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Research report

5-HT2A/C receptors do not mediate the attenuation of compulsive checking by mCPP in the quinpirole sensitization rat model of obsessive–compulsive disorder (OCD)

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HIGHLIGHTS

- The 5-HT agonist mCPP attenuates quinpirole-induced compulsive checking in rats.
- We ask if this effect is mediated by mCPP activity at 5-HT2A/C receptors.
- Blockade of 5-HT2A/C receptors by ritanserin did not inhibit the mCPP effect.
- Attenuation of compulsive checking by mCPP is not mediated by 5-HT2A/C receptors.

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ABSTRACT

There is emerging evidence for a dopamine (DA)–serotonin (5-HT) interaction underlying obsessive–compulsive disorder (OCD). In the quinpirole sensitization rat model of OCD, compulsive checking is induced by chronic treatment with the DA agonist quinpirole, and is attenuated by the 5-HT agonist drug mCPP. However, mCPP has affinity for a number of 5-HT receptor subtypes, and it is unknown by which receptors mCPP exerts its effects on quinpirole-treated animals. The present study tested in rats whether mCPP activity at 5-HT2A/C receptors mediates the attenuation of compulsive checking in quinpirole-treated animals. Rats were chronically treated with quinpirole on the open field for the induction of compulsive checking. Following the induction phase, animals were treated with mCPP (1.25 mg/kg) and the selective 5-HT2A/C receptor antagonist ritanserin (1 mg/kg or 5 mg/kg) to test whether blockade of 5-HT2A/C receptors inhibits attenuation of checking by mCPP. Results showed that as expected, quinpirole induced compulsive checking, and mCPP reduced its performance. However, 5-HT2A/C receptor blockade by ritanserin did not inhibit the attenuation of compulsive checking by mCPP. These results suggest that the reduction in compulsive checking by mCPP is not mediated by activity at 5-HT2A/C receptors, but by another receptor subtype.

1. Introduction

While a large body of research implicates both dopamine (DA) and serotonin (5-HT) in obsessive–compulsive disorder (OCD), there is emerging evidence for a possible DA–5-HT interaction underlying the affliction [1–4]. For example, it has been suggested that hyper DA activity in circuits projecting to cortical and subcortical areas may result in obsessional thoughts or compulsive behaviors, and that such hyperactivity could be calmed by blockade of 5-HT2A, or stimulation of 5-HT1A receptors located on pyramidal cells in the prefrontal cortex [4]. According to the authors [4], such a calming effect may be produced by antipsychotic drugs which in addition to activity at DA D1/D2 receptors, also act as antagonists at 5-HT2A and agonists at 5-HT1A receptors.

In a recent study employing the quinpirole sensitization rat model of OCD, it was reported that acute treatment with the 5-HT agonist drug mCPP reduced the motor vigor of checking and prolonged the post-checking satiety, but did not affect the focus on the task of checking [5]. Vigor, focus, and satiety, had been identified as three separate functional components underlying compulsive checking behavior in the animal model [6,7]. The effect of mCPP on vigor and satiety rendered the quinpirole-treated animals as

Abbreviations: OCD, obsessive–compulsive disorder; 5-HT, serotonin; DA, dopamine; mCPP, 1-(3-chlorophenyl)-piperazine hydrochloride; NAc, nucleus accumbens core; OFC, orbitofrontal cortex; DPAT, 8-hydroxy-2-(di-*n*-propylamino) tetralin hydrochloride.

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no longer meeting criteria for 'compulsive' checking behavior [5]. Given the induction of compulsive checking by the DA agonist drug quinpirole and its reduction by the 5-HT agonist drug mCPP, the authors suggested the presence of a putative DA–5-HT interaction underlying the model compulsive behavior. However, it is unknown which serotonergic receptor subtype(s) stimulated by mCPP mediate the effects of the drug on compulsive checking. Identifying a role for specific 5-HT receptor subtypes would strengthen the argument for a DA–5-HT interaction underlying the performance of compulsive checking behavior in the model.

mCPP acts on several 5-HT receptor subtypes including: 1A, 1B, 1D, 2A, 2C, and 3 [8–11]. mCPP has been reported to have high affinity for the 5-HT2A/C receptor subtype [8,10,11] and has widely been used as a probe of 5-HT function [12], despite having also an affinity for a number of non-5-HT receptor subtypes including: adrenergic $\alpha 1/2$ and β receptors, and to a lesser extent, dopamine and cholinergic receptors [8,13]. Stimulation of 5-HT2 receptors has been suggested by some to mediate the therapeutic effects produced by selective serotonin reuptake inhibitors (SSRIs) on OCD [14,15]. Hence, it is possible that the attenuating effects produced by mCPP on quinpirole-induced compulsive checking may be mediated by stimulation of 5-HT2A/C receptors.

Accordingly, the present study tested whether the selective 5-HT2A/C receptor antagonist drug ritanserin blocks the attenuating effects produced by mCPP on the exaggerated vigor and satiety induced by quinpirole treatment. We hypothesized that mCPP attenuates these constitutive functional components through stimulation of 5-HT2A/C receptors, and hence predicted that ritanserin co-treatment will block the effects of mCPP. However, if mCPP attenuates these functional components by stimulating other receptor subtypes, then ritanserin co-treatment would not block the attenuating effects of mCPP.

2. Materials and methods

2.1. Animals

Subjects were 56 experimentally naïve adult male rats of the Long Evans strain that weighed 250–300 g at the onset of the experiment. Animals were housed in a climate controlled colony room with a 12 h light/dark cycle (6 AM lights on; 6 PM lights off). Testing occurred during the light phase. Food and water were freely available during the experiment. Upon arrival, animals were given a period of 7 days to acclimatize to the facility. This was followed by 5 days of handling for 2–5 min each day before the onset of behavioral testing. Animals were housed and tested in compliance with the regulations set forth by the guidelines of the Canadian Council on Animal Care and approved by the Animal Research Ethics Board, McMaster University.

2.2. Drugs

All drugs were obtained from Sigma-Aldrich, USA. Quinpirole hydrochloride was administered at a dose of 0.125 mg/kg. This dose was chosen because it was shown in a previous study [5] to induce compulsive checking behavior in rats. mCPP was administered at a dose of 1.25 mg/kg. This dose was chosen because Tucci et al. [5] found that 1.25 mg/kg of mCPP attenuated quinpirole effects on vigor of checking and on satiety. Both quinpirole and mCPP were administered in a 0.9% physiological saline vehicle at a volume of 1 ml/kg. For control treatments, animals received 0.9% physiological saline at a similar volume. Ritanserin was administered at doses of either 1 mg/kg or 5 mg/kg in a vehicle containing 67% ethanol and 33% saline at a volume of 0.3 ml/kg. These doses of ritanserin were chosen based on a report in the literature that they significantly reversed hypolocomotion induced by a similar dose of mCPP as used here [35]. For control treatments, animals received vehicle at a similar volume. All injections were made sub-cutaneously under the nape of the neck.

2.3. Apparatus

Behavioral testing of animals occurred on a large open field (160×160 cm table without walls) that was located in a non-colony experiment room, as described previously [6,18]. The table was divided into a grid of 25 virtual rectangular places (locales); no lines were actually marked on the table surface. Four small Plexiglas/glass boxes (approximately $8 \times 8 \times 7.5$ cm) were located at the same fixed location on the open field for the duration of the experiment: two were located at corners and two were located at places near the center of the open field. Following each trial on the open field, the table and objects were wiped clean with a diluted solution of an antibacterial cleaner (Lysol). Behavior of animals on the open field was videotaped continuously by a camera affixed to the ceiling (providing a stationary top view of the entire open field and the rat in it). 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, Netherlands) software [16,17].

2.4. Data analysis

From the digitized video files, EthoVision 3.1 software was used to extract the time series of x, y coordinates of the rat in the open field [18]. To remove noise, digitized tracking data were pre-processed (by applying appropriate filters to smooth the x, y coordinates) [19], 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 [20,21,22]. The coordinates were then mapped onto the 25 open field locales (places) [24], and the frequency of visits and duration of stops in each locale were computed (the terms 'visit' and 'stop' are equivalent and are used interchangeably). Checking behavior is defined with reference to the most visited locale (labelled 'key place' or 'key locale'; these terms are equivalent), which in most instances is also the locale with the longest total duration of stops [23,24]. A visit to the key place is also referred to as a 'check' or 'checking'. Four criteria measures of checking behavior are computed. (1) Frequency of checking: total number of visits to the key locale. (2) Length of check: total duration of stay at the key locale divided by the frequency of visits there; this measure is also an indirect index of ritual-like behavior as the appearance of motor rituals in quinpirole-treated rats is associated with a very short duration of stay in the key locale [24,25]. (3) Recurrence time of checking: mean duration of return times to the key locale ('return time' is the interval from departure to next arrival at the locale). (4) Stops before returning to check: mean number of places visited between returns to the key locale. Animals are considered to be showing compulsive checking behavior when all four criteria measures differ significantly from saline-treated control animals [24], and hence the group of these four measures is termed 'criteria measures' for compulsive checking. Because blockade of 5-HT2C receptors has been reported to alter the hypolocomotor effects produced by mCPP [26,27,28], we also assessed locomotor activity by measuring 'total distance traveled'.

The behavioral profile of compulsive checking behavior has been empirically dissociated into a set of functional components [6]. Three functional components have been identified, each of which is greatly exaggerated in compulsive animals: the *vigor* with which checking is performed, the *focus* on the task of checking and the amount of rest or *satiety* following a bout of checking. Lesions