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Contributions of basolateral amygdala and nucleus accumbens subregions to mediating motivational conflict during punished reward-seeking

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ABSTRACT

The involvement of different nodes within meso-cortico-limbic-striatal circuitry in mediating reward-seeking has been well described, yet comparatively less is known about how such circuitry may regulate appetitively-motivated behaviors that may be punished. The basolateral amygdala (BLA) is one nucleus that has been implicated in suppressing punished reward-seeking, and this structure can modulate goal-directed behavior via projections to subregions of the nucleus accumbens (NAc). Here, we examined the effects of reversible inactivations of the BLA, NAc Shell (NAcS), and core (NAcC) on performance of a "Conflict" task where rats pressed a lever for sucrose reinforcement during three distinct 5 min phases. During the first and last phases of a session, rats lever-pressed for food reward delivered on a VI-15/FR5 schedule. In between these phases was a signaled "Conflict" period, where each lever-press yielded food, but 50% of presses were also punished with foot-shock. Under control conditions, well-trained rats responded vigorously during the two "safe" VI-15/FR5 periods, but reduced responding during the punished Conflict period. Inactivation of either the BLA or the NAcS via infusions of baclofen/muscimol disinhibited punished seeking, increasing lever-pressing during the conflict period, while attenuating pressing during VI-15/FR5 phases. In contrast, NAcC inactivation markedly decreased responding across all three phases. Similar inactivation of the BLA or NAcS did not alter responding in a separate control experiment where rats pressed for food on schedules identical to the Conflict task in the absence of any punishment, while NAcC inactivation again suppressed responding. These results imply that BLA and NAcS are part of a circuit that suppresses reward-seeking in the face of danger, which in turn may have implications for disorders characterized by punishment resistance, including substance abuse and obsessive–compulsive disorder.

1. Introduction

The ability to use information regarding the aversive or rewarding consequences of actions to guide subsequent behavior is a key function of the nervous system. Considerable research has been dedicated to clarifying the influence of positive reinforcement on decision-making, implicating meso-cortico-limbic-striatal circuitry in such reinforcement learning (Cardinal, Parkinson, Hall, & Everitt, 2002; Floresco, 2015; Parkinson, Cardinal, & Everitt, 2000). In contrast, less is known about how this system guides behavior in response to punishment, a process by which an instrumental action co-occurs with a negative consequence, such as a lever-press-contingent foot-shock in rodents. In a majority of individuals, punishment serves to suppress the instrumental action with which it occurs. However, neuropsychiatric conditions such as obsessive–compulsive disorder and substance abuse are characterized by compulsivity, whereby punishment is less effective in curtailing detrimental behavioral patterns (Everitt, 2014; Feil et al., 2010; Figee et al., 2016; Jentsch & Taylor, 1999; Lubman, Yücel, & Pantelis, 2004; Perry & Carroll, 2008). As such, investigation of the circuitry underlying punishment-induced inhibitory control may provide insight into the pathophysiological underpinnings of these symptoms in various disease states.

Compulsivity in the face of punishment is recognized by the DSM-5 as a core symptom of substance abuse and other disorders, and pre-clinical findings suggests that these symptoms may be driven by alterations within cortico-limbic circuitry (Chen et al., 2013; Limpens, Schut, Voorn, & Vanderschuren, 2014; Pelloux,

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Murray, & Everitt, 2013; Radke, Jury, et al., 2015; Radke, Nakazawa, & Holmes, 2015). Prolonged access to cocaine produces punishment-resistant drug seeking, concomitant with hypofunction of medial prefrontal cortex (mPFC) (Chen et al., 2013). Optogenetic inhibition or activation of mPFC decreases or increases, respectively, the impact of punishment on cocaine seeking (but see Pelloux et al., 2013), suggesting that mPFC activity may be causally related to the punishment-mediated inhibition of seeking. Similarly, pharmacological inactivation or lesions of the mPFC produces operant responding for both cocaine and sucrose that is insensitive to potential punishment (Limpens, Damsteegt, Broekhoven, Voorn, & Vanderschuren, 2015; Resstel, Souza, & Guimarães, 2008). Prefrontal regions seem to perform a top-down inhibitory function, acting as a break when responding is directly punished, or in the presence of a fear-inducing stimulus. Likewise, the basolateral amygdala (BLA) promotes behavioral suppression during punishment. Jean-Richard-Dit-Bressel and McNally (2015) recently showed that inactivation of caudal (but not rostral) BLA eliminated the inhibition of lever-pressing produced by contingent foot-shock. Inactivated rats made more lever-presses during punishment, and did not display the typical increase in latency to press caused by punishment. Thus, both mPFC and BLA may contribute to punishment avoidance during appetitively-motivated behavior in a similar manner.

Although the BLA and PFC appear to subserve complementary roles in punishment avoidance, the downstream structure mediating this effect is currently unknown. The nucleus accumbens (NAc) receives dense glutamatergic input from both mPFC and BLA, and is known to regulate various forms of appetitive conditioning via its meso-cortico-limbic efferents (Cardinal et al., 2002; Floresco, 2015; Sesack & Grace, 2010). The NAc is primarily composed of two functionally and anatomically distinct subregions, the more lateral Core (NAcC) and more medial Shell (NAcS) (Heimer et al., 1997; Zahm & Brog, 1992). These two subregions have been suggested to serve dissociable yet complementary functions during reward-seeking, with the NAcC driving approach towards motivationally-relevant stimuli, and the NAcS facilitating inhibition of inappropriate behaviors (Ambroggi, Ghazizadeh, Nicola, & Fields, 2011; Floresco, 2015). In this regard, the ventral regions of the mPFC and caudal BLA project strongly to the medial NAcS (Berendse, Galis-de Graaf, & Groenewegen, 1992; Brog, Salyapongse, Deutch, & Zahm, 1993; Groenewegen, Wright, Beijer, & Voorn, 1999; Heilbronner, Rodriguez-Romaguera, Quirk, Groenewegen, & Haber, 2016; Kita & Kitai, 1990; Wright, Beijer, & Groenewegen, 1996), suggesting that this nucleus may facilitate inhibition of punished behavior regulated by these upstream cortical and limbic regions. It is therefore possible that NAc subregions may differentially contribute to adjusting behavior in response to punishment, with NAcS suppressing reward-seeking in the face of punishment in a manner similar to the BLA or PFC, and NAcC generally promoting action.

The present series of experiments were designed to both confirm a role for BLA in mediating reward/punishment conflict, and explore the potential differential contribution of NAcS versus NAcC to the same behavior. To this end, separate groups of well-trained rats received reversible inactivation of BLA, NAcS, or NAcC while performing an operant-based "Conflict" task. During this task, sucrose reward was available on a lean reinforcement schedule, without punishment, during two safe "Safe/Reward" periods. Interspersed between these periods was a separate "Conflict" period, wherein sucrose was available on a richer schedule, but 50% of lever-presses triggered a foot-shock punishment. Results using this Conflict task, and a "No-Conflict" (identical schedules of reinforcement, but no punishment) control variant, suggested that BLA and NAcS promote punishment-induced behavioral suppression, while NAcC plays a more general role in driving reward-seeking.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by the Animal Care Committee, University of British Columbia, and conducted in compliance with guidelines provided by the Canadian Council on Animal Care. All reasonable efforts were made to minimize the number and suffering of animals used. Male Long-Evans rats arrived weighing 225–350 g (Charles River) and were group housed (4–5 per cage) and allowed 6–7 d of acclimation to the colony. Colony temperature (21 °C) and light cycle (12-h light/dark) were kept constant. Prior to operant training, all rats were individually housed and food-restricted to approximately 90% of their free-feeding weight, and allowed to gain weight throughout the course of the experiment on a delayed-growth curve.

2.2. Apparatus

Behavioral testing was conducted in eight Med Associates (St Albans, VT, USA) operant conditioning chambers. Each chamber (30.5 cm × 24 cm × 21 cm) was contained in a sound-attenuating enclosure, ventilated by a fan that also served to mask external noise. Within each chamber were two retractable levers along one wall, separated by a food receptacle from which sucrose pellet reinforcement was delivered (45 mg pellet, BioServ, Frenchtown, NJ). For all experiments, only the left lever was extended into the chamber. Each box was outfitted with three 100 mA cue lights, one over each retractable lever, and one over the food receptacle. A single 100 mA house light was situated on the wall opposite the food receptacle. Four infrared photobeans located just above the grid floors were used to index locomotor activity. The chamber floor consisted of 19 stainless steel rods spaced 1.5 cm apart. The rods were wired to a shock source and solid-state grid scrambler for the delivery of foot-shock.

2.3. Surgery

Rodent anesthesia was conducted slightly differently for BLA and NAc placements, due to changes in institutional policies regarding anesthetic techniques. Animals receiving BLA cannula were anesthetized with a combination of ketamine/xylazine (100 and 20 mg/ml at 100 and 10 mg/kg, i.p.), exclusively. Animals receiving NAc cannula were first anesthetized with a half-dose of ketamine/xylazine (same mg/ml, i.p.), and then maintained on Isoflurane anesthetic (2–3% Isoflurane concentration) throughout surgery. Twenty-three gauge bilateral stainless-steel guide cannula were aimed at the BLA, NAcS, or NAcC according to the following stereotaxic coordinates (in mm):

BLA – from bregma, AP: −2.7, ML: ±5.3, from dura, DV: −7.0
NAcS – from bregma, AP: +1.3, ML: ±1.0, from dura, DV: −6.3
NAcC – from bregma, AP: +1.6, ML: ±1.8, from dura, DV: −6.3

Dental acrylic adhered to four stainless-steel skull screws held cannula in place. Stainless-steel obturators flush with the end of the guide cannula were inserted immediately following surgery, and remained in place throughout the experiment. Rats were given approximately 1 wk to recover from surgery before beginning behavioral training.

2.4. Training

Twenty-four hours before their first operant training session, rats were provided with ~30 sucrose pellets in their home cage, to reduce potential food neophobia. Subsequently, 15 min training sessions