Amygdala-Prefrontal Cortical Circuity Regulates Effort-Based Decision Making

The basolateral amygdala (BLA) and the anterior cingulate cortex (ACC) region of the prefrontal cortex form an interconnected neural circuit that may mediate certain types of decision-making processes. The present study assessed the role of this pathway in effort-based decision making using a cost-benefit T-maze task. Rats were given a choice of obtaining a high reward by climbing a 30-cm barrier in 1 arm (4 pellets; high-reward [HR] arm) or a small reward in the other arm with no barrier (2 pellets; low-reward [LR] arm). In Experiment 1, bilateral inactivation of the BLA via infusion of bupivacaine impaired decision making, reducing the preference for the HR arm. This effect was not due to spatial or motor deficits because BLA inactivation did not alter behavior when the amount of effort required to obtain either reward was equalized by placing a 2nd barrier in the LR arm. In Experiment 2, disconnection between the BLA and ACC, entailing a unilateral BLA inactivation combined with a contralateral ACC inactivation also impaired decision making. These data suggest that the serial transfer of information between the BLA and ACC guides response selection when evaluating the value of an expected outcome relative to the costs of performing a particular action.

Keywords: anterior cingulate, disconnection, frontal lobe, rat, reward

Introduction

Neuropsychological studies using brain-damaged patients and functional imaging approaches have implicated multiple subregions of the prefrontal cortex (PFC) in mediating certain decision-making processes. Recently developed tests of decision making have employed different variants of “gambling tasks,” designed to simulate real-life decisions in terms of uncertainty, reward, and punishment (Damasio 1994; Bechara and others 1999; Clark and others 2004). Patients with damage to the PFC display impairments on a variety of tasks requiring subjects to choose between options that yield high immediate monetary gain but larger future loss and those that yield lower gain but smaller future loss. Specifically, impairments in decision making have been observed in patients with damage to medial regions of the ventral and orbital PFC (Bechara and others 1998, 1999), the dorsolateral PFC (Manes and others 2002; Fellows and Farah 2005), and the dorsomedial sections of the PFC, including the anterior cingulate cortex (ACC) (Manes and others 2002; Naccache and others 2005). Likewise, performance of decision-making tasks in normal subjects is associated with widespread frontal activations in orbitofrontal and dorsolateral PFC, as well as the ACC (Rogers and others 1999; Ernst and others 2002; Clark and others 2004; Ersche and others 2005; Fukui and others 2005).

One component of decision making that can be assessed in rodents is the evaluation of response costs relative to the potential reward that may be obtained. For example, delay-discounting tasks have been used as a measure of impulsive decision making, where response costs are varied by imposing a delay before delivery of a larger reward versus obtaining an immediate, smaller reward. The neural circuitry that underlies this form of decision making in rodents has been studied in some detail. Excitotoxic lesions of the core region of the nucleus accumbens (NAc), a main efferent connection of the PFC, increase delay discounting and cause a preference for a smaller immediate reward (Cardinal and others 2001). With respect to cortical involvement in this form of decision making, lesions to the orbital PFC either decrease or increase impulsive choice, depending on whether training occurred pre- or postoperatively (Mohini and others 2002; Winstanley and others 2004). However, lesions to either the prelimbic or the ACC subregions of the medial PFC do not alter this form of decision making (Cardinal and others 2001).

An alternative way of varying response costs is to increase the effort required to obtain a larger reward. In studies of effort-based decision making, rats choose between a small reward in 1 arm of a T-maze and a larger reward placed behind a barrier in the other arm (Fig. 1A). Over the course of training, rats exhibit a preference to exert a greater effort and climb the barrier to obtain a larger reward. Initial investigations regarding the neural basis of this form of decision making focused on the mesolimbic dopamine system, revealing that destruction of dopamine terminals in the NAc reduces the preference to exert more effort to acquire a larger reward (Salamone and others 1994). Moreover, recent studies have also displayed a role for the medial PFC in this form of decision making. Specifically, lesions of the ACC impair effort-based decision making in a manner similar to dopamine depletion of the NAc, whereas lesions of the prelimbic cortex or dopamine terminals in the ACC have no effect (Walton and others 2002, 2003, 2005; Schweimer and Hauber 2005). Thus, both the ACC and mesoaccumbens dopamine are components of a neural circuit that assesses the value of an expected outcome relative to the costs of performing a particular action (Rushworth and others 2004).

Another subcortical region that may interact with the PFC to facilitate decision making about costs and benefits of different actions is the basolateral nucleus of the amygdala (BLA). The BLA shares reciprocal connections with the orbital and medial PFC including the ACC (Kita and Kitai 1990; McDonald 1991; McDonald and others 1996; Bouwmeester and others 2002) and also sends projections to the NAc (McDonald 1991). The BLA has long been known to mediate the effects that conditioned reinforcers exert over the control of behavior (Robbins and Everitt 1992, 1996) but has also been implicated in guiding instrumental action in response to changes in reward value.

Stan B. Floresco and Sarvin Ghods-Shariﬁ

Department of Psychology and Brain Research Center, University of British Columbia, 2136 West Mall, Vancouver, British Columbia, V6T 1Z4, Canada
Inactivations of the amygdala complex (including the BLA) alter behavior on tasks where animals are subjected to changes in the magnitude of an expected reward (Salinas and others 1993). Similarly, lesions of the BLA impair performance on a differential outcome procedure, where rats are insensitive to changes in the incentive value of instrumental outcome (Balleine and others 2003; Corbit and Balleine 2005). This finding indicates that another function of the BLA is to mediate processes through which outcome value is integrated within the action-outcome association to guide performance.

The BLA has also been implicated in impulsive decision making. Lesions of this structure alter behavior on a delayed discounting task, similar to rats with lesions to the NAc core or pretraining lesions of the orbital PFC, where rats show a reduced preference for larger, delayed rewards (Cardinal and others 2001; Mobini and others 2002; Winstanley and others 2004). However, the role of the BLA in mediating other types of decision making, such as those regulated by medial regions of the PFC remains unknown. In this regard, it is interesting to note that patients with damage to BLA display impaired decision making on the Iowa gambling task, similar to those with damage to the ventromedial or ACC regions of the PFC (Bechara and others 1999; Manes and others 2002; Naccache and others 2005). Given the anatomical connectivity between the BLA and PFC, it is possible that these 2 brain regions may interact to mediate decision making in situations where an animal must evaluate different options in terms of response cost and reward magnitude. In order to further clarify these issues, the present study was conducted to assess 1) the contribution of the BLA in effort-based decision making and 2) the role of the BLA-PFC pathway in mediating these processes. In doing so, we utilized an asymmetric disconnection procedure to block the transmission of information within specific pathways in each hemisphere. Specifically, a unilateral reversible inactivation of the BLA in one hemisphere was used in combination with a contralateral inactivation of the ACC in the other hemisphere. This procedure has proven to be particularly useful in defining the route of serial information transfer between different brain regions in both rats (Everitt and others 1991; Floresco and others 1997, 1999) and primates (Gaffan and Harrison 1987; Gaffan and others 1988).

Materials and Methods

Apparatus

A 4-arm cross maze was used in both experiments. The maze was made of 1.5 cm thick plywood and painted white. Each arm was 60 cm long and 10 cm wide, encased in 20 cm high walls, and contained a cylindrical food well (2 cm wide × 1 cm deep), drilled 2 cm from the end wall of the arm. Four removable table legs attached to the ends of each arm elevated the maze 70 cm above the floor. Removable pieces of white opaque plastic (20 × 10 × 10 cm) were used to block one of the arms of the maze to form a "T" configuration throughout the duration of the experiment. In "forced" trials, an identical piece of plastic was used to prevent access to one of the goal arms. In both experiments, and for every trial throughout the experiments, rats were released from the stem arm located in the same spatial location in the testing room. One or both of the arms of the maze could be blocked with barriers over which the animals had to climb to obtain rewards. These barriers were constructed in the shape of a 3-dimensional right-angled triangle, using galvanized wire mesh. Four such barriers were constructed, with the height of each being 15, 20, 25, and 30 cm. In order to obtain access to the food in 1 arm, rats had to scale up the vertical side and descend down a slope of varying incline (depending on the size of barrier). The mesh was mounted on a 30 × 10 × 1.5-cm wooden board, and the sides of the barriers were mounted with white plastic 30 cm in length, with the height being complementary to that of the wire mesh. Thus, even though the height of the maze...
arms was only 20 cm, the plastic sides of the barriers prevented animals from climbing onto the sides of the maze arms when barriers of 25 or 30 cm were used.

Subjects and Surgery
Long-Evans rats (275–300g, Charles River Laboratories, St Constant, Quebec, Canada) were used in both experiments. The rats were housed in a colony room with a temperature of 21 °C and a 12-h light-dark cycle (lights on at 7:30 AM). Prior to surgery, each rat shared a steel-hanging cage with 3 other rats. Postsurgery, they were housed individually in a plastic bin filled with Aspen-woodchip bedding and given free access to water. Rats were anesthetized with 100 mg/kg of ketamine hydrochloride and 7 mg/kg xylazine and implanted with bilateral 23 gauge stainless-steel guide cannulae. Rats in Experiment 1 were implanted with 1 pair of cannulae into the BLA (flat skull: anterior-posterior [AP] = –3.1 mm, mediolateral [ML] = 5.0 mm from bregma, and dorsal-ventral [DV] = –6.3 mm from dura). Rats in Experiment 2 were implanted with 2 sets of cannulae, one aimed at the BLA and the other implanted into the ACC region of the PFC (flat skull: AP = +2.0 mm, ML = +30.7 mm from bregma, and DV = –1.2 mm from dura) (Paxinos and Watson 1998). Each rat was given at least 7 days to recover from surgery prior to training. During this recovery period, animals were handled at least 5 min each day, and food restricted to 8% of their free-feeding weight. Body weights were monitored daily to ensure a steady weight loss during this recovery period.

Behavioral Procedure

Habituation Phase
We used a modified version of the habituation and training procedures described by Walton and others (2002). Figure 1D diagrams the timeline of habituation, training, and testing for Experiment 1. On the day prior to initial habituation to the maze environment, rats were introduced to the 45 mg food-reward pellets they would encounter on the maze (Bioserv, Frenchtown, NJ). On the first 2 days of habituation, a total of 20 pellets were placed in the 3 arms of the T-maze (6 down the stem arm and 7 down each of the other arms, 2 pellets in the food well). Each rat was given at least 7 days to explore the maze for 10 min and consume the pellets. If all pellets were consumed prior to the 10-min period, the rat was removed from the maze and placed in its home cage, while the maze was rebaited in the same manner.

The first phase of discrimination training involved putting 4 pellets in the feeding well of one goal arm (the HR arm) and 2 pellets in the other goal arm (LR arm). For half of the rats, the HR arm was the left arm, and for the others, it was the right. The baiting scheme for a particular animal remained constant for the entire duration of the experiment. Initially, each rat was placed in the start arm and was allowed to sample from both arms on each trial. The rats were cycled in groups of four with an intertrial interval of ~3 min. Five such trials were administered for the first 2 days of discrimination training. On the next 2 days, the rats were moved onto the second phase of discrimination training, in which access to one of the goal arms was prevented by blocking the arm with one of the white opaque plastic block at its entrance (forced trials), forcing the rat to sample a particular arm on each trial. The order of the forced trials was determined pseudorandomly so that they never had more than 2 consecutive turns in either direction. Each animal received 10 trials per day. Subsequent habituation trials were similar to the free-choice habituation days except that 1) the rats were removed from the maze after eating the food in the selected arm without being able to sample from the other arm and 2) rats received 16 trials instead of 5. On trials 6 and 12 of these sessions, the white plastic block was placed in the arm chosen by the rat on the previous trial to prevent potential turn biases from interfering with discrimination training. In this last phase of habituation, as well as all subsequent phases of the experiment, criterion performance was defined as choosing the HR arm on ≥75% of trials in a daily session. When a rat achieved criterion performance for 2 consecutive days, it was taken to the next phase of the task where the wire mesh barriers were introduced.

Barrier Training
Over the first 2 days of barrier training, rats received a total of 16 training trials with a 15-cm barrier placed in the HR arm. On the first 8 trials, they were allowed to sample from both arms if they chose the LR arm first, whereas if they climbed over the barrier and entered the HR arm, they were removed from the maze and placed in their home cage. For the remaining 8 trials, rats were removed once they had made their first choice regardless of which arm they entered. On all subsequent training days, rats received a total of 18 trials per day, the first two of which were forced choice; on all subsequent trials rats were free to choose either the LR or the HR arm (see Fig. 1A). Rats were removed from the maze once they chose an arm. Each rat received daily training sessions until it achieved criterion performance (12 or more entries into the HR arm, or 75%) for 2 consecutive days. When this occurred, the barrier size was increased by 5 cm on the following day, and training continued until the rat again achieved criterion performance for 2 consecutive days. The height of the barrier was gradually increased in this manner until the final height of 30 cm was reached. At this point in training, if a rat persisted in choosing the HR arm on less than 60% of trials for an extended period (>10 days), it received 2–4 sessions where the barrier was removed, after which training continued with the 30-cm barrier. The day after achieving criterion performance with the 30-cm barrier in the HR arm for 2 consecutive days, a rat received an infusion test day. Following this test day, the rat was retrained until it again achieved 2 days of criterion performance. This procedure was repeated until a rat had received the entire sequence of infusion treatments (see below). In Experiment 1, rats received additional training with a second, identical 30-cm barrier placed in the LR arm.

Microinfusion and Experimental Design
A within-subjects design was used for both Experiments 1 and 2. Inactivation of the BLA and ACC was achieved via an infusion of the local anesthetic bupivacaine hydrochloride (0.58 µg in 0.5 µl of saline; Abbott Laboratories, Saint-Laurent, Quebec). The functional spread and duration of local anesthetics infused into the brain have been studied in greater detail compared with other methods for inducing reversible inactivation, such as infusion of γ-aminobutyric acid agonists. Electrophysiological studies have estimated that an infusion of 0.5 µl of lidocaine would exert a functional spread of no greater than 1 mm in diameter and would last approximately 20 min (Boeijinga and others 1993; Tehovnik and Sommer 1997). Based on these estimates, it could be expected that infusions of local anesthetics into the region of the amygdala targeted in the present study would inactivate the lateral nuclei of this region (including the BLA) but would leave regions such as the central nucleus relatively intact. Bupivacaine has a longer duration of action compared with lidocaine (~75–90 min, Alam and Mallick 1990; Caterall and Mackie 1996), and we chose this local anesthetic to ensure a functional inactivation for the duration of our test sessions, which typically took ~60 min.

Infusions of bupivacaine and saline were administered into the BLA and ACC via 30 gauge injection cannulae that protruded 0.8 mm past the end of the guide cannula, at a rate of 0.5 µl/90 s by a microsyringe pump (Sage Instruments Model 341). Injection cannulae were left in place for an additional 1 min to allow for diffusion. Each rat remained in its home cage for a further 5 min prior to behavioral testing. On infusion test days, each rat was tested individually, with an intertrial interval of ~3 min. The number of choices directed toward the HR arm was recorded, as were the latencies to reach the end of either arm on each of the 16 free-choice trials. In Experiment 1, microinfusions of saline and bupivacaine were administered bilaterally into the BLA. In the first phase of Experiment 1, each rat was trained until it achieved criterion performance for 2 consecutive days. On the following day, the rat received an infusion of either saline or bupivacaine 5 min prior to a test session with a 30-cm barrier placed in the HR arm. It then received daily training sessions until it again achieved criterion choosing the HR arm on ≥75% of trials for 2 consecutive days, after which it received a second, counter-balanced infusion of saline or bupivacaine the following day. In the second phase of Experiment 1, rats continued to receive daily training sessions with a second, identical 30-cm barrier placed in the LR arm. Thus, rats had to climb over a barrier to obtain any reward, and the effort expended on each trial was the same, regardless of which arm was chosen. This was done to assess if deficits induced by BLA inactivation were due to simple motor or spatial impairment, or an inability to process reward quantity information. Rats were trained to the same
criterion as before and received counterbalanced intra-BLA infusions of either saline or bupivacaine on separate days. The protocol for Experiment 2 was similar to that of Experiment 1, except that rats received a total of 4 infusion test days with a 30-cm barrier placed in the HR arm. As in Experiment 1, following each infusion test day, a rat received daily training trials with a 30-cm barrier placed in the HR arm until it reached criterion for 2 consecutive days.

The logic underlying the use of disconnection lesions to identify components of a functional neural circuit is based on the assumption that information is transferred serially from one structure to an efferent region, on both sides of the brain in parallel (see Fig. 4A). Furthermore, the design assumes that dysfunction will result from blockade of neural activity at the origin of a pathway in one hemisphere and the termination of the efferent pathway in the contralateral hemisphere. It follows that a unilateral inactivation at either site should have no effect on behavior. The following combinations of asymmetrical bilateral infusions were used: 1) a unilateral inactivation of the BLA in combination with a contralateral inactivation of the ACC (disconnection), 2) a unilateral inactivation of the BLA in combination with a contralateral saline injection into ACC, 3) a unilateral inactivation of the ACC in combination with a saline injection into the contralateral BLA, and 4) unilateral injections of saline into the BLA and saline injections into contralateral ACC. We did not include a combined ipsilateral inactivation of the BLA and ACC treatment 1) to minimize the number of infusions that each animal would receive and 2) numerous studies using disconnection designs have shown that ipsilateral lesions of 2 interconnected structures in the same hemisphere do not impair behavior relative to the effect of crossed, disconnection lesions (Olton and others 1982; Warburton and others 2000; Chudasama and others 2003; Dunnett and others 2005). The order of injections was counterbalanced between animals using a quasi-Latin square design. The counterbalancing was designed to ensure that a given sequence of injections was not repeated. The hemisphere (left or right) used for the first injection was also counterbalanced and was alternated for subsequent injections. Each animal was tested separately, and the injection procedure was repeated until the rat had been tested 4 times with each sequence of injections.

**Histology**

Upon completion of behavioral testing, the rats were sacrificed in a carbon dioxide chamber. Brains were removed and fixed in a 4% formalin solution. The brains were frozen and sliced in 50 μm sections prior to being mounted and stained with Cresyl Violet. Placements were verified with reference to the neuroanatomical atlas of Paxinos and Watson (1998).

**Data Analysis**

The choice data from the 1- and 2-barrier test sessions in Experiment 1 were analyzed using separate 2-way between-within-subjects analyses of variance (ANOVAs), with Treatment (baseline, saline, or bupivacaine) as the within-subjects factor and Order of injection as the between-subjects factor. For this analysis, baseline was calculated as the mean percentage of choices of the HR arm on the day prior to each infusion test day. Latency data were analyzed using a 1-way repeated measure ANOVA, with Treatment as the within-subjects factor. Choice and latency data from Experiment 2 were analyzed in a similar manner. In this experiment, baseline was calculated as the mean percentage of HR arm choices on the day prior to each of the 4 infusion test days. For Experiment 2, 3 additional 1-way ANOVAs were conducted analyzing the number of entries into the HR arm made on test days after a unilateral inactivation of the BLA and ACC, as well as after disconnection inactivations, with the side of the injection as a between-subjects factor. This was conducted to rule out the possibility that unilateral inactivations in one hemisphere caused a greater disruption of behavior than inactivations of the other hemisphere.

**Results**

**Experiment 1: Bilateral BLA Inactivation**

Initially, 11 rats were tested in Experiment 1. Three of these animals had placements that were outside of the BLA, and their data were excluded from the analyses (see below). The remaining rats (n = 8) required 35.4 ± 7 training days before receiving counterbalanced infusions of saline or bupivacaine into the BLA. Two of these animals did not achieve criterion performance even with extended training (>50 days), but did exhibit stable levels of performance, choosing the HR arm on 9 of the 16 free-choice trials for at least 5 consecutive days of training. Thus, we chose to test these animals even though they did not achieve our set criterion, although the baseline group mean was still greater than 75%. Analysis of the choice behavior in the 1-barrier test following bilateral infusions of bupivacaine or saline into the BLA and prior to infusion test days (baseline) revealed a significant main effect of Treatment (F2,12 = 28.82, P < 0.001). There was no significant main effect of Order of injection (F1,6 = 0.75, not significant [n.s.]) or significant Treatment × Order interaction (F2,12 = 0.76, n.s.). Multiple comparisons with Tukey’s test confirmed that bilateral inactivation of the BLA caused a significant decrease in the proportion of choices of the HR arm (P < 0.01) relative to their choice behavior following saline infusions or on baseline days, which did not differ from each other (Fig. 1B). It is important to note that regardless of the baseline level of performance, each rat made substantially fewer choices toward the HR arm following BLA inactivation when compared with both baseline and saline test days. This includes the 2 rats that chose the HR arm on <75% of the free-choice trials on baseline days (saline = 59.4 ± 3%, bupivacaine = 21.9 ± 9%). Inactivation of the BLA caused a slight, nonsignificant increase in choice latencies relative to saline treatment (F1,7 = 2.43, n.s.), with rats taking an average of 12.1 ± 2 s per choice on saline test days as compared with 14.8 ± 2 s following infusions of bupivacaine.

In contrast to the effects observed in the 1-barrier test, inactivation of the BLA did not alter choice behavior when a second, identical 30-cm barrier was placed in the LR arm (F2,12 = 2.51, n.s.). Again, there was no significant main effect of Order of injection (F1,6 = 0.45, n.s.) or Treatment × Order interaction (F2,12 = 2.51, n.s.; Fig. 1C). In addition, infusion of bupivacaine into the BLA did not alter choice latencies relative to saline infusions (F1,7 = 0.14, n.s.).

The locations of infusions for all rats in Experiment 1, including those with acceptable placements in the BLA and those deemed unacceptable are displayed in Figure 2A. B. Infusions were localized primarily in the mid to caudal regions of the BLA. As noted above, the data from 3 rats were excluded from the data analyses. One rat had infusions that were located ventrally in the piriform cortex, and 2 rats had infusions located medial to the BLA, encroaching on the central nucleus of the amygdala. In these animals, the percentage of choices directed toward the HR arm 1-barrier task did not differ between bupivacaine (mean = 83.3 ± 5%) and saline (mean = 81.3 ± 2%) treatments. In light of these findings, it is likely that the impairments in decision making induced by bilateral infusions of bupivacaine were due primarily to inactivation of the BLA and not adjacent structures.

**Experiment 2: Disconnection between the BLA and ACC**

Initially, 8 rats were tested in Experiment 2. One of these rats had placements rostral to the BLA, and the data from this animal were excluded from the analyses. The remaining rats (n = 7) required 42.4 ± 6 training days to achieve criterion performance and choose the HR arm on ≥75% of trials for 2 consecutive days.
62.5% following infusions of bupivacaine. Individual rats chose the HR arm on 100% of trials following saline infusions, but only 3% following infusions of bupivacaine into either the right or the left BLA or ACC on unilateral inactivation test days (all P values <0.01 except for saline, P = 0.05). Importantly, neither type of unilateral inactivation caused a statistically significant alteration in performance relative to baseline or saline infusion test days (P > 0.10 for both unilateral inactivation treatments). Moreover, as in Experiment 1, each rat made fewer choices of the HR arm on disconnection test days compared with their performance on saline or unilateral inactivation test days, indicating that the impairments following disconnection of the BLA and ACC were not merely due to an additive effect of both unilateral inactivations.

Analysis of the choice latency data with a 1-way repeated measures ANOVA revealed no significant differences between treatment conditions (F3,18 = 0.41, n.s.). Finally, a separate series of analyses confirmed that there were no differences in performance between rats that received infusions of bupivacaine into either the right or the left BLA or ACC on unilateral inactivation or disconnection test days (all P values <0.01, n.s.).

The location of infusions for all rats in Experiment 2 with acceptable placements is displayed in Figure 3A. BLA infusions were in the same region as were observed in Experiment 1, whereas infusions into the PFC were in the region of ACC anterior to the genu of the corpus callosum. One rat had placements rostral to the BLA, and data from this animal were excluded from the analyses. This rat displayed a reduced preference for the HR arm on disconnection test days, but the magnitude of this effect was substantially smaller than the group mean.

**Discussion**

In Experiment 1, bilateral inactivation of the BLA disrupted effort-based decision making, reducing the preference to exert a greater effort to obtain a larger reward. Similarly, in Experiment 2, disconnection between the BLA and the ACC using an asymmetrical inactivation procedure also disrupted performance in a manner similar to that observed following either bilateral inactivation of the BLA or lesions of the ACC (Walton and others 2002, 2003; Schweimer and Hauber 2005). These effects cannot be attributed to deficits in motivation, motor, spatial, or reward discrimination processes because lesions or inactivation of either the BLA (Experiment 1) or the ACC (Walton and others 2002, 2003; Schweimer and Hauber 2005) did not alter the preference for a larger reward when a 2nd barrier was placed in the LR arm. In addition, it is unlikely that these effects, observed after extensive training, were due to a disruption of responding for conditioned reinforcement because lesions of the BLA only impair the acquisition, but not expression of appetitive conditioned approach, whereas lesions of the ACC do not alter responding for conditioned reinforcement.
reinforcement (Setlow and others 2002; Cardinal and others 2003). Thus, the present data indicate that the serial transfer of information between the BLA and ACC is required for effective decision making in situations requiring an evaluation of the cost of a particular action relative to the potential reward of a particular choice.

The Role of the BLA in Effort-Based Decision Making

The finding that bilateral inactivation of the BLA altered effort-based decision making is similar to a recent study demonstrating the role of this brain region in impulsive decision making. In delay-discounting tasks, rats are given the choice between a smaller immediate and a larger delayed reward (Evenden and Ryan 1996; Cardinal and others 2000, 2001). Excitotoxic lesions of the BLA or the NAc increase impulsive choice and reduce the preference for a larger delayed reward, suggesting that this region of the amygdala facilitates decision making by mediating the representation of larger rewards in its absence over a delay (Cardinal and others 2001; Winstanely and others 2004). In the present study, inactivation of the BLA also reduced the preference for the larger reward, but only when rats were required to climb over a 30-cm barrier to obtain it. Thus, it may be argued that the impairments in decision making observed here may be due to an increased tendency to make impulsive choices following BLA inactivation. However, this explanation seems unlikely for 2 reasons. First, although we did not explicitly record the time it took for rats to receive reward on each trial, given that the animals were well experienced at climbing, the delay to obtain the large reward incurred by scaling over the barrier in the HR arm was negligible (2–3 s). In contrast, the effects of lesions to the BLA on delay discounting only emerge when substantially longer delays are imposed between a response and food delivery (>10 s; Winstanely and others 2004). Second, BLA inactivation resulted in a slight, nonsignificant increase in response latencies despite a decrease in entries into the HR arm. Nevertheless, the present data, in combination with these previous findings indicate that the BLA biases behavior toward response options that lead to larger rewards that come at a greater cost. These response costs may take the form of increased physical effort to obtain the larger reward, or a delay imposed between a response and the delivery of the reward (Winstanely and others 2004), suggesting that these are related, but independent aspects of reinforcement processing. However, although the BLA mediates the direction of instrumental choice in both of these situations, the forebrain circuitry regulating these types of decision-making processes differ. Whereas lesions of the ACC or dopaminergic terminals in the NAc impair effort-based decision making (Salamone and others 1994; Walton and others 2002, 2003), these manipulations do not alter instrumental choice during delay-discounting tasks (Cardinal and others 2001; Winstanley and others 2005).

The present data complement previous findings implicating the BLA in guiding behavior when animals must choose a particular response based on the incentive value of a reward. For example, in the differential outcome procedure, rats learn that one instrumental response delivers a particular food reward (e.g., sucrose pellets), whereas another response delivers a different reward (e.g., maltose solution). In the devaluation component of the task, rats are prefed one of these rewards, which in turn reduce the proportion of responses directed toward the manipulandum associated with the now-devalued food. However, lesions of the BLA abolish this preference, and rats make responses that result in delivery of both the devalued and nondevalued reward with equal frequency. These findings indicate that BLA lesions impair the ability to form associations between a particular response and the specific incentive properties of the outcomes of those responses (Balleine and others 2003; Corbit and Balleine 2005). In the present study, inactivation of the BLA also resulted in rats choosing both the high- and low-value arms with equal frequency. Nevertheless, the present data, in combination with these previous findings indicate that the BLA biases behavior toward response options that lead to larger rewards that come at a greater cost. These response costs may take the form of increased physical effort to obtain the larger reward, or a delay imposed between a response and the delivery of the reward (Winstanely and others 2004), suggesting that these are related, but independent aspects of reinforcement processing. However, although the BLA mediates the direction of instrumental choice in both of these situations, the forebrain circuitry regulating these types of decision-making processes differ. Whereas lesions of the ACC or dopaminergic terminals in the NAc impair effort-based decision making (Salamone and others 1994; Walton and others 2002, 2003), these manipulations do not alter instrumental choice during delay-discounting tasks (Cardinal and others 2001; Winstanley and others 2005).

The present data complement previous findings implicating the BLA in guiding behavior when animals must choose a particular response based on the incentive value of a reward. For example, in the differential outcome procedure, rats learn that one instrumental response delivers a particular food reward (e.g., sucrose pellets), whereas another response delivers a different reward (e.g., maltose solution). In the devaluation component of the task, rats are prefed one of these rewards, which in turn reduce the proportion of responses directed toward the manipulandum associated with the now-devalued food. However, lesions of the BLA abolish this preference, and rats make responses that result in delivery of both the devalued and nondevalued reward with equal frequency. These findings indicate that BLA lesions impair the ability to form associations between a particular response and the specific incentive properties of the outcomes of those responses (Balleine and others 2003; Corbit and Balleine 2005). In the present study, inactivation of the BLA also resulted in rats choosing both the high- and low-value arms with equal frequency, but only when 1 barrier was placed in the HR arm, indicating that rats were indifferent to the larger reward when a greater effort was required to obtain it. From these data, it is apparent that the BLA also mediates action–outcome associations when response options vary with respect to the amount of effort required and the incentive properties (i.e., relative magnitude) of the reward.
Further insight into the neural mechanisms that underlie the contributions of the BLA to effort-based decision making comes from electrophysiological recordings from freely behaving animals. Recordings from rats foraging on a radial maze baited consistently with differential amounts of reward reveal that BLA neurons display anticipatory increases in activity prior to entering a baited arm, as well as increases in activity when the animal encounters a reward (Pratt and Mizumori 1998). Furthermore, the reward-correlated firing rate of these neurons is proportional to the expected magnitude of reward. Thus, approach toward an arm baited with a larger reward is correlated with increased firing of BLA neurons that is greater than when the animal enters an arm known to contain a smaller amount of reward. In a similar vein, BLA neurons recorded from rats performing olfactory discriminations display differential changes in activity between trials with a negative outcome and trials with a positive outcome, reflecting an expectation about the impending consequences of making a response (Schoenbaum and others 1998; Saddoris and others 2005). These findings suggest that neurons in the BLA encode anticipatory information and features relating to the magnitude of reward (Pratt and Mizumori 1998). Support for this notion comes from the findings that posttraining inactivation of the BLA attenuates the normal increase in latency to approach the end of a runway containing food when expected magnitude of reward is reduced (Salinas and others 1993). These results indicate that the amygdala complex (including the BLA) "modulates the storage of memory ... for changes in reward magnitude" (Salinas and others 1993, p. 157). Thus, it is plausible that impairments in decision making induced by BLA inactivation were due to a disruption in the ability of BLA neurons to convey information to forebrain regions about the expected magnitude of reward located in each arm of the maze.

The BLA also sends a dense glutamatergic projection to the ventral striatum (Kelley and others 1982), and 6-hydroxydopamine lesions of the NAc impair effort-based decision making in a manner similar to inactivation of the BLA. In this regard, previous neurochemical studies have shown that stimulation of the BLA can increase dopamine release in the NAc via local glutamate receptor-dependent mechanisms (Floresco and others 1998; Howland and others 2002). Thus, it is possible that impairments in decision making induced by inactivation of the BLA may be due to a disruption of mesoaccumbens dopamine transmission. However, it is important to note that inactivation of the BLA neither alters basal levels of mesoaccumbens dopamine nor attenuates feeding-induced increases in NAc dopamine release (Ahn and Phillips 2002, 2003). Based on these findings, it is unlikely that inactivation of the BLA in the present study would have perturbed dopamine release in the NAc. Furthermore, disconnection between the BLA and the ACC impaired decision making to degree similar to that induced by bilateral BLA inactivation. Thus, although the BLA may interact with the NAc to regulate mesoaccumbens dopamine transmission, it is likely that regulation of effort-based decision making by the BLA is mediated primarily via interactions with the PFC.

**Amygdalar-Prefrontal Cortical Circuits Underlying Decision Making**

In Experiment 2, a unilateral infusion of bupivacaine into the BLA combined with a contralateral infusion into the ACC significantly impaired effort-based decision making to a similar degree as was observed following bilateral inactivation of the BLA alone. Importantly, unilateral inactivation of either structure did not alter choice behavior. Thus, if efficient decision making is dependent on a serial connection linking the BLA to the ACC, then a unilateral inactivation of the BLA would prevent the ACC in the ipsilateral hemisphere from gaining access to reward-related information needed to guide behavior toward the HR arm. In the other hemisphere, information would be relayed from the intact BLA; however, it would go to a dysfunctional ACC. Following an asymmetric disconnection, the ACC on both sides of the brain would be deprived of information essential to evaluate response options relative to their costs (Fig. 4A). The finding that disconnection between the BLA and ACC impaired effort-based decision making indicates that the serial flow of information between the BLA and ACC is essential for guiding efficient choice behavior in these situations. It is notable that the estimated extent of our inactivation of the ACC was substantially smaller than that induced by the excitotoxic lesions of this region by Walton and others (2003); however, the magnitude of the impairments in these studies was comparable. This finding highlights the power of the disconnection approach and suggests that the region of the ACC anterior to the genu of the corpus callosum (which receive input from the BLA) may be a key cortical locus, which mediates this form of decision making.

The BLA shares reciprocal connections with the ACC (Kita and Kitai 1990; McDonald 1991; McDonald and others 1996; Bouwmeester and others 2002). As such, the disconnection procedure used in the present study may have impeded the serial flow of information from the BLA to the ACC and vice versa. It is therefore unclear whether the impairments in decision making induced by disconnection between these 2 regions were due to disruptions in information transfer via corticopetal BLA→ACC connections, corticofugal ACC→BLA
projections, or both. Analysis of the topographical organization of BLA-ACC connectivity provides important insight that may resolve this issue. Ascending projections to the ACC originate primarily in the caudal regions of the BLA and are almost exclusively unilateral (Kita and Kitai 1990; McDonald 1991; Conde and others 1995), whereas descending projections from the ACC terminate in the more rostral regions of the BLA (Sesack and others 1989; McDonald and others 1996; Fig. 4A). In the present study, infusions of bupivacaine into the BLA were localized primarily in the caudal regions of this nucleus. Therefore, this manipulation would be expected to hamper the outflow of information from the BLA to the ACC to a greater degree than its effect on incoming signals originating in the ACC and terminating in the more rostral aspects of the BLA. Furthermore, the ACC sends both ipsilateral and contralateral projections to the BLA (Sesack and others 1989; McDonald and others 1996; Vertes 2004; Gabbott and others 2005). This point is of particular relevance because disconnection between brain regions are not effective in disrupting behavior if they share contralateral projections, as intact sides of each brain region may still interact via contralateral connections (Olton and others 1982; Floresco and others 1997; Dunnett and others 2005). The fact that BLA-ACC disconnection and bilateral BLA inactivation both reduced the preference for the HR arm to a similar degree argues against the notion that these impairments were due to a disruption of the transfer of information originating from the ACC and terminating in the BLA. Instead, it is more likely that the impairments in decision making induced by disconnection between the BLA and ACC were due primarily to a disruption of the transfer of BLA-mediated reward-related information via ascending projections to ACC (Fig. 4B).

The question remains as to the specific contributions that the BLA and ACC make to guiding effort-based decision making. As discussed above, it is likely that activity of BLA neurons encodes information about the expected magnitude of reward associated with a particular response. On the other hand, the ACC in rats facilitates the discrimination of similar stimuli on the basis of their differential association with reinforcement (Cardinal and others 2003). Thus, the ACC may utilize reward-related information from the BLA to facilitate discriminations between stimuli associated with differential magnitudes of reward. Further insight into the role of the ACC in decision making comes from functional imaging studies in humans. These studies demonstrate that the ACC plays an important role in monitoring response conflicts and the coding of action outcomes (Rushworth and others 2004). Moreover, the magnitude of event-related potentials recorded from the ACC is associated with the magnitude of reward obtained by participants performing a gambling task (Gehring and Willoughby 2002). These findings have been interpreted by Rushworth and others (2004) to indicate that the ACC encodes whether a particular action is worth performing. In light of these findings, we propose that in situations where an organism must choose between response options associated with differential magnitudes of reward, increased activity of BLA neurons encodes the expected magnitude of reward that each choice may provide. This reward-related information may be relayed to the ACC via ascending glutamatergic projections. In turn, the ACC may bias behavior in a particular direction by integrating these signals with other information about the response costs associated with each action, possibly mediated via connections with motor cortices (Sesack and others 1989; Reep and others 1990). Once a particular course of action has been determined, the transformation of this strategy into the appropriate behavioral output is likely mediated by corticostriatal connections linking the ACC to the NAc (Floresco and others 1999; Parkinson and others 2000), although the effect of excitotoxic lesions or inactivation of the NAc on effort-based decision making remains to be explored.

Conclusions

The findings that effort-based decision making is dependent on the serial transfer of information from the BLA to the ACC complement human neuropsychological studies implicating interactions between the temporal and frontal lobes in the mediation of decision making. Humans with BLA lesions display poor decision making on the Iowa gambling task, choosing more frequently from high-risk decks that ultimately lead to a long-term loss (Bechara and others 1999). This pattern of choice is also observed in humans with damage to the ACC or ventromedial regional of the PFC (Bechara and others 1999; Manes and others 2002; Naccache and others 2005). Interestingly, although decision making in the Iowa gambling task and effort-based task used in the present study are both dependent on the integrity of the ACC, the ventromedial prelimal PFC does not appear to mediate effort-based decision making in rats (Walton and others 2003). From these data, it is tempting to speculate that dissociable PPC circuits, incorporating anatomically distinct regions of the frontal lobes, may mediate different type of decision making where the risk or effort associated with a choice must be evaluated in terms of the reward that may follow that choice. However, even though these different decision-making processes may potentially be regulated by dissociable frontal lobe circuitry, interactions between the BLA and the PFC play an essential role in mediating both types of decisions.

Notes

We wish to thank Ms Orsolya Magyar for her helpful comments on our manuscript and Ms Jodi Pawluski for her assistance in preparation of our histology figure. This work was supported by an operating grant from the Canadian Institutes of Health Research to SBF. SBF is a Canadian Institutes of Health Research New Investigator and a Michael Smith Foundation for Health Research Scholar. Conflict of Interest None declared.

Address correspondence to Stan B. Floresco, Department of Psychology and Brain Research Center, University of British Columbia, 2136 West Mall, Vancouver, British Columbia, V6T 1Z4, Canada. Email: floresco@psych.ubc.ca.

References


