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Medial prefrontal cortex involvement in the expression of extinction and ABA renewal of instrumental behavior for a food reinforcer

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ABSTRACT

Instrumental renewal, the return of extinguished instrumental responding after removal from the extinction context, is an important model of behavioral relapse that is poorly understood at the neural level. In two experiments, we examined the role of the dorsomedial prefrontal cortex (dmPFC) and the ventromedial prefrontal cortex (vmPFC) in extinction and ABA renewal of instrumental responding for a sucrose reinforcer. Previous work, exclusively using drug reinforcers, has suggested that the roles of the dmPFC and vmPFC in expression of extinction and ABA renewal may depend at least in part on the type of drug reinforcer used. The current experiments used a food reinforcer because the behavioral mechanisms underlying the extinction and renewal of instrumental responding are especially well worked out in this paradigm. After instrumental conditioning in context A and extinction in context B, we inactivated dmPFC, vmPFC, or a more ventral medial prefrontal cortex region by infusing baclofen/muscimol (B/M) just prior to testing in both contexts. In rats with inactivated dmPFC, ABA renewal was still present (i.e., responding increased when returned to context A); however responding was lower (less renewal) than controls. Inactivation of vmPFC increased responding in context B (the extinction context) and decreased responding in context A, indicating no renewal in these animals. There was no effect of B/M infusion on rats with cannula placements ventral to the vmPFC. Fluorophore-conjugated muscimol was infused in a subset of rats following test to visualize infusion spread. Imaging suggested that the infusion spread was minimal and mainly constrained to the targeted area. Together, these experiments suggest that there is a region of medial prefrontal cortex encompassing both dmPFC and vmPFC that is important for ABA renewal of extinguished instrumental responding for a food reinforcer. In addition, vmPFC, but not dmPFC, is important for expression of extinction of responding for a food reinforcer. The role of the medial prefrontal cortex in renewal in the original conditioning context may depend in part on control over excitatory context-response or context-(response-outcome) relations that might be learned in acquisition. The role of the vmPFC in expression of extinction may depend on its control over inhibitory context-response or context-(response-outcome) relations that are learned in extinction.

1. Introduction

Conditioned instrumental (operant) behaviors are voluntary actions that are controlled by their consequences. Animals readily acquire behaviors (e.g., lever pressing) to obtain a desirable outcome (e.g., food pellet or drug delivery) and likewise learn to suppress behavior when the reinforcer is withheld. This extinction of instrumental responding is an important and fundamental component of behavioral change (Bouton, 2014; Bouton & Todd, 2014). However, extinguished behaviors can re-emerge through several manipulations and mechanisms, including renewal. Renewal occurs when an animal is tested in a context different from the extinction context (Bouton & Bolles, 1979), resulting in a return of extinguished responding. This return of responding demonstrates that extinction is not erasure of the original learning. A major challenge in successful treatment of behavioral disorders in humans (e.g., addiction) is the susceptibility of these behaviors to relapse.

Instrumental renewal has been reliably demonstrated with several different reinforcers and across different paradigms. ABA renewal (conditioning in context A, extinction in context B, testing in context A) has been shown with food reinforcers (e.g., Nakajima, Tanaka, Urushihara, & Imada, 2000) and different drug reinforcers (e.g., alcohol, cocaine, heroin) (e.g. (Bossert, Liu, Lu, & Shaham, 2004; Chaudhri, Sahuque, & Janak, 2009; Fuchs, Eaddy, Su, & Bell,

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2007; Hamlin, Clemens, & McNally, 2008). Additionally, AAB and ABC renewal have also been observed in instrumental conditioning (Bouton, Todd, Vurbic, & Winterbauer, 2011) and show that removal from the extinction context is sufficient to elicit renewal of responding; return to the acquisition context is not necessary. Such results suggest that extinction at least partly involves learning to inhibit the response in the extinction context.

There are several possible mechanisms that may underlie renewal (e.g., Bouton, 1993). However, Todd (2013) found that when the renewal test occurred in a context that was associated with extinction of a separate response, renewal was not affected. Additionally, in a discriminated operant situation in which responses were controlled by different discriminative stimuli, renewal of a response was reduced if the same response, but not a different response, had previously been extinguished in the test context (Todd, Vurbic, & Bouton, 2014b). Thus, renewal is due at least partly to the release from context specific *response* inhibition that develops during extinction, rather than negative occasion setting or context-outcome inhibition. It is worth noting that when renewal tests are conducted in context A, the original acquisition context, the test context might also engage excitatory mechanisms learned there during acquisition (see Section 4).

A number of brain regions appear to play a role in ABA renewal of extinguished instrumental behavior for drug reinforcers (Bossert, Marchant, Calu, & Shaham, 2013). Many of these brain regions are important regardless of the type of drug reinforcer. For example, inactivation of the nucleus accumbens shell, or infusion of a D1R antagonist there, reduced ABA renewal of extinguished lever-pressing for cocaine (Fuchs, Ramirez, & Bell, 2008), alcohol (Chaudhri et al., 2009), or heroin (Bossert, Poles, Wihbey, Koya, & Shaham, 2007). However, the picture is not as clear for the medial prefrontal cortex. For example, inactivation of the dorsomedial prefrontal cortex (primarily the prelimbic cortex) reduced expression of ABA renewal of extinguished lever pressing for cocaine; inactivation of ventromedial prefrontal cortex (primarily the infralmbic cortex) had no effect (Fuchs et al., 2005). Similarly, inactivation of the dmPFC reduced expression of ABA renewal of extinguished nose-poking for alcoholic beer; inactivation of vmPFC had no effect (Willcocks & McNally, 2013). In contrast, inactivation of dmPFC had no effect on the expression of ABA renewal of extinguished lever pressing for heroin, whereas inactivation of the vmPFC reduced it (Bossert et al., 2011). Thus, inactivation of dmPFC (but not vmPFC) attenuated ABA renewal of extinguished instrumental behavior for cocaine or alcohol, while inactivation of vmPFC (but not dmPFC) attenuated ABA renewal of extinguished instrumental behavior for heroin. Further evidence that different drug reinforcers might involve different brain regions is provided by results showing that the renewal context activates vmPFC neurons projecting to the nucleus accumbens shell when heroin has been the reinforcer (Bossert et al., 2012) but not when alcohol has been the reinforcer (Hamlin, Clemens, Choi, & McNally, 2009).

While neither the dmPFC nor vmPFC appears to be important for the learning of instrumental extinction (Mendoza, Sanio, & Chaudhri, 2015; Peters, LaLumiere, & Kalivas, 2008), there is some evidence that vmPFC is important for its *consolidation* and *expression*. For example, Peters et al. (2008) gave rats 11 sessions of extinction of lever-pressing for cocaine. Inactivation of vmPFC prior to session 12 of extinction impaired expression of extinguished lever-pressing (i.e., increased responding) (Peters et al., 2008). In contrast, inactivation of dmPFC did not produce the same increase in extinguished responding and inactivation of nucleus accumbens shell increased responding on both an extinguished and an inactive lever. LaLumiere, Niehoff, and Kalivas (2010) performed post-session inactivation of the dmPFC or the vmPFC after each of five short (30 min) sessions of extinction of lever-pressing for cocaine; vmPFC inactivation impaired expression of extinction during subsequent longer (2 h), inactivation-free sessions of extinction, suggesting impaired consolidation of extinction learning, while dmPFC inactivation was without effect. Although the results of Peters et al. (2008) and LaLumiere et al. (2010) suggest that the vmPFC may be required for suppression of responding in extinction after cocaine reinforcement, Willcocks and McNally (2013) did not find any effect of vmPFC inactivation on expression of extinguished nose-poking for alcoholic beer, and Bossert et al. (2011) did not find any effect of vmPFC inactivation on expression of extinguished lever-pressing for heroin.

In comparison to renewal of extinguished instrumental responding for drug reinforcers, we are reaching a relatively good understanding of the behavioral mechanisms that underlie renewal of extinguished instrumental responding for a food reinforcer (e.g., Bouton & Todd, 2014). This might make the food reinforcement paradigm more analytically powerful for exploring the contributions of different brain regions. Here we therefore extend the investigation of the role of the dmPFC and the vmPFC in relapse by inactivating each of these regions just prior to test in an appetitive instrumental ABA renewal paradigm, using a sucrose pellet reinforcer. Our studies include several controls (e.g., off-site cannula placements; use of fluorescent muscimol to map infusion spread; angled cannula placement in the vmPFC to avoid dmPFC damage) that improve interpretation and application of our results. It was hypothesized that if the vmPFC and dmPFC are underlying suppression and promotion (respectively) of appetitive instrumental behavior, then inactivation of the vmPFC would attenuate expression of extinction, while dmPFC inactivation would attenuate ABA renewal. Both of these predictions were confirmed in the current experiments using a food reinforcer, which represents the first time that both effects have been observed in a single study. However, given previous results showing that vmPFC is important for ABA renewal of extinguished lever-pressing for heroin (Bossert et al., 2011), we were not surprised to also observe an attenuation of ABA renewal with inactivation of the vmPFC.

2. Material and methods

2.1. Subjects

A total of 95 adult male Wistar rats (57–61 days old at delivery) obtained from Charles River Canada were used. Of this total, 4 rats were eliminated based on an inability to locate one or both cannulas, leaving a total of 91 rats (25 rats in Experiment 1, 42 rats in Experiment 2, 25 rats as off-site controls). Animals were housed in a temperature and humidity controlled colony room, and kept on a 12/12 hr light/dark schedule. Rats were maintained at approximately 90% of their free-feeding weight throughout the experiment.

2.2. Apparatus

Two sets of four operant chambers were used in these experiments, which served as context A and context B (counterbalanced). For a detailed description of the apparatus used, see Todd, Winterbauer, and Bouton (2012). Briefly, the chambers were slightly modified versions of Med Associates (St. Albans, VT) model ENV-008-VP chambers. They measured $30.5 \times 24.1 \times 21.0$ cm ($l \times w \times h$) and were individually housed in sound attenuation chambers. Chambers in context A and B differed in tactile (staggered stainless steel grid floor/flat stainless steel grid floor), olfactory (lemon scent/pine scent), and visual cues (black stripes on chamber sides/clear chamber sides). Ventilation fans provided background noise of 65 dB. A recessed food cup was centered in the front wall, with retractable levers on either side of the food