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Differential effect of viral overexpression of nucleus accumbens shell 5-HT_{1B} receptors on stress- and cocaine priming-induced reinstatement of cocaine seeking

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ABSTRACT

5-HT_{1B} receptors are densely expressed on terminals of medium spiny neurons projecting from the nucleus accumbens shell (NAccSh) to the ventral tegmental area, where 5-HT_{1B} receptors modulate GABA release directly, and firing of dopaminergic neurons indirectly. While interactions between NAccSh 5-HT_{1B} receptors and stress have been reported in early stages of psychostimulant-induced neuroadaptations, specifically psychomotor sensitization, the effect of this interaction on later stages of drug seeking is currently unknown. Here, we examined the effect of herpes simplex virus (HSV)-mediated overexpression of NAccSh 5-HT_{1B} receptors on reinstatement of cocaine seeking induced by exposure to stress or a cocaine prime. Rats were trained to self-administer cocaine (0.75 mg/kg/infusion) and the operant response was extinguished. Rats were then injected with viral vector expressing 5-HT_{1B} and green fluorescent protein (GFP) or GFP alone into the NAccSh. The effect of 5-HT_{1B} receptor overexpression was assessed on reinstatement induced by intermittent footshock (0.5 mA for 15 min) or a cocaine prime (10 mg/kg, ip). Results indicate that NAccSh 5-HT_{1B} receptor overexpression had no effect on footshock reinstatement while significantly decreasing cocaine priming-induced reinstatement. We also found that NAccSh overexpression of 5-HT_{1B} receptors had no effect on saccharin intake following social defeat stress. These results suggest that the efficacy of pharmacological agents targeting 5-HT_{1B} receptors for the treatment of cocaine relapse will depend largely on the nature of the reinstating stimulus. Taken together with previous results, it appears that NAccSh 5-HT_{1B} receptors influence stress responses in early, but not in the later stages of psychostimulant-induced neuroadaptations.

1. Introduction

Addiction to psychostimulant drugs such as cocaine has become a worldwide epidemic with major social and economic burdens on society. An important problem in the treatment of addiction to cocaine and other drugs of abuse is the vulnerability of individuals to relapse to drugs months or even years after cessation of drug use (Dackis and O'Brien, 2001; Gossop et al., 1989). Indeed, up to 90% of previously addicted individuals relapse to drug use within twelve months of abstinence (DeJong, 1994). Relapse in addicts can be induced by exposure to the drug itself, drug-associated cues or by exposure to stressful stimuli (Childress et al., 1988, 1993; Sinha et al., 2000; Sinha and Li, 2007).

The serotonergic neuronal system plays an important role in relapse to cocaine seeking (Filip et al., 2005, 2010). Interestingly, both enhancing (Burmeister et al., 2003) and decreasing (Tran-Nguyen et al., 2001) serotonin (5-HT) levels attenuate reinstatement of cocaine seeking induced by exposure to cues previously associated with cocaine self-administration, and have variable effects on priming-induced reinstatement of cocaine seeking. These inconsistencies in delineating the effects of serotonergic neuronal modulation on relapse to cocaine seeking are most likely due to the complexity of 5-HT receptor sub-types (Filip et al., 2010).

5-HT_{1B} receptors are known to regulate the behavioral effects of cocaine (Miszkiel et al., 2011). These receptors are $G_{i/o}$-coupled receptors that negatively couple with adenylate cyclase, resulting in an inhibitory effect on neuronal activity (Morikawa et al., 2000). *In situ* hybridization histochemistry studies indicate that 5-HT_{1B} receptors are expressed on medium spiny neurons throughout the striatum (Bruinvels et al., 1994); these GABAergic neurons project to several brain regions including the ventral tegmental area (VTA), ventral pallidum, and globus pallidus externa (Groenewegen et al., 1999). Physiological studies indicate that 5-HT_{1B} receptors on these neurons are translocated to axon terminals where they negatively regulate GABA release (O'Dell and Parsons, 2004). The level of expression for these receptors is quite dynamic and is differentially regulated by stress, novelty, and both cocaine exposure and withdrawal (Furay et al., 2011; Hoplight et al., 2007; Neumaier et al., 2002a, 2009). Stimulation of VTA 5-HT_{1B} receptors

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potentiates the effects of cocaine, likely via disinhibition of GABA release (O'Dell and Parsons, 2004; Yan et al., 2004). Previously we demonstrated in a conditioned place preference assay, that increased expression of 5-HT_{1B} receptors in nucleus accumbens shell (NAccSh) neuronal terminals in the VTA shifts the cocaine dose–response curve to the left (Neumaier et al., 2002b). However, we later observed that these receptors can enhance or inhibit cocaine preference depending on whether the procedure was optimized to detect preference or aversion, respectively (Barot et al., 2007), suggesting a more complicated role of these receptors.

While many investigators have examined the effect of 5-HT_{1B} receptor modulation on reinstatement of cocaine seeking induced by a cocaine prime or conditioned cues (Acosta et al., 2005; Pentkowski et al., 2009; Przegalinski et al., 2007, 2008), the effect of 5-HT_{1B} receptor modulation on stress-induced reinstatement is currently unknown. We have previously observed an interaction between coincident exposure to mild stress and increased 5-HT_{1B} receptor expression in NAccSh projection neurons that facilitates the behavioral effects of exposure to drugs of abuse (Ferguson et al., 2009), suggesting an interplay between the stress response and 5-HT_{1B} receptor overexpression. These interactions between 5-HT_{1B} receptors and stress have been observed in the early stages of psychostimulant-related behavioral plasticity (Neumaier et al., 2002b, 2009; Ferguson et al., 2009; Hoplight et al., 2007; Furay et al., 2011) and we are now examining the effect of 5-HT_{1B} receptors in stress-induced reinstatement of cocaine seeking.

In the present study, we examined the effect of transiently increasing 5-HT_{1B} receptor expression in the NAccSh using herpes simplex virus-mediated gene transfer on footshock stress-induced cocaine reinstatement. The optimal way to ascertain the behavioral specificity of an experimental manipulation is to determine its effect on more than one reinstating stimulus (Nair et al., 2009). Hence, we also examined the effect of NAccSh 5-HT_{1B} receptor expression on cocaine-priming induced reinstatement of cocaine seeking. To further study the role of NAccSh 5-HT_{1B} receptors in stress responses, we examined the effect of 5-HT_{1B} overexpression on saccharin preference following exposure of rats to social defeat stress.

2. Materials and methods

2.1. Subjects

For cocaine reinstatement experiments, male Long-Evans rats (Charles River, Raleigh, NC), weighing 350–425 g were used. These rats were initially double-housed and allowed to acclimate for at least one week to the vivarium prior to the experiment. The temperature- and humidity-controlled vivarium was under a 12-h light–dark cycle (lights on at 6 a.m.). Following the acclimation period, rats were handled every other day for at least 6 days prior to surgery. After surgery, the rats were housed individually in the vivarium. *For the social defeat experiment*, rats were procured from Harlan Laboratories (Indianapolis, IN). Resident pairs consisted of one male (375–425 g) and one female Long-Evans rat (150–200 g). No dependent measures were collected from these rats. Saccharin preference was performed on male, Sprague–Dawley rats (225–250 g), which were individually housed throughout the experiment. Food and water were available *ad libitum* for all rats, with the exception of the 3 h training, extinction and reinstatement test duration/day for cocaine reinstatement experiments when rats had access to water *ad libitum*, but not food. All experimental procedures were approved by the University of Washington Institutional Animal Care and Use Committee and were conducted in accordance to the guidelines of the "Principles of Laboratory Animal Care" (NIH publication no. 86–23, 1996). A total of 72 rats were used for these experiments, of which 22 were excluded due to failure to learn to self-administer cocaine ($n = 5$), virus-induced gene expression outside the target brain region ($n = 12$), active lever responding that was more than 3 standard deviations away from the mean ($n = 1$) or failure to meet an extinction criterion of less than an average of 20 active lever presses per 3 h over 3 consecutive days ($n = 4$).

2.2. Intravenous surgery

Intravenous catheters were made in the laboratory using silastic tubing (Dow Corning, Midland, MI) with a silicon 'bead' at the proximal end of the catheter to aid in anchoring to the jugular vein. Rats were anesthetized with isoflurane (3% for induction and 1–3% for maintenance of anesthesia). The neck and intrascapular regions were shaved and prepared with 10% povidone iodine and 70% ethanol. An incision (~10 mm) was made in the skin and fascia overlying the right jugular vein. The fascia and tissue surrounding the vein was cleared using two pairs of straight forceps, following which the vein was lifted using a pair of curved forceps. An incision (~0.5 mm) was made in the jugular approximately 5 mm proximal to the point where the vein enters the pectoralis major muscle, the catheter was fed into the vein under strict aseptic conditions and held in place with 2–3 surgical knots using 4-0 silk. Optimal positioning of the catheter was verified by drawing blood into it with negative pressure. A second incision (~25 mm) was then made in the intrascapular region. A pair of straight hemostats was used to form a subcutaneous path from this incision ventrally around the right forelimb to the neck incision. The intravenous catheter was fed through this subcutaneous path to the intrascapular region where it was connected to a vascular access harness (Instech Laboratories Inc., Plymouth Meeting, PA). Rats were closely monitored following surgery and kept warm until ambulatory. Buprenorphine (0.1 mg/kg, s.c.) (Exp.1) or meloxicam (0.2 mg/kg, s.c.) (Exp. 2) was administered for analgesia and rats were allowed to recover for 7–10 days before cocaine self-administration training. During the recovery and training phases, catheters were flushed every 24–48 h with sterile gentamicin (0.08 mg/ml). To verify catheter patency, rats received intravenous injections of methohexital sodium (10 mg/ml; injection volume: 0.12 ml), a short-acting barbiturate that induces a rapid loss of muscle tone when administered intravenously. We performed the catheter patency test in 3 rats when their lever responding during self-administration training became erratic. These rats did not demonstrate momentary loss of muscle tone and were excluded from the study.

2.3. Intracranial surgery and virus-mediated gene transfer

We used replication-deficient herpes-simplex viral vectors to modulate 5-HT_{1B} receptor expression. The experimental viral vector contains two cassettes: one that expresses fully functional hemagglutinin-tagged 5-HT_{1B} and one that expresses green fluorescent protein (5HT_{1B}/GFP); a virus expressing GFP alone was used as the control vector (Clark et al., 2002). We have previously confirmed that this viral vector system produces hemagglutinin-tagged 5-HT_{1B} receptors in NAccSh neurons and not glia, is translocated to axon terminals in the VTA, and shows peak receptor expression in the terminals at around four days after infection (Barot et al., 2007). Viral vectors were injected using surgical procedures previously described (Barot et al., 2007; Ferguson et al., 2009; Neumaier et al., 2002b). Briefly, each rat was placed in a Stoelting stereotaxic apparatus, the scalp incised, skull landmarks visualized by scraping the periosteum and burr holes were drilled at the injection sites. A 27 gauge needle was directed to the NAccSh stereotaxically (AP: +1.8 mm, ML: ±0.8 mm with injector bevel pointing inwards, DV: −6.8 mm) (Paxinos and Watson, 2005). 5-HT_{1B}/GFP or GFP viral vector (2 μl containing ~10^8 infective units) was injected over 10 min, after which the needle was left in place for 5 min and then slowly withdrawn. This volume of viral vector was chosen based on previous studies in our laboratory to induce discrete infection in the target region (Mitchell et al., 2007; Neumaier et al., 2002a) producing infection rates of approximately 20% (Clark et al., 2002). The skin incision was closed with 3-0 monofilament nylon sutures in Exp. 1 and in Exps. 2 and 3 skin closure was augmented with sterile n-butyl cyanoacrylate glue.