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Nucleus accumbens mu opioid receptors regulate context-specific social preferences in the juvenile rat

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ABSTRACT

The μ opioid receptor (MOR) in the nucleus accumbens (NAc) is involved in assigning pleasurable, or hedonic value to rewarding stimuli. Importantly, the hedonic value of a given rewarding stimulus likely depends on an individual's current motivational state. Here, we examined the involvement of MORs in the motivation to interact with a novel or a familiar (cage mate) conspecific in juvenile rats. First, we demonstrated that the selective MOR antagonist CTAP administered into the NAc reduces social novelty preference of juvenile males, by decreasing the interaction time with the novel conspecific and increasing the interaction time with the cage mate. Next, we found that a 3-h separation period from the cage mate reduces social novelty preference in both juvenile males and females, which was primarily driven by an increase in interaction time with the cage mate. Last, we showed that MOR agonism (intracerebroventricularly or in the NAc) restored social novelty preference in juvenile males that did not show social novelty preference following social isolation. Taken together, these data support a model in which endogenous MOR activation in the NAc facilitates the relative hedonic value of novel over familiar social stimuli. Our results may implicate the MOR in neuropsychiatric disorders characterized by altered social motivation, such as major depression and autism spectrum disorder.

1. Introduction

Across species, the juvenile period (here defined as immaturity and the transition period from weaning to adulthood) is characterized by increased interactions with peers compared to younger and older ages (Spear, 2000; Doremus-Fitzwater et al., 2010; Hunt et al., 2016). Such peer interactions are highly rewarding and are modulated by the μ-opioid receptor (MOR). For example, juvenile MOR knockout mice show reduced social interest and no preference for socially rewarding environments (Cinque et al., 2012). Furthermore, systemic administration of MOR agonists enhances, while MOR antagonists reduce, social play (a highly rewarding behavior) in juvenile male rats (Panksepp et al., 1980, 1985; Vanderschuren et al., 1995).

Importantly, the MOR has been implicated in the assignment of pleasurableness or 'hedonic' value to rewarding stimuli (Berridge and Kringelbach, 2015; Laurent et al., 2015). We recently showed that this might also be true for socially rewarding stimuli. In detail, when given the choice, juvenile rats interact more with a novel conspecific than with their cage mate and central MOR blockade reduced this social novelty preference (Smith et al., 2015). This suggests that central MOR activation plays a role in the encoding of the relative rewarding value of novel *versus* familiar social stimuli. Here, our first aim was to determine where in the brain this effect is mediated. The nucleus accumbens (NAc) and basolateral amygdala (BLA) are two candidate regions because of their well-established roles in the regulation of reward-related and social behaviors (Stuber et al., 2012; Ambroggi et al., 2008; Peciña and Berridge, 2000; Katayama et al., 2009; Trezza et al., 2011; Lichtenberg and Wassum, 2017) and the abundant expression of MORs in these brain regions (Kornblum et al., 1987; Smith et al., 2017). Therefore, we aimed to determine whether MOR activation in the NAc and/or BLA is causally involved in the regulation of social novelty preference in juvenile male rats. We predicted that pharmacological blockade of MORs in either the NAc or the BLA would reduce social novelty preference.

An individuals' motivation to seek social contact likely depends on social context, which, in turn, may shift the relative reward value of novel *versus* familiar conspecifics. For example, when housed with a cage mate, juvenile male and female rats interacted more with a novel conspecific than with their cage mate when given the choice (Smith et al., 2015). However, when juvenile male and female rats were isolated from their cage mate, they engaged more in interactions with their cage mate upon reunion than did juveniles that were united with a

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novel individual (Cirulli et al., 1996; Terranova et al., 1999). Importantly, the MOR system plays a role in the motivation to seek out social interaction upon social separation. For example, systemic MOR agonism reduced distress vocalizations upon maternal separation in puppies, chicks, rat pups, and infant rhesus monkeys, and this effect was reversed by administration of an MOR antagonist (Panksepp et al., 1978, 1980; Carden and Hofer, 1990; Kalin et al., 1988). Therefore, our second aim was to determine the impact of acute separation from a cage mate on social novelty preference in juvenile rats. We hypothesized that social separation would decrease social novelty preference and that this would be restored by MOR agonist administration.

2. Methods

2.1. Animals

Male and female Wistar rats were obtained from Charles River Laboratories (Raleigh, NC) at 22 or 23 days of age and housed in standard rat cages (26.7 × 48.3 × 20.3 cm) under standard laboratory conditions (12-h light/dark cycle, lights on at 7:00 am, food and water available *ad libitum*, 22 °C, 60% humidity). Experimental rats (subjects and cage mates) were one day older than novel stimulus rats to ensure that they were unrelated. All animals were housed with same-sex cage mates. All experiments were conducted in accordance with the NIH *Guide to the Care and Use of Laboratory Animals* and approved by the Boston College Institutional Animal Care and Use Committee (IACUC).

2.2. Social novelty preference test

Experimental rats (29–32 days of age) were exposed to the social novelty preference test in accordance with Smith et al. (2015). Briefly, experimental and stimulus rats were moved to the testing room 1 h prior to the onset of behavioral testing. All testing took place in the latter half of the light phase. Light intensity in the testing apparatus was ~240 lux. The testing apparatus consisted of a 3-chambered Plexiglass box (each chamber: 40 × 27 × 40 cm) with openings to allow for passage between chambers. The apparatus was cleaned with a dilute soap solution prior to each test. One day prior to testing, experimental and stimulus rats were acclimated to the testing room for 1 h and to the apparatus for 10 min. For testing, novel and familiar (cage mate) stimulus rats were placed in opposite ends of the apparatus and confined to Plexiglass rod containers (18 × 10 × 21 cm) to restrict their movement while still allowing for investigation by the experimental rat. The experimental rat was placed in the middle chamber and allowed to freely explore the three chambers and the social stimuli for 10 min. Behavior was recorded and scored using JWatcher (http://www.jwatcher.ucla.edu) by an experimenter blind to the treatment and sex of the rat. Time spent investigating each stimulus rat, frequency of investigation, time spent in each chamber, and number of entries into the middle chamber were measured. Number of entries into the middle chamber was taken as a measure of general locomotor activity. Investigation of the stimulus rats was defined as direct nose poking through the container rods. The percentage of time investigating the novel stimulus rat [(time investigating the novel stimulus rat/time investigating the novel + familiar rat) x 100] was calculated as measure of social novelty preference. Experimental animals were considered to exhibit a preference for social novelty when the percentage of time spent investigating the novel conspecific was significantly different from chance (50%). The percentage of time spent investigating the novel stimulus rat, the difference score, and time spent interacting with each stimulus were used as the main outcome measures.

2.3. Cannulation and injection procedures

At 27 or 28 days of age, experimental rats were anesthetized with isoflurane (Henry Schein, Dublin, OH) and positioned into a stereotaxic frame with the incisor bar set at −4.5 mm (Stoelting, Wood Dale, IL). Guide cannulas (Plastics One, Roanoke, VA) were implanted 2 mm dorsal to the target region using coordinates based on Paxinos and Watson (2007) and adapted for use in juveniles. Guide cannulae (22 gauge) were implanted bilaterally to target the NAc (1.6 mm rostral to bregma, +/− 2.4 mm lateral to the midline, 4.3 mm ventral to the skull surface, angle of 10° from midline) or BLA (2.6 mm caudal to bregma, +/−4.3 mm lateral to the midline, 6.0 mm ventral to the skull surface). For the NAc, we targeted the dorsomedial sub-region (core and shell) because this was where we previously observed the highest MOR binding density in the juvenile rat (Smith et al., 2017a). Cannula placements were considered to be hits between 2.0 and 2.5 mm rostral to Bregma for the NAc and between 2.64 and 3.36 mm caudal to Bregma for the BLA (See Supplementary Fig. S1 for all individual cannula placements). Of note, because of the smaller size of the juvenile brain, our coordinates yielded placements that were either more anterior (NAc) or more posterior (BLA) than would be expected in the adult brain. For intracerebroventricular (ICV) injections, a guide cannula (21 gauge) was implanted unilaterally to target the lateral ventricle (1.0 mm caudal to bregma, +1.6 mm lateral to the midline, 2.0 mm ventral to the skull surface). Guide cannulae were secured *via* stainless steel screws and dental acrylic adhesive and were closed with a dummy cannula (28/26 gauge for local/ICV injections; Plastics One). Following surgery, rats were singly housed for one hour before rehousing with their cage mate and were given daily injections of Rimadyl analgesic (10 mg/kg; Henry Schein, Dublin OH) for two days post surgery.

Pharmacological manipulations and behavioral testing commenced two days after stereotaxic surgery. We have previously shown that this short recovery period does not affect the expression of social novelty preference or other social behaviors, such as social play in juvenile rats (Veenema et al., 2012, 2013; Bredewold et al., 2014, 2015; Smith et al., 2015, 2017b). Experimental rats were handled for four days prior to testing to habituate them to the injection procedure. Injection systems were composed of polyethylene tubing connected to a Hamilton syringe (10/25 µl for local/ICV injections) and an injector cannula (26/28 gauge for with 2 mm extension beyond the guide cannula for local/ICV injections; Plastics One). Injections were given over the course of 1 min, and then kept in place for 30 s following injection to allow for tissue uptake. After the experiments, rats were euthanized with CO_2, and charcoal (local injections) or blue ink (ICV) was injected through the guide cannula as a marker to check proper placement of the cannula histologically (on Nissl stained coronal brain sections; Fig. 1A & E) for local placement or visually for ICV placement.

2.4. Experimental procedures

2.4.1. Experiment 1: MOR blockade in the NAc or BLA

The effect of MOR blockade in the NAc or the BLA on social novelty preference was assessed in juvenile male rats (separate cohorts; NAc, n = 19; BLA, n = 21). We limited our investigation to males, because no sex differences were observed in social novelty preference (Smith et al., 2015) and in MOR binding density in either the NAc or the BLA (Smith et al., 2017a) in juveniles. Experimental male rats were housed in pairs, and stimulus male rats were housed in groups of 3–4. At 29 or 30 days of age, rats received bilateral injections of vehicle (0.9% saline; 0.3 µl/side) or the MOR antagonist CTAP (3.3 µg/0.3 µl/side; Sigma-Aldrich, St. Louis, MO, USA) into either the NAc or the BLA 20 min prior to social novelty preference testing. One to two days later, rats were again exposed to the social novelty preference test, this time with the opposite drug treatment, in counter-balanced order. The dose of CTAP was consistent with a previous study demonstrating an effect of CTAP in the NAc on social play behavior in juvenile male rats (Trezza et al., 2011).

2.4.2. Experiment 2: social context manipulations

To assess the effect of social context on social novelty preference,