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FUNCTIONAL INTERACTION BETWEEN THE BASOLATERAL AMYGDALA AND THE NUCLEUS ACCUMBENS UNDERLIES INCENTIVE MOTIVATION FOR FOOD REWARD ON A FIXED RATIO SCHEDULE

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Abstract—The ability for incentive properties of reward stimuli to maintain motivated behavior in the absence of the rewards themselves may be reliant in part on a glutamatergic projection from the basolateral (BLA) amygdala to the nucleus accumbens septi (NAS). The present work examined this idea in regard to food reward. In the first part of this study, lever pressing by rats on a fixed ratio 16 (FR16) schedule of food reinforcement was suppressed in a dose-dependent manner following bilateral infusion of the $GABA_A$ agonist muscimol to the BLA. Consumption of food when freely available was unaffected by the highest dose of muscimol, suggesting no change in the primary reward value of the food. Bilateral infusion of the broad-spectrum dopamine (DA) receptor antagonist flupenthixol to the NAS also resulted in a significant decrease in FR16 performance. As with the amygdala, consumption of freely available food was not affected by flupenthixol injections into the NAS. When unilateral injection of flupenthixol to the NAS was combined with contralateral injection of muscimol to the BLA, FR16 performance was suppressed. No significant change in lever press performance was observed following unilateral NAS injection of flupenthixol combined with ipsilateral injection of muscimol to the BLA. The results of this study support the idea that a functional connection between the BLA and NAS transmits incentive information necessary for the maintenance of responding in the absence of primary reward.

Key words: amygdala, reward, incentive motivation, dopamine, nucleus accumbens, muscimol, GABA.

The amygdala is well known for involvement in mediating fear and anxiety (LeDoux et al., 1992; Davis, 1992; Fendt and Fanselow, 1999). However, a long history of research (e.g., Weiskrantz, 1956) suggests the amygdala is more generally involved in assigning emotional valence, including reward (Robbins and Everitt, 1996; Baxter and Murray, 2002).

The nature of amygdalar involvement in reward has yet to be determined. The basolateral amygdala (BLA), for example, has been the subject of numerous behavioral studies examining how this structure might play a role in reward processes. While BLA neurons are responsive to cues paired with reward (e.g., Paton et al., 2006; Tye et al., 2008; Ambroggi et al., 2008), lesions of the BLA do not affect simple stimulus–reward conditioning, as shown in tests with nonhuman primates such as visual-discrimination learning, visuomotor conditional learning, food-cup approach, and food and object preferences (reviewed in Baxter and Murray, 2002). A similar pattern has been seen in work with rodents (e.g., autoshaping, Parkinson et al., 2000; conditioned magazine approach, Hatfield et al., 1996; simple instrumental conditioning, Balleine et al., 2003). On the other hand, BLA damage produces major deficiencies in the ability of stimuli paired with primary reward to maintain responding when the primary reward is remote in time. This has been seen in procedures such as appetitive pavlovian second-order conditioning, in which a stimulus paired with a stimulus previously paired with a primary reward does not readily maintain responding in BLA-lesioned rats (Everitt et al., 1989; Hatfield et al., 1996; Setlow et al., 2002). Robbins and Everitt (1996) encompassed much of the above in suggesting the amygdala contributes to incentive motivation, which allows stimuli associated with primary rewards to maintain behavior in the absence of the rewards themselves.

Another brain structure strongly implicated in incentive motivation is the nucleus accumbens septi (NAS), particularly in regard to its dopamine (DA) innervation from the ventral tegmental area (VTA—see Berridge, 1996; Robbins and Everitt, 1996; Berridge and Robinson, 1998; Ikemoto and Panksepp, 1999). Dopaminergic transmission in the NAS is important for maintaining responding in conditions of intermittent reward (Neill et al., 2002; Salamone et al., 2003). The importance of NAS DA for performance under high work requirements is suggested in work by Sokolowski et al. (1998), who showed a significant positive correlation between food-rewarded fixed-ratio operant response rate and extracellular NAS DA in rats. Depletion of NAS DA decreases responding at high work requirements engendered by an FR schedule of food reinforcement while having little effect on FR1 responding (Aberman et al., 1998; Aberman and Salamone, 1999; Hamill et al., 1999). In general, these investigators and others (e.g., Neill et al., 1981) have suggested an involvement of NAS DA in the capacity for overcoming work-related costs.

Given the probable involvement of both BLA and NAS in incentive motivation, an amygdalo-accumbens system has been proposed (Cador et al., 1989; Everitt et al., 1989, 1991; Phillips et al., 2003). A projection from the BLA to the NAS medial core region, shown by anterograde (Krettek and Price, 1978) as well as retrograde (Brog et al., 1993)

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Abbreviations: BLA, basolateral amygdala; DA, dopamine; FR16, fixed ratio 16; NAS, nucleus accumbens septi; VTA, ventral tegmental area.

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transport studies, may influence NAS DA release via an action on mesolimbic DA terminals (Floresco et al., 1998; Howland et al., 2002). It is plausible that manipulations of the BLA could influence incentive motivation via this NAS connection. Although inactivation of BLA does not decrease basal extracellular NAS DA (Louilot and Besson, 2000; Ahn and Phillips, 2003), it remains possible such treatment could decrease NAS DA efflux evoked by a behavioral condition. One such condition may be sustained responding in the absence of primary reward. This idea is supported by the finding of Neill et al. (2002) that DA metabolites in NAS were higher in rats responding on a FR10 than a FR1 schedule of intracranial self-stimulation. Consistent with this thinking, BLA lesions were found to decrease lever-press responding by male rats on a second-order schedule for access to female rats (Everitt et al., 1989), indicating the reduction of incentive motivated behavior. Interestingly, BLA lesions did not interfere with subsequent mating, suggesting that primary reward is regulated by a separate neural substrate.

In the current study, we investigated a similar distinction in effects of BLA manipulation. Motivated behavior in a high-ratio (fixed ratio 16, FR16) operant task was measured in food-deprived rats lever-pressing for food reward. We temporarily inactivated the BLA via local infusion of the $GABA_A$ agonist muscimol, which we selected because other studies (e.g., Muller et al., 1997) have demonstrated the ability of intra-amygdaloid muscimol to produce behavioral effects consistent with local inactivation. Our expectation was that muscimol injection into the BLA would decrease FR performance similar to results reported following NAS DA depletion (e.g., Aberman and Salamone, 1999) or BLA lesion (Levine et al., 1974), but not affect intake where food was made freely available. To examine the hypothesis that decreased FR performance came about via disruption of BLA–NAS communication, functional disconnection of BLA and NAS was performed by way of simultaneous unilateral inactivation of BLA with muscimol and contralateral DA blockade in the NAS with the D1/D2 antagonist flupenthixol. This “disconnection” or “asymmetric lesion” approach has been used successfully in previous experiments to examine functional connectivity between amygdala and other brain regions, including NAS (Gaffan and Harrison, 1987; Gaffan et al., 1988; Everitt et al., 1991; Han et al., 1997; Setlow et al., 2002; Di Ciano and Everitt, 2004; Floresco and Ghods-Sharifi, 2007; Ambroggi et al., 2008).

EXPERIMENTAL PROCEDURES

Subjects

Adult male Sprague–Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 350–400 g at the beginning of the study were used for all experimental procedures. An initial group of 16 animals was used for bilateral BLA inactivation during FR16 and free-feeding experiments. Six rats bearing bilateral NAS cannulae were used to examine the effect of DA receptor blockade at this site on free feeding. FR16 experiments involving drug infusions to both NAS and BLA targets used nine animals. All animals were maintained at 90% free-feeding body-weight by a rationed diet, and were individually housed in a temperature- and humidity-controlled colony on a 12-h light/dark cycle (lights on at 07:00 h) with water available *ad libitum*. Behavioral testing was conducted between 09:00 and 15:00 h.

Preoperative training

Each rat was trained to lever-press for the delivery of 45 mg food pellets (Bio-Serv, Frenchtown, NJ, USA) in one of two (31×31×25 cm) operant conditioning chambers constructed of clear Plexiglas©. A single lever was located 3 cm above the floor, 6 cm from the front wall of the chamber. A computer program controlled a daily 20-min session, where each rat could earn food pellets by depressing the lever. After initial training, the number of responses required to deliver a pellet was slowly increased to 16 (FR16). All rats were run daily on FR16 for several weeks, until the total number of lever presses performed per session stabilized (varying by less than 100 across sessions). After this initial training, all rats underwent surgery for intracranial implantation of guide cannulae.

Surgical procedures

Surgical procedures were approved by the Emory University Animal Care and Use Committee and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (revised 1996). In conducting these experiments, we attempted to minimize the number of animals used and their suffering. Surgery was performed with rats under pentobarbital (50 mg/kg) anesthesia in a Kopf (Tujunga, CA, USA) stereotaxic frame. Stainless steel 22 ga guide cannulae (Plastics One, Inc., Roanoke, VA, USA) were secured to the skull with stainless steel screws and dental cement. Following surgery, 30 ga stainless steel stylets, cut flush with the bottom of the cannulae, were inserted to prevent blockage. A recovery period of 1 week was allowed with unrestricted access to food before reducing body weight to 90% of the postoperative weight and resuming operant training.

Intracranial infusion cannula. For the combined FR16 and free-feeding experiments, guide cannulae were bilaterally implanted in a group of nine rats with tips aimed 1.5 mm above the center of the BLA: 6.4 mm anterior and 3.0 mm dorsal to the interaural line, and 5.0 mm lateral to the midline (Paxinos and Watson, 1998). This placement allowed for infusion cannulae to extend 1.5 mm beyond the guides to reach the BLA injection site. Guide cannulae were implanted at an injection control site in six rats, with tips 1.5 mm dorsal to a target in the ventral aspect of posterolateral dorsal striatum (same AP and L coordinates as BLA implants, but 4.5 mm dorsal to the interaural line).

The examination of the effect of intra-accumbens flupenthixol on free feeding used six rats not tested in the FR16 procedure. Guide tubes were bilaterally implanted to terminate at the dorsal surface of the NAS medial core region: 10.5 mm anterior and 3.5 mm dorsal to the interaural line, and 1.5 mm lateral to the midline. This placement allowed for infusion cannulae to extend 1.0 mm beyond the guides to reach the NAS injection site.

For the BLA/NAS disconnection experiments, guide cannulae were bilaterally implanted in nine rats with tips aimed as above at the dorsal surface of the BLA: 6.4 mm anterior and 3.0 mm dorsal to the interaural line, and 5.0 mm lateral to the midline, as well as bilateral guide cannulae aimed at the dorsal surface of the NAS medial core region: 10.5 mm anterior, 3.5 mm dorsal to the interaural line, and 1.5 mm lateral. As before, injectors extended 1.5 mm beyond the tips of the BLA guides and 1 mm beyond the tips of the NAS guides.

Drug infusions

Injections were performed with 30 ga stainless steel injectors placed directly into the target zones. The injectors were connected