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Role of ventral subiculum in context-induced reinstatement of heroin seeking in rats

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Abstract

In rats, re-exposure to heroin-paired contexts after extinction of lever-responding in a different context reinstates heroin seeking. Previous reports indicate that ventral hippocampus/Ca1 region plays a critical role in cocaine-, cue-, and context-induced reinstatement of cocaine seeking. Here, we examined whether ventral subiculum, the output region of ventral hippocampus, is involved in context-induced reinstatement of heroin seeking. We found that reversible inactivation of ventral, but not posterior Ca1, with the GABAergic agonists muscimol+baclofen decreased context-induced reinstatement of heroin seeking. Our findings, together with previous studies on cocaine seeking, indicate a critical role of ventral subiculum in context-induced relapse across drug classes.

Keywords

Conditioned cues; drug environment; heroin self-administration; hippocampus; muscimol +baclofen; relapse; reinstatement

Exposure to environmental contexts associated with heroin intake can provoke drug relapse in humans (Wikler, 1973). We and others have adapted an ABA renewal procedure (Bouton and Bolles, 1979) to study the role of drug-associated contexts in drug-seeking (Crombag and Shaham, 2002). Using this procedure, several different labs have found that re-exposing rats to drug-associated contexts after extinction of drug-reinforced responding in different contexts reinstates heroin, cocaine, alcohol, and nicotine-seeking (Crombag et al., 2008; Diergaarde et al., 2008; Fuchs et al., 2008; Janak and Chaudhri, 2010; Millan et al., 2011).

We previously identified roles of dopamine and glutamate transmission in accumbens shell, but not core, in context-induced reinstatement of heroin seeking (Bossert et al., 2006; Bossert et al., 2007). More recently, we found that reversible inactivation of ventral mPFC, a brain area that sends glutamatergic projections to accumbens shell (Sesack et al., 1989), decreases context-induced reinstatement (Bossert et al., 2011). Here, we sought to examine

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the role of another major accumbens shell afferent projection area, the ventral subiculum (Groenewegen et al., 1987). Ventral subiculum is a primary output region of the hippocampus (Groenewegen et al., 1987; Naber and Witter, 1998) and is involved in acquisition and expression of contextual fear conditioning (Maren, 1999). Electrical stimulation of ventral subiculum reinstates cocaine seeking (Taepavarapruk and Phillips, 2003; Vorel et al., 2001) and inactivation of ventral subiculum, using the sodium channel blocker lidocaine, decreases discrete-cue- and cocaine-priming-induced reinstatement (Sun and Rebec, 2003).

Experimental procedures followed the guidelines of the “Principles of Laboratory Animal Care” (8th edition, 2012; <http://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf>) and are described in detail in the Supplemental Online Material section. In Experiment 1, we determined whether bilateral injections of muscimol (0.03 nmol/0.5µl/side)+baclofen (0.3 nmol/0.5 µl/side) into ventral subiculum would decrease context-induced reinstatement of heroin seeking. Rats were trained to self-administer heroin (diacetylmorphine HCl; NIDA) in Context A for 3 h/d for 12 days (0.1 mg/kg/infusion for the first 6 sessions and 0.05 for the last 6 sessions). During training, heroin infusions were earned under a fixed-ratio-1 (FR1), 2.3 sec timeout reinforcement schedule and were paired with a compound tone-light cue for 2.3 sec. During the extinction phase in Context B, responses on the previously active lever led to presentation of the tone-light cue but not heroin. Tests for context-induced reinstatement were conducted under extinction conditions (lever presses led to the presentation of the tone-light cue but not heroin) and began after a minimum of 14 daily extinction sessions. Each rat was injected with the assigned dose (vehicle or drug) prior to testing; the rats were tested once in Context A (heroin context) and once in Context B (extinction context). Data were analysed by repeated measures ANCOVA (inactive lever presses as a covariate), using the between-subjects factor of Drug (vehicle or muscimol+baclofen) and the within-subjects factor of Test Context (heroin [A] or extinction [B]). As a neuroanatomical control, we repeated this procedure except that vehicle or muscimol+baclofen was injected into the posterior Ca1 area (3 mm dorsal to ventral subiculum).

In Experiment 2, we tested whether the effect of muscimol+baclofen on reinstatement in Experiment 1 was due to performance deficits. Rats were implanted with cannula aimed at the ventral subiculum and were then trained to lever press for 45 mg pellets under an FR1 20-sec timeout reinforcement schedule for nine 30-min sessions until lever responding was stable. The rats were then injected with vehicle or muscimol+baclofen prior to each of two sessions separated by 48 h; rats were run in the food self-administration session in between the tests (no injections were delivered). Data were analyzed by repeated measures ANOVA for active lever presses using the within-subjects factor of Drug (vehicle or muscimol +baclofen).

The rats demonstrated reliable heroin self-administration, as indicated by the increase in lever presses when the heroin dose was decreased from 0.1 to 0.05 mg/kg/infusion on training day 7 ($p<0.01$; Figure S1A). During the extinction phase, response rates decreased over time as evidenced by a main effect of session ($p<0.01$; Figure S1B). During the reinstatement tests, bilateral injections of muscimol+baclofen into ventral subiculum

decreased context-induced reinstatement of heroin seeking (Fig. 1A). The statistical analysis demonstrated a significant interaction of Drug $\times$ Test Context ($F_{(1,12)}=6.13$, $p<0.05$). Bilateral injections into the posterior Ca1 area (a dorsal anatomical control) did not decrease context-induced reinstatement. The statistical analysis demonstrated a significant effect of Test Context ($F_{(1,14)}=9.1$, $p<0.01$) but no significant effects of Drug or Test Context $\times$ Drug (p values > 0.2) (Fig. 1B).

Injections of muscimol+baclofen had no effect on food-reinforced responding (p values > 0.2 ; $n=4$). The mean $\pm$ SEM number of pellets, active, and inactive lever presses were, respectively, 112 $\pm$ 22, 194 $\pm$ 46, and 10 $\pm$ 4 for vehicle; and 102 $\pm$ 21, 171 $\pm$ 56, and 11 $\pm$ 89 for muscimol+baclofen.

We found that bilateral injections of muscimol+baclofen into ventral subiculum, but not posterior Ca1, decreased context-induced reinstatement of heroin seeking. It is unlikely that this decrease in reinstatement is due to drug diffusion to areas outside the ventral subiculum because injections of muscimol+baclofen 3 mm dorsal to ventral subiculum had no effect on reinstatement. We can also rule out the possibility that this decrease in reinstatement is due to motor deficits, because ventral subiculum muscimol+baclofen injections did not decrease high rates of food-maintained responding. The lack of effect of muscimol+baclofen into posterior Ca1 is at first surprising since ventral subiculum/ventral hippocampus also includes area Ca1. However, projections from posterior Ca1 are topographically different than those from ventral subiculum/Ca1 with the former projecting to rostral nucleus accumbens and the latter projecting to caudal nucleus accumbens (Groenewegen et al., 1987), an area we previously reported to play a critical role in this reinstatement (Bossert et al., 2006; Bossert et al., 2007; Bossert et al., 2012). Furthermore, the lack of effect of posterior Ca1 injections is consistent with those of Lasseter et al. (2010) who found that muscimol+baclofen injections into this area had no effect context-induced reinstatement of cocaine seeking.

The literature on the functional role of ventral subiculum or ventral hippocampus (which includes subfields Ca1 and Ca3 (Swanson and Cowan, 1977)) in drug relapse has thus far been limited to studies on relapse to psychostimulant seeking. Vorel et al. (2001) demonstrated that electrical stimulation of ventral subiculum reinstates cocaine seeking after extinction of cocaine self-administration and Taepavarapruk et al. (2003) found that such stimulation causes resumption of *d*-amphetamine seeking after a 2-h voluntary abstinence period. Furthermore, reversible inactivation of ventral subiculum or ventral hippocampus using the sodium channel blocker lidocaine decreases, respectively, discrete-cue- and cocaine-priming-induced reinstatement (Sun and Rebec, 2003), as well as discrete-cue- and methamphetamine-priming-induced reinstatement (Hiranita et al., 2006). Lastly, Rogers et al. (2007) and Lasseter et al. (2010) found that inactivation of ventral hippocampus using muscimol+baclofen, which unlike lidocaine only inactivates local cell bodies, decreases cue-, context, and cocaine-priming-induced reinstatement. Our findings now expand on this literature to include a role for ventral subiculum in context-induced reinstatement of opiate seeking.

Ventral subiculum sends ipsilateral and contralateral glutamatergic afferents to principle terminal regions of the mesocorticolimbic dopamine system, including the accumbens shell (Groenewegen et al., 1987), a region we have shown to play an important role in context-induced reinstatement of heroin seeking (Bossert et al., 2006; Bossert et al., 2007; Bossert et al., 2012). We have also found that the projection from ventral mPFC to accumbens shell plays a critical role in context-induced reinstatement of heroin seeking (Bossert et al., 2012). Therefore, it is possible that ventral subiculum-accumbens shell pathway also be important for this reinstatement. Indeed, the hippocampus is the major source of input to accumbens, and hippocampal and tyrosine hydroxylase-labeled terminals in accumbens converge on the same postsynaptic dendrites (Sesack and Pickel, 1990). Future research is needed to determine the role of this pathway in context-induced reinstatement of opiate and psychostimulant seeking.

Finally, our recent studies (Bossert et al., 2011; Bossert et al., 2012) as well as others' studies (Badiani et al., 2011; Caprioli et al., 2008) have demonstrated important neurobiological differences in opiate-versus psychostimulant-seeking behaviors. Here, in contrast, we identified the ventral subiculum/ventral hippocampal area, previously implicated in cue-, context- and drug-induced reinstatement of psychostimulant seeking (Hiranita et al., 2006; Lasseter et al., 2010; Taepavarapruk and Phillips, 2003; Vorel et al., 2001), as a critical site for reinstatement of heroin seeking. Taken together, these findings suggest a general role of ventral subiculum/ventral hippocampus in relapse to drug seeking across different drug classes, as well as different types of relapse-provoking stimuli.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A. Ventral subiculum

B. Posterior Ca1

Figure 1. Effect of bilateral injections of muscimol+baclofen into (A) ventral subiculum or (B) posterior Ca1 on context-induced reinstatement of heroin seeking

Left panels show Mean±SEM number of presses on the previously active lever after bilateral injections of muscimol+baclofen into ventral subiculum (n=8 per group) or **posterior Ca1** (n=9 per group) prior to exposure to the Heroin context or the Extinction context. Right panels show approximate placements (mm from Bregma) of the injector tips for ventral subiculum or **posterior Ca1** (Paxinos and Watson, 2005) and representative photomicrographs of cannula placements. * Different from the Vehicle condition, $p < 0.01$.