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Mirtazapine and ketanserin alter preference for gambling-like schedules of reinforcement in rats

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ABSTRACT

Drug and behavioral addictions have overlapping features, *e.g.*, both manifest preference for larger, albeit costlier, reinforcement options in cost/benefit decision-making tasks. Our prior work revealed that the mixed-function serotonergic compound, mirtazapine, attenuates behaviors by rats motivated by abused drugs. To extend this work to behavioral addictions, here we determined if mirtazapine and/or ketanserin, another mixed-function serotonin-acting compound, can alter decision-making in rats that is independent of drug (or food)-motivated reward. Accordingly, we developed a novel variable-ratio task in rats wherein intracranial self-stimulation was used as the positive reinforcer. Using lever pressing for various levels of brain stimulation, the operant task provided choices between a small brain stimulation current delivered on a fixed-ratio schedule (*i.e.*, a predictable reward) and a large brain stimulation delivered following an unpredictable number of responses (*i.e.*, a variable-ratio schedule). This task allowed for demonstration of individualized preference and detection of shifts in motivational influences during a pharmacological treatment. Once baseline preference was established, we determined that pretreatment with mirtazapine or ketanserin significantly decreased preference for the large reinforcer presented after gambling-like schedules of reinforcement. When the rats were tested the next day without drug, preference for the unpredictable large reinforcer option was restored. These data demonstrate that mirtazapine and ketanserin can reduce preference for larger, costlier reinforcement options, and illustrate the potential for these drugs to alter behavior.

1. Introduction

The ability to assess risk and to estimate costs and benefits associated with different choice options helps to determine advantageous courses of action (Baarendse et al., 2013; Orsini et al., 2015). Suboptimal/disadvantageous decision-making can have severe consequences, such as is observed in individuals who exhibit drug and/or behavioral addictions (Bechara et al., 2001; Rogers and Robbins, 2001; Ernst et al., 2003; Brand et al., 2005). Gambling disorders are examples of behavioral addiction that are highly relevant to modern society, yet there is no government-approved therapy for these disorders. We are interested in identifying efficacious pharmacotherapeutics for gambling disorders. To enhance translational value, we selected compounds already deemed safe for human use.

Serotonin (5-HT) is involved in normal executive function (Floresco and Jentsch, 2011), and dysregulation of this transmitter system is associated with risky decision-making (Nordin and Eklundh, 1999; Pallanti et al., 2006; Potenza et al., 2013), measures of impulsivity (Dalley and Roiser, 2012), and drug addiction (Muller and Homberg, 2015). Mirtazapine is an atypical antidepressant with a complex pharmacological profile that includes antagonism at 5-$HT_{2A/2C}$ receptors (De Boer, 1995; Wikstrom et al., 2002). Mirtazapine attenuates various behaviors motivated by abused drugs, including methamphetamine (Herrold et al., 2009; Voigt et al., 2011; Graves and Napier, 2011; Voigt and Napier, 2011) and morphine (Graves et al., 2012a) in rats, and reduces cocaine intake in humans (Graves et al., 2012b). We recently revealed that mirtazapine reduces the capacity of the dopamine D2/D3 receptor agonist, pramipexole, to induce risky decision-making by rats performing a probability discounting task (Holtz et al., 2016). The potential for mirtazapine to influence risky behaviors

Abbreviations: 5-HT, serotonin; FR, fixed-ratio; ICSS, intracranial self-stimulation; VR, variable-ratio

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independent of drug-provoked effects has not been tested.

Ketanserin is another mixed-function antagonist with high affinity for 5-$HT_{2A/2C}$ receptors (Hoyer, 1988; Bonhaus et al., 1995). Ketanserin reduces behaviors by rats that are motivated by cocaine (Burmeister et al., 2004), nicotine (Levin et al., 2008) and methamphetamine (Bhatia et al., 2011), as well as neurophysiological effects of methamphetamine (McDaid et al., 2007). In studies of impulsivity, ketanserin reduces impulsive motor behavior (Passetti et al., 2003; Talpos et al., 2006; Fletcher et al., 2007). Thus, ketanserin, like mirtazapine, may be beneficial for the treatment of behavioral addiction.

Based on this background, we sought to determine if mirtazapine and ketanserin would influence risky decision-making in rats using a task that approximated key features of gambling disorders, such as weighing cost *versus* benefit and the uncertainty of reward delivery, and was independent of drug-motivated reward. We also sought to avoid food-reinforcement protocols, for serotonin regulates feeding and satiety, and both mirtazapine and ketanserin alter these functions in humans (Risselada et al., 2010; Jeong and Bahk, 2014) and rats (Pratt et al., 2016). Accordingly, we implemented a modified choice task developed by Johnson et al. (Johnson et al., 2011; Johnson et al., 2012) using intracranial self-stimulation (ICSS) as the positive reinforcer. This task utilizes gambling-like schedules of reinforcement to model features of human cost/benefit decision-making including the choice between the unpredictable occurrences of a high effort/large reinforcer option, and the predictable occurrence of a low effort/small reinforcer option. In this paradigm, the response cost placed on obtaining the large reinforcer is the exertion of greater physical effort (*i.e.*, increased number of lever presses) necessary to obtain the reward; the average number of responses to obtain brain stimulation reinforcement on the high effort/large reinforcer schedule was always greater than the number or responses required on the low effort/small reinforcer alternative. The first feature was modeled by a variable-ratio (VR) schedule of ICSS-mediated reinforcement, which was contrasted to the second feature wherein ICSS was available using a predictable, fixed-ratio (FR) schedule. Using this task, we tested the effects of mirtazapine and ketanserin on preference for the VR option of reinforcement.

2. Methods and materials

2.1. Animals

Male Sprague-Dawley rats (n = 31) were purchased from Envigo Laboratories (Indianapolis, IN) weighing 250–274 g upon arrival. They were housed in pairs under a 12 h light/dark cycle (lights on at 7 AM) in an environmentally controlled facility with food and water available *ad libitum*. Procedures were in accordance with those established in the *Guide for the Care and Use of Laboratory Animals* (National Research Council, Washington DC) as approved by the Rush University Medical Center Institutional Animal Care and Use Committee.

2.2. Test drugs

Mirtazapine (isolated from tablet form by Plantex, Hackensack, NJ; a division of Teva Pharmaceuticals Ltd., North Wales, PA) was dissolved in 1 N HCl, diluted with sterile H_2O, and the pH was adjusted to ~6.3–6.8 using 1 N NaOH. Mirtazapine was administered intraperitoneally (ip) as 5.0 mg/ml/kg. This dose was selected based on our extensive prior studies showing that it is sufficient to reduce several forms of methamphetamine- (Graves and Napier, 2011; Herrold et al., 2009; McDaid et al., 2007; Voigt et al., 2011; Voigt and Napier, 2011), and morphine- (Graves et al., 2012a) motivated behaviors in rats without increasing latency to lever press in cue reactivity paradigm or altering coordinated motor function on a rotarod (Graves and Napier, 2011). Ketanserin tartrate (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile H_2O and administered ip at doses of 1.0, 2.5, or 5.0 mg/ml/kg (as the free base). Ketanserin vehicle (sterile H_2O) was administered as 1 ml/kg, ip. The doses used for both mirtazapine and ketanserin convert to a "Human Equivalency Dose" that provides efficacious treatment in humans (Fawcett and Barkin, 1998b; Fawcett and Barkin, 1998a; Liechti et al., 2000; Graham et al., 2002). Mirtazapine and ketanserin were tested in separate groups of rats.

2.3. Stimulating electrode implantation

Rats were anesthetized with isoflurane and placed into a stereotaxic frame (David Kopf, Tujunga, CA) with the nose piece set at − 3.3 mm. A midline scalp incision was made, and a burr hole was drilled through the skull at the following coordinates (from Bregma): − 2.8 mm AP and − 1.8 mm ML. A bipolar stimulating electrode (MS303/3-B/SPC; Plastics One, Roanoke, VA) was lowered 8.4 mm from the top of the skull into the lateral hypothalamus. Electrodes were secured to the skull using stainless steel screws and dental acrylic, and the incision was sutured. Rats were returned to the home cage following recovery from anesthesia; one week later, operant training began. Three rats were removed from the study due to loss of headpiece during training or lack of task acquisition due to electrode placement outside of the lateral hypothalamus.

2.4. Testing apparatus

Rats were tested in operant chambers (30.5 cm × 24.1 cm × 21.0 cm; Med-Associates, St. Albans, VT), enclosed in ventilated, sound-attenuating boxes. Each chamber was fitted with two retractable levers on one wall with a stimulus light above each lever. On the opposite wall, a single 100 mA house light was located in the top center. Intracranial stimulation was delivered by constant current stimulators (PHM-152/2 dual programmable ICSS stimulator) *via* bipolar leads connected to 2-channel commutators (Plastics One) mounted above the chamber. All experimental data were recorded by a PC connected to the operant chambers *via* an interface.

2.5. Behavioral testing protocol

The ICSS testing protocol was modified from those previously established in our laboratory using probability discounting (Rokosik and Napier, 2011; Rokosik and Napier, 2012; Holtz et al., 2016) and delay discounting (Tedford et al., 2015) tasks.

2.5.1. Phase 1: shaping/fixed-ratio 1 (FR1)

Rats were trained to associate a reinforcing electrical stimulation of the brain with pressing a lever using a forepaw. Rats were primed with 100 μA at 100 Hz, then "guided" towards the extended lever with subsequent stimulation. Current parameters were incremented as needed to train the rats to associate the stimulation with pressing the lever, then we verified that the rats would consistently self-initiate lever pressing and maintain a stable lever pressing on both levers on a FR1 schedule of reinforcement (to ensure that a lever bias did not develop).

2.5.2. Phase 2: fixed-ratio 3 (FR3)

This phase verified that the current parameters determined in Phase 1 were sufficient to maintain a stable FR3 schedule of reinforcement within a session. To do so, rats were placed in the operant chamber with one lever (either the right or left) extended for 10 min, and they were trained to press the lever three times in order to obtain brain stimulation. Rats were trained on both levers to ensure that a lever bias did not develop.

2.5.3. Phase 3: choice fixed-ratio task

This phase verified that the rats could discriminate between the small and large reinforcers. The stimulation current values for small and large reinforcements were determined from a previously published current frequency *vs.* lever pressing response curve (Tedford et al., 2015); the small reinforcer was set at 50 Hz, a value slightly above the threshold for stimulation current levels that supported responding. The