem, 2013). For the challenge tests, a dose of 0.1 mg/kg was used as it is lower than the highest dose of the drug administered here to induce sensitization and yet high enough to have an effect on locomotion based on prior studies (Eilam and Szechtman, 1989b; Tucci *et al*, 2014).

DATA ANALYSIS

Evidence of sensitization is often given from a significantly higher performance at end of chronic treatment compared to the acute effects of the drug at start of treatment. However, a more stringent demonstration is with a test for sensitization at the end of chronic treatment where both the saline controls and the drug-treated group are administered a challenge dose of the drug (usually a lower dose than used during chronic treatment) and evidence for sensitization is provided by a significantly higher response in the chronic treatment group compared to the acute drug response of the saline controls. Such a test for sensitization controls for changes in drug response due to increased familiarity with the test procedures (Stewart and Vezina, 1988; Stewart and Badiani, 1993; Einat *et al*, 1996). Hence, in the presence study, sensitization is assessed at end of chronic treatment with a test for sensitization to quinpirole and a test for sensitization to 8-OH-DPAT. A test for conditioned activity where all groups are administered saline is also included although the design of the present study does not permit assessment of the contributions of associational and non-associational mechanisms. Because the present study is focused on presentation and analysis of the challenge tests, the prior phase of chronic drug treatment is referred to as "pretreatment" in the results section.

For statistical analysis of the dependent variable "distance travelled" a 4 x 4 x 3 ANOVA was used with Chronic Pretreatment Quinpirole Dose (0, 0.03125, 0.625, 0.125 mg/kg) and Chronic Pretreatment 8-OH-DPAT Dose (0, 0.03125, 0.625, 0.125 mg/kg) as the between-group factors and Acute Challenge Drug (quinpirole, 8-OH-DPAT, saline) as a within-group factor. Huynh-Feldt adjustment was employed for violation of the sphericity assumption as indicated by Mauchly's Test of Sphericity. A significant triple interaction was found and is presented in Fig. 1; simple effects were evaluated by comparing the relevant marginal means and 95% confidence intervals shown in Fig. 1. The chosen level of significance was *P* less than 0.05. Calculations were carried out using IBM SPSS Statistics version 22.

RESULTS

After the prescribed course of 10 drug injections, all groups were challenged with: (a) quinpirole (0.1 mg/kg), to evaluate the presence of sensitization to quinpirole (Fig. 1a); (b) 8-OH-DPAT (0. 1 mg/kg), to evaluate the presence of sensitization to 8-OH-DPAT (Fig. 1b); and (c) saline, to evaluate baseline or conditioned locomotion (Fig. 1c). The three challenge tests were analyzed with a Chronic Pretreatment QNP Dose by Chronic Pretreatment DPAT Dose by Acute Challenge Drug ANOVA and Figure 1 shows graphically the significant triple interaction ($F_{12.8, 271.7}$ = 2.7, *P* = 0.001, partial eta squared = 0.113). *Solid* circles represent the group means and floating bars display the 95% confidence intervals. Groups with non-overlapping confidence intervals are considered as significantly different from each other (*P* < 0.05). Results of the relevant comparisons are presented below.

CROSS-SENSITIZATION

Cross-sensitization would be evidenced by the group sensitized to quinpirole showing a sensitized response also to a challenge with 8-OH-DPAT, and *vice versa* for the group sensitized to 8-OH-DPAT. Fig. 1a shows that of the 3 groups pretreated chronically with quinpirole-alone, only the group pretreated with the highest dose of quinpirole (0.125 mg/kg) showed a sensitized response, as only this group had a significantly higher response than the acute quinpirole response shown by the saline control group. This can be seen readily by inspection of Fig. 1a where the *green short dashed* horizontal line denotes the upper bound of the 95% confidence interval of the acute quinpirole response shown by saline controls and the group pretreated with 0.125 mg/kg (first floating bar in the last cluster of bars) is clearly much above that horizontal line. However, as shown in Fig. 1b, this quinpirole sensitized group did not show a sensitized response to 8-OH-DPAT since their response to the challenge with 8-OH-DPAT was no

different from the acute 8-OH-DPAT response of saline controls (the 95% confidence interval crosses the *blue long dashed* horizontal line which denotes the upper bound of the 95% confidence interval of the saline group's acute 8-OH-DPAT response). For ease of exposition and facilitate direct comparisons, these data are shown also in table format in Table 1 where the relevant comparisons are juxtaposed to each other to reveal clearly that the group pretreated with 0.125 mg/kg of QNP was sensitized to QNP but not to DPAT.

Similarly, as shown in Table 1 and Fig. 1b, the group pretreated chronically with 0.125 mg/kg of 8-OH-DPAT showed a sensitized response to 8-OH-DPAT (0.1 mg/kg) compared to the saline control group injected acutely with the same dose of 8-OH-DPAT. Moreover, although sensitized to 8-OH-DPAT, this group did not show a sensitized response to quinpirole as their locomotor response overlapped with the acute quinpirole performance of the saline controls.

Absence of cross-sensitization is suggested also from the time course profiles of locomotion under quinpirole and 8-OH-DPAT shown in Fig. 2. Specifically, as shown in Fig. 2b, when quinpirole sensitized rats were challenged with 8-OH-DPAT (Fig. 2b, *open triangles*), the time course of locomotor activity did not have the shape of sensitized locomotion induced by quinpirole (Fig. 2b, *open circles*); instead, it resembled the profile of an acute response to 8-OH-DPAT (Fig. 2a, *open triangles*). Similarly, the QNP challenge to 8-OH-DPAT sensitized rats (Fig. 2c, *open circles*) yielded the typical acute quinpirole time course profile (Fig. 2a, *open circles*).

Thus, chronic treatments with quinpirole (0.125 mg/kg) and 8-OH-DPAT (0.125 mg/kg) each induced locomotor sensitization, but the sensitization effects of the two drugs did not show cross-sensitization.

8-OH-DPAT CO-TREATMENT EFFECTS ON SENSITIZATION TO QUINPIROLE

Fig. 1a displays the impact of co-treatment with various doses of 8-OH-DPAT on the induction of sensitization to quinpirole. As is evident from the fourth cluster of floating bars, the sensitized response induced by chronic injections of 0.125 mg/kg of quinpirole-alone, was attenuated by 8-OH-DPAT. Specifically, the addition of various doses of 8-OH-DPAT to the regimen of chronic treatment with quinpirole (0.125 mg/kg) produced a dose-dependent reduction in the sensitized quinpirole response, with the response of the group treated chronically with quinpirole (0.125 mg/kg) plus 8-OH-DPAT (0.125 mg/kg) being significantly smaller than the group treated chronically with quinpirole only. Table 2 shows these findings in tabular form, together with the data for groups

administered a lower dose of QNP (0.0625 mg/kg) plus DPAT (all doses), to highlight the contrasting effects of chronic DPAT on the two doses of quinpirole, as described below.

Specifically, in contrast to the above attenuation of quinpirole sensitization, 8-OH-DPAT co-treatment had the opposite effect when combined with chronic injections of 0.0625 mg/kg of quinpirole. Chronic injections of this dose of quinpirole did not yield locomotor sensitization to quinpirole challenge, but, in combination with 8-OH-DPAT (all doses) this co-treatment regimen induced a sensitized response to quinpirole as the amount of locomotion shown by the co-treated groups was significantly higher than the acute QNP response shown by saline controls (Table 2 and Fig. 1a). However, it should be noted that none of the co-treated groups were significantly different from the 0.0625 mg/kg quinpirole-alone group (Table 2 and Fig. 1a). This may suggest that the effect of DPAT co-treatment was to merely push the QNP-alone effect over a threshold for sensitization to emerge.

Finally, as shown in Fig 1a, chronic injections of 0.03125 mg/kg of quinpirole did not induce sensitization and co-injections of 8-OH-DPAT did not change the amount of locomotion compared to quinpirole-only.

Thus, the effects of co-treatment with 8-OH-DPAT depended on the dose of quinpirole used to induce quinpirole sensitization: 8-OH-DPAT reduced the effect on sensitization of the higher dose of quinpirole (0.125 mg/kg), increased the effect of the middle dose quinpirole (0.0625 mg/kg), and did not modify the effects of the lowest dose of quinpirole (0.03125 mg/kg).

QUINPIROLE CO-TREATMENT EFFECTS ON SENSITIZATION TO 8-OH-DPAT

The impact of co-treatment with various doses of quinpirole on sensitization to 8-OH-DPAT is graphed in Fig. 1b and presented in tabular form in Table 3. In Fig. 1b, the floating bars above the *blue long dashed* horizontal line reflect groups showing sensitization to 8-OHDPAT as for these groups the lower bound of the 95% confidence interval is significantly above the acute 8-OH-DPAT response shown by the saline control group. As is evident in Fig. 1b, and highlighted in Table 3, chronic treatment with 0.125 mg/kg of 8-OH-DPAT induced a sensitized 8-OH-DPAT response. Co-treatment with the two highest doses of quinpirole (0.125 and 0.0625 mg/kg) did not alter the sensitized response to 8-OH-DPAT but co-treatment with the lowest dose of quinpirole (0.03125 mg/kg) did prevent sensitization to 8-OHDPAT as this group was not significantly higher than the acute 8-OH-DPAT response shown by saline controls (Table 3 and Fig. 3b).

In contrast to the reducing effects of lowest dose quinpirole on sensitization to 8-OH-DPAT, co-treatment with the highest dose of quinpirole (0.125 mg/kg) may promote 8-OH-DPAT sensitization. Specifically, comparison of the groups treated chronically with 0.0625 mg/kg of 8-OH-DPAT and various doses of quinpirole shows that 8-OH-DPAT-alone (0.0625 mg/kg) did not induce sensitization to 8-OH-DPAT but the combination of 8-OH-DPAT (0.0625 mg/kg) and quinpirole (0.125 mg/kg) did, as locomotion in this group was significantly higher to the challenge dose of 8-OH-DPAT than in saline controls administered the same dose of 8-OH-DPAT (Table 3 and Fig. 3b).

Finally, chronic injections of 0.03125 mg/kg of 8-OH-DPAT, with or without co-injections of quinpirole (all doses), were ineffective in inducing sensitization to 8-OH-DPAT.

Thus, lowest but not the highest dose quinpirole co-treatment attenuated sensitization to 8-OH-DPAT. However, the highest dose quinpirole co-treatment had sensitization-promoting effects on sensitization to 8-OH-DPAT.

SALINE CHALLENGE TEST

As shown in Fig. 1c, after challenge with saline, 10 of the 15 chronic drug pretreated groups are above the *black solid* horizontal line, indicating that their locomotor activity is significantly elevated compared to saline controls injected with saline. Hence, a history of chronic drug experience produced conditioned activity in these rats. Specifically, chronic injections of quinpirole (0.125 mg/kg) or chronic injections of 8-OH-DPAT (0.0625, 0.125 mg/kg) resulted in elevated baseline locomotor activity. Moreover, all groups co-treated with quinpirole (0.0625, 0.125 mg/kg) and 8-OH-DPAT (all doses) had significantly elevated locomotor activity as did the group co-treated with quinpirole (0.03125 mg/kg) and 8-OH-DPAT (0.125 mg/kg). In all, conditioned locomotion was induced by chronic exposure to the highest dose of either drug, but all doses of 8-OH-DPAT were effective in inducing conditioned locomotion if combined with chronic exposure to quinpirole.

DISCUSSION

Locomotor sensitization can be induced by a number of psychostimulant drugs, including quinpirole (Willner *et al*, 1992; Szechtman *et al*, 1993; Szechtman *et al*, 1994a; Coscina *et al*, 1998; Szumlinski *et al*, 2000; Lomanowska *et al*, 2004; Foley *et al*, 2006; Perreault *et al*, 2006) and 8-OH-DPAT (De La Garza *et al*, 2000; Alkhatib *et al*, 2013). The sensitization produced by quinpirole and 8-OH-DPAT could result from changes in separate and independent pathways. Alternatively, the sensitization could result from changes at a common site that is altered by the action of both drugs. The latter model

predicts that regardless of which one of the drugs induced sensitization, the expression of it can be evoked by the other drug; that is, the effects of the two drugs would show cross-sensitization. The present study does not support the common site model because no cross-sensitization between quinpirole and 8-OH-DPAT was found. The lack of cross-sensitization is consistent with similar finding in another study where one dose of quinpirole (0.2 mg/kg) and another dose of 8-OHDPAT (1 mg/kg) were used to induce sensitization (Alkhatib *et al*, 2013). The present dose-response study establishes that the absence of cross-sensitization is not an artifact of comparing inappropriate doses of quinpirole and 8-OH-DPAT. Thus, regardless of the chronic treatment dose, quinpirole and 8-OH-DPAT each induces sensitization by altering a separate and distinct pathway.

Nevertheless, chronic co-administration of quinpirole together with 8-OH-DPAT induced sensitization to each drug that was significantly different from the sensitization induced by chronic injections of each drug alone. As summarized in Table 2, co-treatment with 8-OH-DPAT either attenuated or enhanced sensitization to quinpirole, depending on the chronic dose of quinpirole. Specifically, 8-OH-DPAT dose dependently attenuated the sensitization induced by co-treatment with the highest dose of quinpirole (0.125 mg/kg). However, when co-administered with an ineffective dose of quinpirole (0.0625 mg/kg), 8-OH-DPAT had instead the opposite effect and promoted the induction of sensitization, regardless of what dose of 8-OH-DPAT was co-administered (0.03125, 0.0625, or 0.125 mg/kg). In a similar manner, as summarized in Table 3, the effects of quinpirole co-treatment on the induction of sensitization to 8-OH-DPAT depended also on the chronic dose of 8-OH-DPAT. However, the quinpirole dose-response profile was inverted: the lowest (0.03125 mg/kg) but not the highest dose of quinpirole (0.125 mg/kg) attenuated the sensitization induced by co-treatment with the highest dose of 8-OH-DPAT (0.125 mg/kg). And yet, when co-administered with an ineffective chronic dose of 8-OH-DPAT (0.0625 mg/kg), only the highest dose of quinpirole (0.125 mg/kg) promoted the induction of sensitization to 8-OH-DPAT. These findings reveal that activation of 5-HT_{1A} receptors can modulate the induction of sensitization by the D2/D3 dopamine receptor agonist quinpirole; and conversely, that the activation of D2/D3 receptors can modulate the induction of sensitization by the 5-HT_{1A} serotonin receptor agonist 8-OH-DPAT. Below we first suggest a plausible mechanism by which 5-HT_{1A} activity may modulate sensitization to quinpirole and then consider the converse phenomenon.

EFFECTS OF 5-HT_{1A} ACTIVITY ON SENSITIZATION TO QUINPIROLE

Locomotor sensitization induced by quinpirole is proposed to result from the repeated actions of quinpirole on pre-synaptic and post-synaptic mechanisms, producing

necessary changes at both sites (Szechtman *et al*, 1994b; Perreault *et al*, 2006). The pre-synaptic changes are such that dopamine neurotransmission is shut-down by quinpirole more rapidly and more completely. The post-synaptic changes are such that the efficacy of post-synaptic D2 receptors is increased. Together, these effects of repeated quinpirole produce sensitized responding as follows:

Dopamine D2 receptors are located pre-synaptically on dopamine cell bodies, dendrites, and axon terminals, as well as on the post-synaptic targets of dopamine innervation. Quinpirole acts at pre- and post-synaptic D2 sites, although low doses of quinpirole are biased towards the pre-synaptic receptors (Skirboll *et al*, 1979; Starke, 1981; Kelland *et al*, 1990). The acute pre-synaptic effects of quinpirole include inhibition of dopamine release (Boyar and Altar, 1987; Koeltzow *et al*, 1998; Rouge-Pont *et al*, 2002) and reduction of dopamine neuron firing (Skirboll *et al*, 1979; Starke, 1981). These acute effects result in a depletion of extracellular dopamine at 40-60 min after injection of quinpirole, as measured by microdialysis (Imperato *et al*, 1988; Rouge-Pont *et al*, 2002). However, a regimen of repeated quinpirole injections yields in quinpirole-sensitized rats a reduction in dopamine cell burst firing (Sesia *et al*, 2013) and a decline in phasic and tonic dopamine release (Koeltzow *et al*, 2003; Escobar *et al*, 2015), but no desensitization of pre-synaptic autoreceptors (Szumlinski *et al*, 1997; Koeltzow *et al*, 2003; Lomanowska *et al*, 2004; Perreault *et al*, 2006; Escobar *et al*, 2015). It had been proposed that such a change in the profile of dopamine neurotransmission is one of the necessary components in the induction of quinpirole sensitization because the development of it would reflect a process of successively quicker and greater shut-down of dopamine neurotransmission by quinpirole (Perreault *et al*, 2006). In other words, given a progressive reduction in baseline dopamine activity, successive quinpirole injections would deplete extracellular dopamine faster and for longer.

The quinpirole-induced extracellular dopamine depletion is proposed to enable the necessary second component to develop, namely, an increase in efficacy of post-synaptic D2 receptors (Eilam *et al*, 1992; Szechtman *et al*, 1994b; Szumlinski *et al*, 1997; Perreault *et al*, 2006). In particular, it had been noted that the time course of acute quinpirole on locomotion is biphasic, with depression of activity for up to 40-60 min after drug injection followed by excitation thereafter (Eilam *et al*, 1989a; Eilam *et al*, 1989b; Eilam *et al*, 1992; Van Hartesveldt *et al*, 1994). Because locomotor excitation coincided with least extracellular dopamine (Imperato *et al*, 1988; Rouge-Pont *et al*, 2002), it was proposed that locomotion increases via stimulation of post-synaptic D2 receptors—without competition from inhibitory effects of endogenous dopamine (Eilam *et al*, 1991; Eilam *et al*, 1992; Szechtman *et al*, 1994b). Accordingly, locomotor sensitization ensues from the relatively selective, and repeated, activation of post-

synaptic D2 receptors, raising D2 receptor efficacy (Szumlinski *et al*, 1997; Perreault *et al*, 2006). The increase in efficacy may stem from the quinpirole sensitization regimen inducing a higher density of dopamine D2-like receptors in the nucleus accumbens (Culver *et al*, 2008); increasing the proportion of dopamine D2 receptors in the high-affinity state (Seeman *et al*, 2006; Perreault *et al*, 2007); or altering dopamine second messenger transduction pathways (Culm *et al*, 2004; Beaulieu and Gainetdinov, 2011; Chen *et al*, 2012; Liu *et al*, 2015). Furthermore, the increase in efficacy could be more indirect and result from neuroplastic changes produced by repeated quinpirole, such as morphological alterations in post-synaptic dendritic complexity (Dvorkin *et al*, 2008; Lalchandani *et al*, 2013); reduction in prefrontal glutamate neurotransmission (Escobar *et al*, 2015); or inhibition of neuronal activity in several brain regions (Carpenter *et al*, 2003; Richards *et al*, 2005; Richards *et al*, 2007).

In short, quinpirole sensitization involves inhibition of pre-synaptic dopamine release and enhanced efficacy of post-synaptic D2 receptors. Accordingly, treatments that potentiate pre-synaptic dopamine release should attenuate quinpirole sensitization and those that enhance post-synaptic D2 signal transduction should potentiate sensitization. This framework is used below to interpret the present findings of reduction and potentiation of quinpirole sensitization by co-administered 8-OH-DPAT.

One plausible mechanism by which co-administrated 8-OH-DPAT dose-dependently reduced sensitization to quinpirole (0.125 mg/kg) relates to findings that 8-OH-DPAT can increase extracellular DA (Arborelius *et al*, 1993b; Chen and Reith, 1995; Müller *et al*, 2007). The mechanism for increased extracellular DA may involve excitation by 8-OH-DPAT of VTA dopamine neuron firing (Arborelius *et al*, 1993a) and/or diminution of inhibitory serotonergic tone on dopamine activity (Barnes and Sharp, 1999; Fink and Göthert, 2007; Hayes and Greenshaw, 2011). Thus the actions of co-administered 8-OH-DPAT would be opposite to effects of quinpirole on extracellular dopamine. Consequently, co-administered 8-OH-DPAT would be impeding the decline in extracellular dopamine. Accordingly, in the presence of co-administered 8-OH-DPAT, the inhibitory effects of endogenous dopamine would be present for longer than with quinpirole alone, hampering selective post-synaptic D2 activation by quinpirole and thereby retarding the rise in post-synaptic D2 efficacy. It may be expected that by extending the duration of co-treatment, the level of sensitization would be comparable to treatment with quinpirole alone because 5-HT_{1A} receptors show desensitization (Blier and Ward, 2003; Müller *et al*, 2007).

A plausible mechanism by which 8-OH-DPAT co-treatment could enable sensitization to an ineffective dose of quinpirole (0.0625 mg/kg) is likely post-synaptic. Even though the

0.0625 mg/kg dose of quinpirole was inadequate to induce sensitization, nevertheless, it is sufficient to inhibit extracellular dopamine (Imperato *et al*, 1988) and provide the necessary background for selective post-synaptic D2 stimulation. However, the post-synaptic stimulation from this dose of quinpirole is evidently inadequate to sustain the necessary cascade of molecular events for sensitized responding. But, when combined with even a very low dose of 8-OH-DPAT (0.03125 mg/kg), the two drugs together were sufficient to induce the cascade of molecular events necessary for sensitized responding. No further potentiation of sensitization was evident with higher co-administered doses of 8-OH-DPAT (Figure 1), suggesting a convergence on a common molecular site from stimulation of D2 and 5-HT_{1A} receptors. Speculatively, we suggest that this convergence may be on glycogen synthase kinase 3 (GSK-3) signaling pathway as both quinpirole and 8-OH-DPAT trigger cascades for inhibitory regulation of the kinase GSK-3ß (Beaulieu *et al*, 2007). Nevertheless, considering the complexity of D2 signaling (Beaulieu *et al*, 2011), there probably exist a number of pathways through which 5-HT_{1A} ligands could modulate the increase in post-synaptic D2 receptor efficacy induced by repeated injections of quinpirole.

In summary, even though the cross-sensitization results suggest that the induction of sensitization to quinpirole does not involve 5-HT_{1A} receptors, nevertheless 5-HT_{1A} activation can modulate quinpirole sensitization by influencing the key pre-synaptic and post-synaptic events producing sensitization to quinpirole. In this respect, the conclusion here for quinpirole sensitization is similar as for amphetamine sensitization, namely, that induction of amphetamine sensitization does not involve 5-HT_{1A} receptors but could be modulated by stimulation of 5-HT_{1A} receptors (Przegaliński *et al*, 2000).

EFFECTS OF D2/D3 ACTIVITY ON SENSITIZATION TO 8-OH-DPAT

The magnitude of sensitization to 8-OH-DPAT was small compared to that of quinpirole, but present nevertheless (Fig. 1ab and Table 1). There is an extensive literature examining the effects of 8-OH-DPAT on responding to psychostimulant drugs (Przegaliński *et al*, 2000; Carey *et al*, 2004; Müller *et al*, 2007; Haleem, 2013), but little consideration of sensitization to 8-OH-DPAT itself, possibly because the magnitude of the effect is small and seemingly complex (De La Garza *et al*, 2000). Hence, our interpretation of possible mechanisms by which co-administration of quinpirole altered sensitization to 8-OH-DPAT is not guided by a framework as refined as the one for sensitization to quinpirole.

Sensitization to 8-OH-DPAT is not only smaller in magnitude but has also a different form than the sensitization induced by quinpirole. The time course of sensitized

locomotor activity after an injection of quinpirole has a totally different profile than the time course of locomotor activity after an acute injection of quinpirole (Fig. 2ab; Szechtman *et al*, 1994a; Szechtman *et al*, 1994b; Alkhatib *et al*, 2013). However, the time course of locomotion in rats sensitized to 8-OH-DPAT is identical to the profile after an acute injection of 8-OH-DPAT, except for a shift upwards of the time course curve (Fig. 2ac; Alkhatib *et al*, 2013). Interestingly, except for the shift in intercepts, both the acute and sensitized 8-OH-DPAT time course profiles are similar to the typical habituation profile of rats introduced into a testing environment; that is, high activity at start of testing and a monotonic decline to low activity towards end of testing (Mignon and Wolf, 2002). Such a time course profile is consistent with the possibility that 8-OH-DPAT acts to increase the gain on a system that normally is activated when an animal is introduced into a testing environment and which mediates the habituation to it.

One system that mediates locomotion when rats are introduced into a new environment is the dopamine system and hence the increase in locomotion produced by an injection of 8-OH-DPAT can result from higher levels of dopamine activity. Evidence for this possible mechanism are findings that activation of 5-HT_{1A} receptors facilitate dopamine release (Arborelius *et al*, 1993b; Tanda *et al*, 1994; Chen *et al*, 1995; Ichikawa and Meltzer, 1999; Fink *et al*, 2007) and presence of extensive serotonergic innervation of dopamine neurons and terminals (Barnes *et al*, 1999; Alex and Pehek, 2007; Müller *et al*, 2007; Filip and Bader, 2009). 5-HT_{1A} receptors are found on the soma and dendrites of serotonergic raphe neurons, where they serve as autoreceptors to inhibit cell firing, and thus regulate serotonergic tone (Barnes *et al*, 1999; Albert and Le François, 2010). 5-HT_{1A} receptors are also found on non-serotonergic neurons where they serve as heteroreceptors mediating cellular responses to released 5HT and as "pre-synaptic heteroreceptors" (Fink *et al*, 2007) having inhibitory effects on the non-serotonin neurotransmitter release (DA, NA, ACh and GABA). It is noteworthy that because inhibitory GABA interneurons are often interposed between the serotonin terminals and DA, NA or ACh neurons, the functional effect of 5-HT_{1A} stimulation of such GABA interneurons is disinhibition (facilitation) of DA, NA or ACh neurotransmitter release (Fink *et al*, 2007). Accordingly, repeated pharmacological activation of 5-HT_{1A} receptors may yield sensitization to 8-OH-DPAT through neuroplastic changes that result in the diminution of the serotonergic inhibitory tone over dopamine activity. Indeed, the observation that baseline locomotor activity was increased in rats treated chronically with 8-OH-DPAT (Figure 1), is consistent with this possibility. Below we use the outlined framework to interpret the present findings of reduction and potentiation of 8-OH-DPAT sensitization by co-administered quinpirole.

One possible mechanism by which co-treatment with the lower (0.03125 mg/kg), but not the higher dose of quinpirole (0.125 mg/kg), attenuated sensitization induced by 8-OH-DPAT (0.125 mg/kg) may relate to the dose-dependent effects of quinpirole on pre-synaptic *versus* post-synaptic dopamine receptors. The actions of low dose quinpirole are predominantly pre-synaptic, biasing the dopamine neurons towards less firing. In this respect, the actions of co-administered low dose quinpirole are opposite to the excitability-promoting effects of 8-OH-DPAT on dopamine cell firing and release (Arborelius *et al*, 1993a; Arborelius *et al*, 1993b; Tanda *et al*, 1994; Chen *et al*, 1995). Such contrary actions of low dose quinpirole may reduce the gain on dopamine activity produced from repeated stimulation of 5-HT_{1A} receptors and hence a reduction in sensitization to 8-OH-DPAT. However, as is evident in Figure 1, the reduction in sensitization to 8-OH-DPAT is not evident from co-treatment with a higher dose of quinpirole (0.125 mg/kg). This suggests that even though 8-OH-DPAT would evoke less dopamine release in sensitized rats, this reduction could be compensated by the increase in efficacy of post-synaptic D2 receptors produced by co-treatment with the higher dose of quinpirole (discussed in section "Effects of 5-HT_{1A} activity on sensitization to quinpirole"). In other words, the amount of locomotion is as high as with chronic 8-OH-DPAT alone, because even though in quinpirole co-treated rats less dopamine would be released, the neurotransmitter acts on more sensitive post-synaptic receptors to produce an equivalent amount of locomotion.

A plausible mechanism by which quinpirole co-treatment (0.125 mg/kg) could enable sensitization to an ineffective dose of 8-OH-DPAT (0.0625 mg/kg) reflects likely the increase in post-synaptic D2 efficacy produced by co-administered quinpirole. Presumably, the absence of sensitization with the 0.0625 mg/kg dose of 8-OH-DPAT is a quantitative effect; that is, chronic treatment with this dose of 8-OH-DPAT increased the gain on dopamine activity but not high enough to exceed the effects of an acute drug injection. Indeed, an increase in gain is suggested by elevated baseline locomotion in rats treated chronically with 0.0625 mg/kg of 8-OH-DPAT (Figure 1, *right* panel). Considering that co-treatment with the high dose of quinpirole (0.125 mg/kg) would increase the efficacy of D2 post-synaptic receptors, the elevation in dopamine activity evoked by a challenge with 8-OH-DPAT would manifest itself as sensitization to 8-OH-DPAT.

In summary, sensitization to 8-OH-DPAT may reflect attenuated serotonergic inhibitory tone over midbrain dopamine activity. Modulation of this sensitization by co-treatment with quinpirole is probably through direct effects of quinpirole on dopamine neurons and their post-synaptic D2 receptors related to locomotor activity. However, it should be also considered that co-injections of quinpirole could have altered the activity of

serotonin neurons themselves. This possibility is suggested by the presence of D2 receptors in dorsal raphe (Levant *et al*, 1993; Yokoyama *et al*, 1994) and by the finding that blockade of those D2 receptors increased the excitatory effects of acute quinpirole (Szumlinski and Szechtman, 2002).

COMPETING INTERESTS

The authors declare no competing interests.

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Table 1. Test for cross-sensitization between the effects induced by chronic pretreatment with quinpirole (0.125 mg/kg) and chronic pretreatment induced by 8-OH-DPAT (0.125 mg)

Chronic Pretreatment Group (mg/kg)	**Acute Challenge with**			
	QNP (0.1 mg/kg)		**DPAT (0.1 mg/kg)**	
	Mean[1]	95% Confidence Interval[1]	Mean[1]	95% Confidence Interval[1]
QNP (0) + DPAT (0)	78.2	(52.8 – 103.6)	70.9	(60.5 – 81.3)
QNP (0.125) + DPAT (0)	**279.2**[2]	**(244.7 – 313.6)**	82.3	(68.2 – 96.4)
QNP (0) + DPAT (0.125)	66.1	(30.2 – 102.0)	**113.8**[2]	**(99.1 – 128.5)**

[1] Mean distance travelled (and 95% confidence interval) in meters during 60 minutes following an acute injection of quinpirole (QNP, 0.1 mg/kg) or 8-OH-DPAT (DPAT, 0.1 mg/kg) in 3 groups of rats pretreated with 10 injections of either saline (QNP (0) + DPAT (0) group), 0.125 mg/kg of QNP (QNP (0.125) + DPAT (0) group) or 0.125 mg/kg of 8-OH-DPAT (QNP (0) + DPAT (0.125) group). Data are from Fig. 1a and Fig. 1b.

[2] **Bold font** indicates $p<0.05$ compared to the chronic saline control group (QNP (0) + DPAT (0) group). A significantly higher response than the acute response of saline controls when challenged with the same drug as in chronic pretreatment indicates that the chronic pretreatment drug induced sensitization. Lack of a significant effect compared to the acute response of saline controls when challenged with the drug not used for chronic pretreatment shows that a sensitized response is not evoked with the non-pretreatment drug and hence that cross-sensitization is absent.

Table 2. Effects of chronic co-injections of 8-OH-DPAT on the sensitization to QNP.

Chronic QNP dose (mg/kg)	**Co-injected 8-OH-DPAT dose (mg/kg)**[1]				**Effect on sensitization to QNP**[2]
	0	.03125	.0625	.125	
0.125	**279 (245-314)**	**247 (211-283)**	**231 (195-266)**	**185 (148-223)***	↓ dose-dependent
0.0625	113 (78-147)	**157 (122-191)**	**147 (112-181)**	**160 (124-196)**	↑ with all doses

[1] Mean distance travelled (and 95% confidence interval) in meters during 60 minutes following an acute injection of quinpirole (QNP, 0.1 mg/kg) administered to groups of rats pretreated with 10 injections of QNP (0.125 mg/kg or 0.0625 mg/kg) plus 4 doses of 8-OH-DPAT (0, 0.03125, 0.0625 or 0.125 mg/kg). **Bolded** numerals show a sensitized response to the challenge dose of QNP (0.1 mg/kg) as the indicated distance was significantly higher than the acute QNP response of saline controls (mean = 78 m, 95% confidence interval = 53 – 104 m). Data are from Fig. 1a.

[2] Summary of how co-treatment with 8-OH-DPAT affects sensitization to quinpirole. Bolded value in the 0 mg/kg 8-OH-DPAT column indicates that the given dose of chronic QNP induced sensitization and the 3 columns to the right show how co-injections with 8-OH-DPAT modulate the quinpirole-alone effect. DOWN arrow indicates that 8-OH-DPAT co-treatment reduces the effects of quinpirole and the UP arrow that 8-OH-DPAT co-treatment increases/potentiates the quinpirole-alone effect.

*$p < .05$ *vs* quinpirole-alone.

Table 3. Effects of chronic co-injections of QNP on the sensitization to 8-OH-DPAT.

Chronic DPAT dose (mg/kg)	**Co-injected QNP dose (mg/kg)**[1]				**Effect on sensitization to DPAT**[2]
	0	.03125	.0625	.125	
0.125	**114 (99-129)**	90 (75-105)	**96 (82-111)**	**120 (105-136)**	↓ with low dose
0.0625	91 (76-106)	78 (64-93)	90 (75-105)	**106 (92-121)**	↑ with highest dose

[1] Mean distance travelled (and 95% confidence interval) in meters during 60 minutes following a challenge injection of 8-OH-DPAT (DPAT, 0.1 mg/kg) administered to groups of rats pretreated with 10 injections of 8-OH-DPAT (0.125 mg/kg or 0.0625 mg/kg) plus 4 doses of QNP (0, 0.03125, 0.0625 or 0.125 mg/kg). **Bolded** numerals show a sensitized response to the challenge dose of 8-OH-DPAT (0.1 mg/kg) as the indicated distance was significantly higher than the acute 8-OH-DPAT response of saline controls (mean = 71 m, 95% confidence interval = 61 – 81 m). Data are from Figure 1b.

[2] Summary of how co-treatment with QNP affects sensitization to 8-OH-DPAT. Bolded value in the 0 mg/kg QNP column indicates a sensitized response at the given dose of chronic 8-OH-DPAT and the 3 columns to the right show how co-injections with QNP modulate the 8-OH-DPAT-alone effect. DOWN arrow indicates that QNP co-treatment reduces the effects of 8-OH-DPAT and the UP arrow that QNP co-treatment increases/potentiates the 8-OH-DPAT-alone effect.

FIGURE CAPTION

Figure 1 – Locomotor performance by 16 groups of rats pretreated chronically with various doses of quinpirole (0, 0.03125, 0.0625, 0.125 mg/kg), 8-OH-DPAT (0, 0.03125, 0.0625, 0.125 mg/kg), or a combination of the two drugs on 3 challenge tests: **(a)** following an acute injection of quinpirole (QNP, 0.1 mg/kg); **(b)** following an acute injection of 8-OH-DPAT (DPAT, 0.1 mg/kg)) and **(c)** following an acute injection of saline. Chronic pretreatment consisted of 10 administrations of the indicated drugs, two injections per week over the course of 5 weeks. The same schedule was continued without interruption for an additional 3 injections and constitutes the challenge tests shown in the figure. *Solid* circle is the mean value of distance travelled during the 60 min test for the indicated group, and the floating bar represents the 95% confidence intervals. A floating bar entirely above the *black solid* horizontal line indicates the group is significantly different from the saline control group on the saline challenge test; a floating bar entirely above the *green short dashed* horizontal line is significantly higher than the acute quinpirole response of the saline control group; and a floating bar entirely above the *blue long dashed* horizontal line is significantly higher than the acute 8-OH-DPAT response. Because the means and 95% confidence intervals shown in the figure portray a significant triple interaction of Chronic Pretreatment QNP Dose by Chronic Pretreatment DPAT Dose by Acute Challenge Drug ($F_{12.8, 271.7}$ = 2.7, P = 0.001, partial eta squared = 0.113), any two groups from across the 3 panels with non-overlapping floating bars are significantly different from each other by simple effects; however, the only significant comparisons that are marked in the figure are those from comparisons performed within a cluster of bars. *, $p < 0.05$ *vs first* floating bar of the same cluster; **, $p < 0.05$ *vs first* and *second* floating bar of the same cluster.

Figure 2 – Time profile of the acute locomotor response to an injection of saline, QNP (0.1 mg/kg) and DPAT (0.1 mg/kg) in: **(a)** control rats pretreated chronically with saline; **(b)** rats pretreated chronically with 0.125 mg/kg of QNP; and, **(c)** rats pretreated chronically with 0.125 mg/kg of DPAT. Each point is the mean distance travelled in the indicated 5 min interval; estimated standard errors of the mean were generally not larger than the size of the data symbol and are not plotted. Time profiles are shown for 3 of the 16 groups plotted in Figure 1.

Figure 1

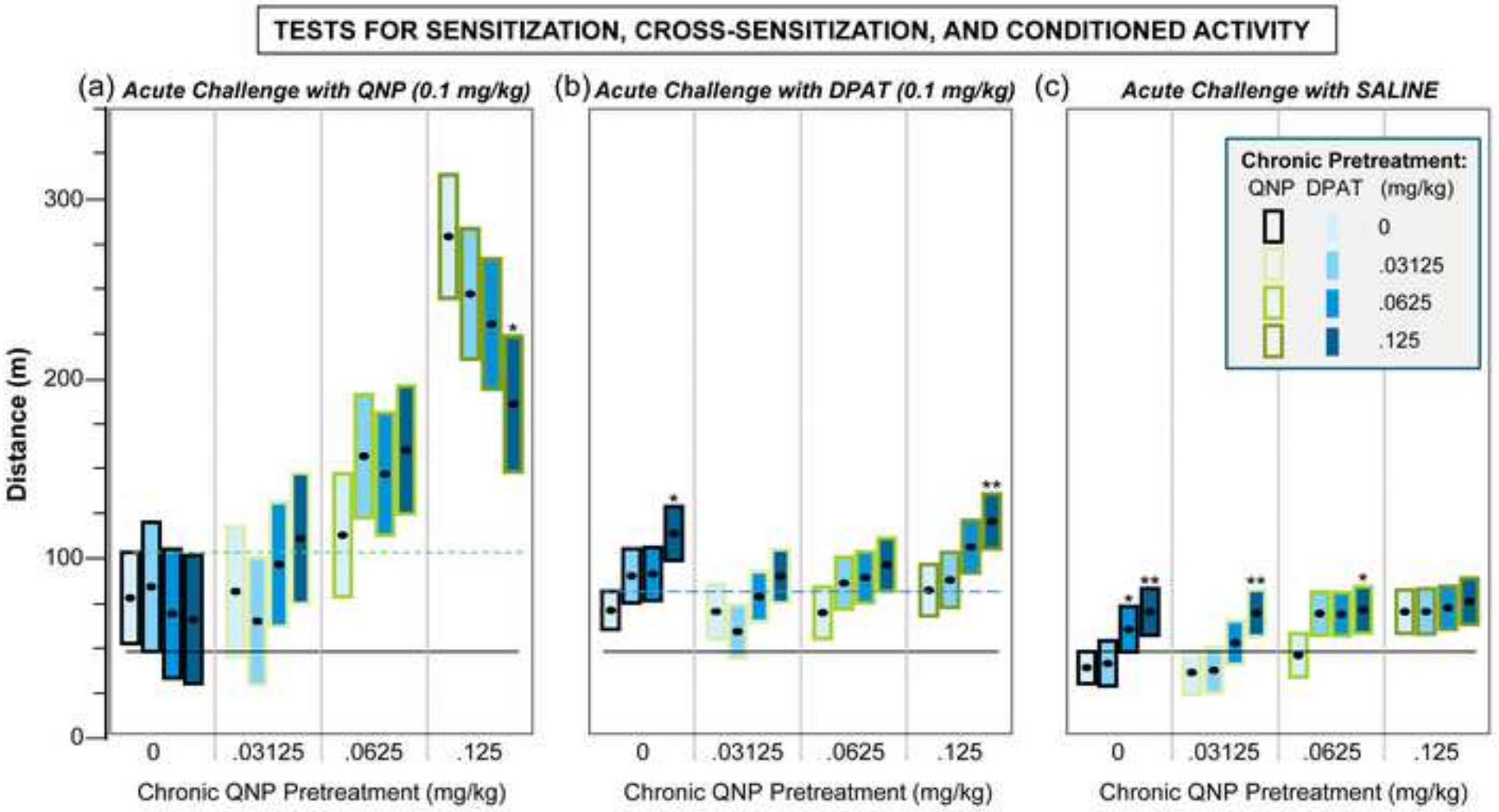

Figure 2