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mGluR5 activation in the nucleus accumbens is not essential for sexual behavior or cross-sensitization of amphetamine responses by sexual experience

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ABSTRACT

Natural rewards and psychostimulants cause similar neural plasticity in the nucleus accumbens (NAc). In addition, sexual experience in male rats causes increased locomotor activity and conditioned place preference (CPP) induced by D-Amphetamine (amph). The latter is dependent on a period of abstinence from sexual reward. In this study, the role of mGluR5 activation in the NAc for expression of mating and the cross-sensitizing effects of sexual experience was tested. First, intra-NAc infusions of mGluR5 antagonists MPEP (1 or 10 μg/μL) or MTEP (1 μg/μL) 15 min prior to mating during 4 daily sessions had no effect on male rat sexual behavior. Subsequently, these sexually experienced males were tested for amph-induced locomotor activity and CPP after one week of abstinence from sexual reward. In addition, sexually naïve males that received MPEP, MTEP or vehicle infusions prior to 4 daily handling sessions were included. Cross-sensitization of locomotion or CPP was not prevented by NAc mGluR5 antagonism during acquisition of sexual experience. Instead, sexually naive animals that received NAc mGluR5 antagonists without mating demonstrated sensitized amph-induced locomotor responses and enhanced CPP on par with sexually experienced males. Finally, we showed that sexual experience caused prolonged down-regulation of mGluR5 protein in the NAc, dependent on abstinence from sexual behavior. Together, these findings suggest that mGluR5 activation in the NAc is not essential for the expression of mating, but that experience-induced reduction in mGluR5 protein may contribute to the cross-sensitization of amph responses by sexual experience and abstinence.

1. Introduction

Natural reward behavior can influence drug responses and drug-associated reward (Balfour et al., 2004; Bradley and Meisel, 2001; Frohmader et al., 2010a; Gipson et al., 2011; Olsen, 2011; Pitchers et al., 2010a, 2014, 2013; Stairs et al., 2011). We have previously demonstrated that sexual experience in male rats causes long-lasting sensitization of behavioral responses to amphetamine (amph), including amph-induced locomotor activity (cross-sensitization) and conditioned place preference (CPP) (Frohmader et al., 2010a, 2011; Pitchers et al., 2010a, 2014, 2010b). The latter is dependent on a period of abstinence from sexual reward (Pitchers et al., 2010a, 2012, 2013). Male rat sexual behavior is rewarding and reinforcing: sexually experienced male rats form a CPP for copulation (Agmo and Berenfeld, 1990; Agmo and Gomez, 1993; Tenk et al., 2009), perform operant tasks to gain access to sexually receptive females (Everitt et al., 1987; Everitt and Stacey, 1987), and develop faster running speeds in straight-arm runway (Lopez and Ettenberg, 2002), T-maze (Kagan, 1955) and hurdle climbing tests (Sheffield et al., 1951). In addition, sexual experience in male rats causes facilitation of subsequent sexual behavior, indicated by increased sexual motivation and performance (Balfour et al., 2004; Beloate et al., 2016; Pitchers et al., 2010a, 2010b, 2012, 2013, 2014).

Studies investigating the mediators of changes in drug responses caused by sexual experience and abstinence have primarily focused on the mesolimbic system, specifically the nucleus

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accumbens (NAc) and ventral tegmental area. Dopaminergic neurons in the ventral tegmental area undergo morphological changes following sexual experience, an effect mediated by endogenous opioids (Pitchers et al., 2014). In the NAc, deltaFosB and dendritic spines are increased, which are D1 dopamine receptor dependent processes (Pitchers et al., 2013). Together, these changes mediate the long-term outcomes of sexual experience on sensitized amph-CPP (Beloate et al., 2016; Pitchers et al., 2014, 2010b, 2013). Neuroplasticity in glutamate signalling in the NAc also seems to play a role in mediating the effects of sexual experience (Pitchers et al., 2012; Wolf, 2010) since it is associated with altered ionotropic receptor trafficking and function in the NAc (Pitchers et al., 2012). In addition to ionotropic glutamate receptors, there is evidence that type 5 metabotropic glutamate receptors (mGluR5) are important mediators of addictive drugs (Carroll, 2008; Kalivas, 2009; Kim et al., 2015; Lou et al., 2014). Yet, mGluR5 remains understudied regarding its role in mediating natural reward behaviors, or the interactions between natural and drug behaviors.

There are 8 subtypes of metabotropic glutamate receptors (mGluR1-8) organized into 3 groups based on sequence homology, pharmacological profile and cell signalling mechanisms (Conn and Pin, 1997). mGluRs act via G-proteins to regulate intracellular processes (G-protein coupled receptors; GPCRs). Of particular interest in the current study, mGluR5 is a group I metabotropic receptor that is coupled to $G\alpha_{(q/11)}$ protein and activates phospholipase C and increases intracellular calcium. There is a high density of mGluR5 in the NAc (Conn and Pin, 1997; Romano et al., 1995; Testa et al., 1994, 1995), located mainly outside the synaptic cleft on the post-synaptic membrane (Mitrano and Smith, 2007). The antagonism of mGluR5 attenuates drug self-administration (Kenny et al., 2005; Platt et al., 2008), psychostimulant-induced locomotor activity (Herzig and Schmidt, 2004; Martinez et al., 2014), alcohol consumption (Cozzoli et al., 2012) and reinstatement of cocaine, methamphetamine, heroin, nicotine and alcohol (Backstrom et al., 2004; Backstrom and Hyytia, 2006; Besheer et al., 2008; Chesworth et al., 2013; Gass et al., 2009; Herrold et al., 2013; Kenny et al., 2005; Kim et al., 2015; Kumaresan et al., 2009; Lee et al., 2005; Lou et al., 2014; Martin-Fardon et al., 2009; McGeehan and Olive, 2003; Paterson and Markou, 2005; Platt et al., 2008). Much less work has been conducted to study the antagonism of mGluR5 on non-drug reward behavior, especially in a brain site-specific manner. To this point, systemic administration of mGluR5 antagonists has been show to inhibit sex-seeking and sexual behavior (Li et al., 2013), but have no effect on food self-administration (Tessari et al., 2004), food-CPP (Herzig et al., 2005), or sweetened or condensed milk self-administration (Martin-Fardon et al., 2009). Therefore, the goal of the current study was to determine whether mGluR5 activation in the NAc during sexual behavior affects the expression of this behavior and its facilitation with experience. The second objective was to test the hypothesis that NAc mGluR5 activation mediates the long-term consequences of sexual experience and abstinence on sensitized amph responses, including enhanced amph-induced locomotor activity and CPP.

2. Methods

2.1. Animals

Adult male (225–250 g upon arrival) and female (210–220 g) Sprague Dawley rats were obtained from Charles River Laboratories (Senneville, QC, Canada) and were housed in Plexiglas cages. The colony room was temperature- and humidity-regulated and maintained on a 12/12 h light dark cycle with food and water available *ad libitum*. Female partners for mating sessions received a subcutaneous implant containing 5% estradiol benzoate (Sigma–Aldrich, St. Louis, Missouri) and 95% cholesterol following bilateral ovariectomy under deep anesthesia (isofluorane; 2% MAC; MWI, Boise, ID, USA) administered using a Surgivet Isotec 4 gas apparatus (Smiths Medical Vet Division, Markham, Ontario, Canada). Sexual receptivity was induced by administration of 500 μg progesterone (Sigma–Aldrich, St. Louis, MO, USA) in 0.1 mL sesame oil (Sigma–Aldrich, St. Louis, Missouri) approximately 4 h before testing. All procedures were approved by the Animal Care and Use Committees of the University of Western Ontario and conformed to Canadian Council on Animal Care guidelines involving vertebrate animals in research.

2.2. Drugs

D-amphetamine (amph) sulfate (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in sterile saline. Animals received an amph dose of 0.5 mg/kg body weight, calculated on the basis of the free base, in a volume of 1 mL/kg body weight. All amph injections were given subcutaneously (s.c.) during the first half of the light phase (2–6 h after lights on), immediately before placement into the behavioral apparatus. We have previously shown that this dose of amph causes CPP in sexually experienced, but not naïve animals (Beloate et al., 2016; Pitchers et al., 2010a, 2013). In the current study, two different mGluR5 antagonists were used: 2-methyl-6-(phenylethynyl)-pyridine (MPEP; Sigma–Aldrich; dissolved in sterile saline (0.9%)) and 3-[(2-methyl-4-thaizolyl)ethynyl]pyridine (MTEP; Sigma–Aldrich; dissolved in 2% DMSO). Both mGluR5 antagonists are non-competitive antagonists, however MTEP is more selective and potent (Lea and Faden, 2006). Animals received MPEP at a dose of 1 μg/μL (low) or 10 μg/μL (high), or MTEP at 1 μg/μL in a 1 μL volume per side into the NAc at 15 min prior to introduction of receptive female. These doses have been used by other studies for local infusion into the NAc and were found to cause no motor impairments (Besheer et al., 2010; Lea and Faden, 2006; Roohi et al., 2014; Sinclair et al., 2012). Vehicle injections were either saline (for MPEP) or 2% DMSO (for MTEP).

2.3. Mating behavior

In all experiments, sexually naïve males were randomly assigned to either of two experimental conditions: sexually naive or sexually experienced. Sexually experienced animals were allowed to mate 4 (Expts 1 and 2) or 5 (Expt 3) times on consecutive days with receptive females in rectangular test cages (60 × 45 × 50 cm) until display of ejaculation. Cages were thoroughly cleaned with 70% ethanol solution and fresh bedding was added between mating sessions. Sexual behavior was performed during the dark phase (2–6 h after onset of dark). All mating sessions were observed and sexual behavior was recorded. The number of mounts (M) and intromissions (IM), mount latency (ML; time from introduction of the female to first mount), intromission latency (IL; time from introduction of the female to first intromission), and ejaculation latency (EL; time from first intromission to ejaculation) were recorded (Agmo, 1997). Naïve animals were placed in a clean test cage for one hour without access to a female concurrently with sexually experienced males mating in the same room, such that naïve controls were exposed to similar levels of disturbance and environmental novelty as experienced males.

2.4. Experiment 1: effects of MPEP during sexual experience on sensitization of amph-induced locomotor activity

To determine the functional significance of mGluR5 activation in the NAc during sex behavior and on sex experience-induced cross-