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Orbitofrontal cortex and basolateral amygdala lesions result in suboptimal and dissociable reward choices on cue-guided effort in rats

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Abstract

The orbitofrontal cortex (OFC) and basolateral nucleus of the amygdala (BLA) are important neural regions in responding adaptively to changes in the incentive value of reward. Recent evidence suggests these structures may be differentially engaged in effort and cue-guided choice behavior. In two t-maze experiments, we examined the effects of bilateral lesions of either BLA or OFC on 1) effortful choices where rats could climb a barrier for a high reward or select a low reward with no effort and 2) effortful choices when a visual cue signaled changes in reward magnitude. In both experiments, BLA rats displayed transient work aversion, choosing the effortless low reward option. OFC rats were work averse only in the no cue conditions, displaying a pattern of attenuated recovery from the cue conditions signaling reward unavailability in the effortful arm. Control measures rule out an inability to discriminate the cue in either lesion group.

Keywords

rat; cost-benefit; cue; reward value; work; decision making

To make good decisions, organisms must evaluate the cost of rewards and flexibly respond to changes in the incentive value of rewards. One experimental manipulation of cost discounts the incentive value of reward by increasing the effort (or work) to obtain the reward (Walton, Bannerman, & Rushworth, 2002). Inactivations of the basolateral nucleus of the amygdala (BLA) bias animals' choices in favor of an effortless small reward over a large reward at greater effort (Floresco & Ghods-Sharifi, 2007; Ghods-Sharifi, St Onge, & Floresco, 2009). These findings corroborate the idea that BLA processes current incentive value and is required in updating reward choices when that value decreases (Baxter & Murray, 2002).

The orbitofrontal cortex (OFC), though also involved in mediating adaptive choices, appears to be uninvolved in responses to effort discounting (Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006). This is seemingly at odds with reports that OFC neuronal activity is

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associated with subjective reward value and preference (Padoa-Schioppa & Assad, 2006) which in turn is affected by effort, probability of reward (Kennerley et al., 2008), reward magnitude (Costa Araujo et al., 2010; van Duuren, Lankelma, & Pennartz, 2008), and delay to reward (Roesch & Olson, 2005). One putative mechanism by which OFC may mediate response selection is by associating salient cues in the environment with their expected outcomes (Schoenbaum & Roesch, 2005).

Historically, the ability to use cues to guide reward choices is heavily attributed to BLA. BLA plays a significant role in the discrimination of stimuli predictive of reward (Burke, Takahashi, Correll, Leon Brown, & Schoenbaum, 2009; Muramoto, Ono, Nishijo, & Fukuda, 1993) and increased synaptic strength in this region is associated with successful cue-reward learning (Tye, Stuber, de Ridder, Bonci, & Janak, 2008). Electrophysiological recording studies in rats (Roesch, Taylor, & Schoenbaum, 2006; Schoenbaum, Setlow, Saddoris, & Gallagher, 2003) and monkeys (Simmons & Richmond, 2008) suggest that OFC may also be important in using cues predictive of reward to guide choices. These cues may help in establishing expected outcomes that guide Pavlovian and instrumental behavior (Burke, Franz, Miller, & Schoenbaum, 2007; Holland & Gallagher, 2004; Ostlund & Balleine, 2007; Pickens, Saddoris, Gallagher, & Holland, 2005; Ramirez & Savage, 2007; Schoenbaum & Roesch, 2005; van Duuren et al., 2008), including updating responses to changes in reward contingency and reward value (Izquierdo, Suda, & Murray, 2004).

The present study seeks to further explore the role of OFC and BLA in effortful choices (Experiment 1) and effortful choices when a cue signals changes in reward magnitude (Experiment 2). Because a large corpus of data supports the idea that BLA updates choices in the face of changing incentives, we hypothesize that BLA lesions will result in a general pattern of work aversion, consistent with a previous report (Floresco & Ghods-Sharifi, 2007). Thus, rather than engaging in greater effort for the larger rewards, we predict BLA-lesioned rats will choose small rewards involving less effort across Experiments 1 and 2. In contrast, we hypothesize that OFC updates choices based on the associative learning of cues predictive of reward. We expect OFC-lesioned rats to be work-tolerant when effort demands increase, but unable to maximize rewards after a cue signals changes in reward magnitude.

Experiment 1

Methods

Subjects—Subjects were twenty-seven male Long Evans rats (Charles River Laboratories), weighing between 275–300g at the start of behavioral testing. All rats were individually housed and food-restricted to 85% of their free-feeding body weight to ensure motivation to work for food rewards, while water was available *ad libitum*. Housing conditions were maintained at a 12-hr light/12-hr dark cycle, with the temperature held constant at 22 C. Rats were randomly assigned to lesion or SHAM groups. All behavioral testing took place between 0800h and 1600h 5–6 days per week. Procedures for this study were reviewed and accepted by the Institution for Animal Care and Use Committee at California State University, Los Angeles.

Apparatus—A t-maze (Stoelting Co., Wood Dale, Illinois) with one start arm and two goal arms was used (Figure 1a). Each goal arm measured 41.9 cm in length, 10.2 cm wide, with walls 20.3 cm high. The start arm measured 50.3 cm in length. The t-maze was placed 94.0 cm above the floor during testing and was not moved for the duration of the experiment. Located at the end of each goal arm was a white ceramic bowl measuring 5.1 cm in diameter in which the food reward was placed. “Froot loops®” (Kellogg NA Co., Battle Creek, MI) were given as food rewards during testing: a “high reward” (HR) consisted of four ½ froot loops, while a “low reward” (LR) consisted of one ½ froot loop. To increase effort for the

HR, wooden triangular blocks of 15, 20, 25 and 30 cm were used to impede access to the food reward in the HR arm (Figure 1b). The wooden triangular blocks were placed in the t-maze such that the rat was required to climb straight up the side (90°) and down (45°) to the food reward located at the end of the goal arm. The blocks were covered in plastic mesh to ease ambulation. To allow access to only one of the goal arms (as done during ‘forced-choice’ trials) a white cardboard insert measuring 17.8 cm × 10.2 cm blocked one of the goal arms, forcing the rat to the preselected arm. Between trials, rats were removed from the t-maze and placed in a cylindrical glass holding tank measuring 30cm in height and 25cm in diameter.

Surgery—Rats received surgery after being handled but before any behavioral training and testing (see Figure 2 for sequence of methods). Anesthesia was induced with 3% isoflurane in 100% oxygen in a tightly sealed induction chamber. Throughout surgery the gas was administered through a nosecone (2–2.5%, to effect) mounted on an ultra precise small animal stereotaxic headframe (Model #963, Kopf Instruments, Tujunga CA). Respiratory rate and body temperature were monitored throughout the surgery. Surgical anesthesia was assessed by a lack of response to toe or foot pinch, at which point, the rat was placed in the earbars. Using a surgical scalpel (size 11) the skin was cut from anterior to posterior, retracted using hemostats, and the skull then leveled at bregma. Small drill holes were made over the target sites and the Hamilton microliter syringe (Hamilton Co., Reno, Nevada) needle was lowered to infuse the neurotoxin, ibotenic acid in 0.02 microliter increments. To mix the drug for surgery, 10 mg of ibotenic acid (Sigma-Aldrich, St. Louis, Missouri) was dissolved in 700 ml 0.1 M PBS, aliquoted to 7 microliter centrifuge tubes and maintained in –80 C until use.

Six rats received bilateral injections of ibotenic acid in the BLA, ten rats received bilateral injections of ibotenic acid in the OFC, and eleven sham-operated animals (SHAM) received all operative procedures but instead of neurotoxin, they received 0.1M PBS in the same sites (n=5 OFC, n=6 BLA). The OFC injections were placed using coordinates from Chudasama & Robbins (2003) relative to bregma: Site 1 (0.2 microliter), AP = +4mm; ML = +/- 0.8; DV = –3.4mm; Site 2 (0.2 microliter), AP = +3.7mm; ML = +/- 2.0mm; DV = –3.6mm; Site 3 (0.2 microliter), AP = +3.2mm; ML = 2.6mm; DV = –4.4mm. The injections, directed at the BLA and using coordinates adapted from a previous report (Burke et al., 2007) were as follows: Site 1 (0.2 microliter) AP = +2.8 mm; ML, +/- 5.0 mm; DV = –8.4 mm; Site 2 (0.1 microliter) AP = +2.8 mm; ML, +/- 5.0 mm; DV = –8.1 mm from bregma.

When injections were complete, the wound was closed using Vicryl absorbable sutures. Each rat was placed in a 100% oxygen atmosphere (in the induction chamber) for 10–20 minutes following closing and removal from the stereotaxic instrument and given a subcutaneous injection of 1.0 cc warmed 0.1M PBS. Rats were continually kept warm using a heating pad and with continued body temperature monitoring until they were ambulatory. The rats were allowed 3–5 d to recover before postoperative training and testing.

Histology—Following Experiment 1 and Experiment 2, rats were humanely euthanized with an overdose of euthasol before being perfused transcardially with 250 ml .1M PBS followed by 250 ml 4% formaldehyde over 10 minutes. Rat brains were extracted and placed in formalin overnight. The following day, brains were removed from the fixative and placed in 30% sucrose solution for at least 5 days. Once brains sank to the bottom of the 5 ml brain jars, the brains were removed and “flash frozen:” brains were placed in –80 deg isopentane and stored in isopentane until two days prior to sectioning, at which point they were removed from this liquid and stored in aluminum foil in a –80 deg freezer. Brains were then sectioned 20 microns thick, using a cryostat (Mikron Instruments, San Marcos CA), mounted onto slides, and stained with a cresyl violet stain for nissl bodies. Target areas were

viewed under a microscope (Axioscop 50, Carl Zeiss Inc. Germany), lesions were plotted onto blank Paxinos & Watson (1996) coronal sections, and then reconstructed using Adobe Photoshop (Figure 3).

Behavioral Testing—One week upon arrival from Charles River Laboratories, each rat was handled for a minimum of 10 min once per day for five days prior to surgery and behavioral testing. During the last two days of handling, animals were transported for 10 min to habituate them to the transport cart prior to the commencement of testing. Upon returning to the vivarium, they were given approximately 10 froot loops in their home cage to accustom them to the food rewards.

Acclimation to the t-maze—A habituation and training protocol adapted from Walton et al. (2002) was used to habituate the rats to the t-maze and familiarize them with the froot loops. Approximately 3–5 d after surgery and during the acclimation phase, each rat was individually placed into the t-maze and allowed to explore and eat froot loops freely for 10 min from the two goal arms and start arm. Criterion for successful completion of this stage (and for advancement to the next phase) was to eat fifteen ½ froot loops within 10 min for two consecutive days.

Discrimination training with free sampling—Upon successful completion of the acclimation phase, rats began 2 d of discrimination training in which they began to learn the reward values associated with each goal arm. In this phase, one goal arm was baited with four ½ froot loops, (HR), and the other with one ½ froot loop, (LR). The rat was allowed to sample freely from both arms for five trials. Each trial lasted until the rat finished all the froot loops. Trials were separated by a 30 sec intertrial interval (ITI) on day 1, and a 60 sec ITI on day 2. HR and LR arm designations were counterbalanced among rats (half the rats received HR in the left goal arm, the other half in the right), and remained constant for the duration of testing in Experiment 1 and Experiment 2.

Discrimination training with forced trials—On the third day, each rat was administered ten “forced” trials, in which either the HR or LR arm was pseudorandomly blocked by a white cardboard insert, forcing the rat to choose the opposite arm. In this phase, rats were allowed only to sample from only one arm per trial, with a 30 sec ITI. This task was administered for two consecutive days.

Discrimination training with free choice—Each rat was allowed to choose either the HR or LR arm, and was removed from the t-maze upon eating the food reward from the chosen arm. A “choice” constituted front paw entry into the goal arm. If rats did not remain in the chosen arm to eat the reward, the tester placed them back into the chosen arm until the reward was eaten. If rats did not begin eating the food reward within 10 sec, they were removed from the maze and placed in the holding tank for a 30 sec ITI without the food reward. A total of 12 trials with a 30 sec ITI were administered, during which time the t-maze was wiped clean with 70% ethanol solution to prevent scent-guided choice. During trials 6 and 12, the rat was forced to choose the arm not chosen during trials 5 and 11, respectively, as a means of preventing side bias. These forced trials did not constitute part of the criterion analysis. Criterion for this phase was choosing the HR arm 90% or more for 2 consecutive days. All groups reached this criterion before commencing the next phase (Figure 4A).

Effort test phases with barriers (Test 1 and Test 2)—During this phase, rats were required to climb subsequently taller barriers to procure the HR. Each session consisted of 12 trials (with trial numbers 6 and 12 as forced trials) where the HR goal arm was impeded

by a 15 cm (or larger) wooden, mesh-covered block. For the first 5 trials of the 15 cm barrier only, the LR was blocked with a cardboard insert to force the rat to choose the HR arm and climb the barrier. For all other trials, the rat could choose freely. Upon eating the food reward, the rat was placed in a holding tank for a 30 sec ITI, during which the maze was wiped clean with 70% ethanol to prevent the use of olfactory cues influencing choice. Irrespective of the rat's choice behavior, barrier heights increased after every second day of testing from 15 cm, 20 cm, 25 and 30 cm.

In order to assess the stability of an impairment should one emerge, and to better approximate the pre-post double testing in other effortful choice studies (Floresco & Ghods-Sharifi, 2007; Walton et al., 2002), rats were given two tests of increasing effort (Test 1 and Test 2). Test 2 was repeated an average of 7 d later for all of the BLA rats, but only a random subset of OFC and SHAM rats ($n = 4$ and $n = 5$, respectively) were retested. Rats not assessed on Test 2 were placed on discrimination reversal learning (not reported here), tested on Experiment 2, and assessed for lesion placement. Between the two tests, animals were allowed to rest in their homecages without handling. In order to progress to Experiment 2, rats were required to choose the HR arm 85% or more for two consecutive days on the 30 cm barrier at the end of Test 2. Additionally, rats were allowed a maximum of 10 sessions to obtain this criterion.

Data Analyses: Data were analyzed using StatView software. Statistical significance was set at $p \leq 0.05$. Effortful choice data (percent HR chosen) were analyzed via a repeated-measures ANOVA for barrier height (15, 20, 25, and 30 cm) by lesion group (OFC, BLA, SHAM), for each Test separately (Test 1 and Test 2).

Results

Test 1 effortful choices—A repeated-measures ANOVA on Test 1 performance across the four barrier heights (15, 20, 25, and 30 cm) by lesion group (OFC, BLA, SHAM) revealed a significant main effect of group ($F_{2,17}=6.1$, $p=0.01$), a significant within-subject effect of barrier height ($F_{7,119}=3.6$, $p<0.01$) and no significant interaction of lesion group by barrier height ($F_{14,119}=1.6$, $p>0.05$). Effortful choices for Test 1 are shown in Figure 4B.

Test 2 effortful choices—A repeated-measures ANOVA on Test 2 performance across the four barrier heights (15, 20, 25, and 30 cm) by lesion group (OFC, BLA, SHAM) revealed no significant effect of lesion group ($F_{2,12}=0.74$, $p=0.50$), a significant within-subject effect of barrier height ($F_{7,84}=9.0$, $p<0.01$), and no significant interaction of lesion group by barrier height ($F_{14,84}=1.7$, $p>0.05$). Effortful choices for Test 2 are shown in Figure 4C. Additionally, though there were no statistically significant differences between lesion groups in the number of sessions to reach the criterion of 85% HR choices across two consecutive days for the 30 cm barrier, two BLA-lesioned rats did not reach this criterion within 10 sessions and were consequently not tested on Experiment 2.

Discussion

Confirming a previous report, rats with pretraining BLA lesions displayed work aversion: they were less likely to climb over increasing barrier heights to procure the high reward and instead selected the low reward involving less effort. This decrease in effortful behavior in the BLA group was only in the presence of the barrier: all rats were able to reach a criterion of 90% high reward choices in the absence of the barrier (before Test 1). Our findings confirm those of a previous report (Floresco & Ghods-Sharifi, 2007) that investigated the effects of post-training inactivation of BLA (versus our pretraining lesions) on choices involving different reward values to select from (4 versus 2 as opposed to our 4 versus 1 paradigm). A novel unexpected finding of the present study is that the work-averse pattern

observed after damage to the BLA (Test 1) could be overcome with repeated training: rats recovered upon retesting which commenced approximately one week later (Test 2). Upon repeated experience with the barriers, BLA-lesioned animals increased their high reward choices, to SHAM levels at the end of Test 2. These findings align well with the idea that BLA may be most important in strengthening the initial association of a neutral stimulus (barrier) with the incentive value of its reward (Ramirez & Savage, 2007) but then becomes less required once reward learning is firmly established with increased training. This recovery may also involve compensatory mechanisms in other areas like the medial frontal cortex, specifically the anterior cingulate cortex which is known to contribute to effortful behavior (Floresco & Ghods-Sharifi, 2007; Walton, Bannerman, Alterescu, & Rushworth, 2003; Walton et al., 2002). Additionally, an intact OFC could also still support the monitoring and updating of expected outcomes necessary to develop an appropriate response strategy later in training (Ramirez & Savage, 2007; Schoenbaum, Chiba, & Gallagher, 1999), which coincides with the recovery of performance in the present experiment. Because histological verification of our lesions confirmed placement to include all of BLA and, in two cases, to extend further to incorporate central and medial nuclei of the amygdala, it is not likely that this recovery of function in BLA-lesioned animals is due to incomplete damage.

The BLA contributes heavily to the processing of changing incentive value (Baxter & Murray, 2002), thus it is likely this region is recruited to a greater extent when the heights of the barriers first increase, when the value of the reward most effectively decreases. Future studies should counterbalance the order of barrier exposure in effortful t-maze experiments, from low-to-high as well as high-to-low presentation. This would address the possibility that elapsed time from surgery mediates recovery of function in the BLA-lesioned animals.

The present results also confirm previous findings that OFC is uninvolved in effortful choices (Rudebeck et al., 2006): OFC and SHAM rats chose the high reward at comparable levels as barrier heights increased on both Test 1 and Test 2. Additionally, because behavioral dissociations between pre- and post- training lesions of OFC have been reported before (Boulougouris & Robbins, 2009; Mobini et al., 2002; Winstanley et al., 2004), and since our pretraining OFC lesions result in the same effects as post-training lesions (Rudebeck et al., 2006), we can conclude that an intact OFC is not necessary for either the learning or the maintenance of effortful choices.

Experiment 2

To explore the impact of cue guidance on effortful choice we compared BLA and OFC lesioned animals on a novel ABA design on the t-maze. For the first Phase A (A1) of Experiment 2, we introduced a posterboard cue behind the t-maze that signaled the HR arm was no longer rewarded, thus the effort to engage in climbing the barrier would not be worthwhile. We compared these trials with trials in which there was no cue present, and the HR arm was baited with the same 4 ½ foot loops reward as in Experiment 1. To be sure that any effects of lesions were related to the effort in climbing the barrier and not due to an inability to discriminate the cue, we subsequently administered Phase B, in which the barrier was removed and the animals experienced both Cue and No Cue trials as before. Lastly, the second Phase A (A2) was identical to the first and involved reintroduction of the 30 cm barrier. We expected that any impairment in cue-guided effort seen in A1, would disappear in B, but reappear in A2. Experiment 2 took place approximately 4.5 weeks after completion of Test 2 (Experiment 1) and an average of 9 weeks after surgery.

Methods

Subjects—Twenty-five subjects were the same as those used in Experiment 1. Because Experiment 2 required impeded access to the goal arm using the tallest 30 cm barrier, only the BLA-lesioned animals ($n=4$ out of 6) that reached a criterion of 85% HR choices for this barrier condition on Experiment 1 were used for Experiment 2. We note that of the two rats that sustained unintended centromedial amygdalar damage, one of the rats chose the HR at 100% at the end of Experiment 1 (with the 30 cm barrier), and the other did not attain criterion and was one of the rats excluded from Experiment 2.

Apparatus—The t-maze was the same as that used in Experiment 1. For Experiment 2, a large black and white posterboard measuring 76.2 cm $\times$ 50.8 cm served as the visual cue predicting changes in reward availability on cued trials and was placed directly behind the T-maze.

Responses to cue-signaled changes in reward availability (30 cm barrier):

Phase A1—Each session consisted of 22 trials: 20 trials where the rat could choose freely between HR arm (as experienced in training) versus LR arm, and 2 forced trials that required the rat to choose the arm not selected on the previous trial.

The first 10-trial block of free choice trials was identical to that of the previous experiment, with the same HR and LR values experienced in training (HR = four $\frac{1}{2}$ froot loops and LR = one $\frac{1}{2}$ froot loop) and using the tallest barrier of 30 cm in the HR arm. On the second 10-trial block of free-choice trials a large black and white posterboard was placed behind the t-maze and served to signal a change in reward availability in the effortful arm. When this cue was present, the LR arm remained rewarded with a $\frac{1}{2}$ froot loop but the HR arm was no longer rewarded. A total of ten sessions of Phase A1 was completed for each rat.

The rat was placed in a holding tank for a 30 sec ITI after obtaining and consuming the food reward(s), during which the maze was wiped clean with 70% ethanol to prevent the use of olfactory cues influencing arm selection.

Responses to cue-signaled changes in reward availability (no barrier): Phase B

—As in the previous phase, each session consisted of 22 trials: 20 trials where the rat could choose freely between HR arm (as experienced in training) versus LR arm, and 2 forced trials that required the rat to choose the arm not selected on the previous trial.

The first 10-trial block of free choice trials was identical to that of the previous phase (A1), with the same HR and LR values experienced in training (HR = four $\frac{1}{2}$ froot loops and LR = one $\frac{1}{2}$ froot loop) except without the 30 cm barrier impeding access to the HR. On the second 10-trial block of free-choice trials a large black and white posterboard was placed behind the t-maze and served to signal a change in reward availability in the HR arm. When this cue was present, the LR arm remained rewarded with a $\frac{1}{2}$ froot loop but the HR arm was no longer rewarded. A total of ten sessions of Phase B was completed for each rat.

The rat was placed in a holding tank for a 30 sec ITI after obtaining and consuming the food reward(s), during which the maze was wiped clean with 70% ethanol to prevent the use of olfactory cues influencing arm selection.

Responses to cue-signaled changes in reward availability (30 cm barrier):

Phase A2—This phase was identical to Phase A1 above with reintroduction of the 30 cm barrier.

Data Analyses: Data were analyzed using StatView software. Statistical significance was set at $p \leq 0.05$. As noted in Experiment 1, after Test 1 a random subset of OFC and SHAM rats were placed on a different task before rejoining the rest of the animals for Experiment 2. No statistically significant differences between these groups were found, so their data were combined here in Experiment 2.

Effortful choice data (percent HR chosen) were analyzed using a repeated-measures ANOVA for session (1 through 10) by lesion group (OFC, BLA, SHAM), for each Phase and Cue condition separately (Cue, No Cue). If an interaction was found for any condition, Fisher's PLSDs were conducted.

Results

Cue-guided effortful choice behavior (Phase A1)—A repeated-measures ANOVA on No Cue sessions by lesion group revealed a significant main effect of lesion group ($F_{2,22}=4.59$, $p=0.02$), no significant within-subject effect of session ($F_{9,198}=1.5$, $p=0.17$), but a significant lesion group by session interaction ($F_{18,198}=2.0$, $p<0.01$). Thus, both the BLA and OFC lesion groups showed reduced HR choices relative to the SHAM group when reward availability was the same as that experienced in the previous Experiment (HR = 4 ½ froot loops), Figure 5A.

A repeated-measures ANOVA on Cue sessions by lesion group revealed no significant lesion group differences ($F_{2,22}=0.83$, $p=0.45$), but a significant within-subject effect of session ($F_{9,198}=10.8$, $p<0.01$), and a significant lesion group by session interaction ($F_{18,198}=3.1$, $p<0.01$). Whereas OFC and SHAM groups gradually decreased their HR choices during the Cue presentation (optimizing their rewards) over sessions, BLA lesioned rats displayed low levels of HR choice from the beginning, Figure 5B.

Cue-guided choice behavior without barrier (Phase B)—A repeated-measures ANOVA on No Cue sessions by lesion group revealed no significant lesion group differences ($F_{2,22}=0.78$, $p=0.49$), no significant within-subject effect of session ($F_{9,198}=0.41$, $p=0.92$), and no significant lesion group by session interaction ($F_{18,198}=0.97$, $p=0.50$). Thus, when there was no work involved, all groups chose the HR arm at a high level when reward availability was the same as that experienced in the previous Experiment (HR = 4 ½ froot loops), Figure 6A.

A repeated-measures ANOVA on Cue sessions by lesion group similarly resulted in no significant lesion group differences ($F_{2,22}=1.2$, $p=0.35$), no significant lesion group by session interaction ($F_{18,198}=1.6$, $p>0.05$), and a significant within-subject effect of session as all rats decreased their choice of the HR arm ($F_{9,198}=13.3$, $p<0.01$). In general, all animals tended to show a reduction of HR arm choices during the Cue presentation, Figure 6B.

Cue-guided effortful choice behavior (Phase A2)—A repeated-measures ANOVA on No Cue sessions by lesion group revealed no significant main effect of lesion group ($F_{2,22}=2.1$, $p=0.18$), but a significant within-subject effect of session ($F_{9,198}=2.6$, $p=0.01$), and though the interaction of lesion group by session failed to reach significance ($F_{18,198}=1.6$, $p=0.09$), there appeared to be a trend for the OFC group to be once again work-averse on later sessions upon retest with the 30 cm barrier. The BLA group, conversely, performed no differently than SHAM and again showed evidence of improvement (as in Test 1 to Test 2, Experiment 1), Figure 6C.

A repeated-measures ANOVA on Cue sessions by lesion group revealed no significant difference by lesion group ($F_{2,22}=3.2$, $p>0.05$), a significant within-subject effect of session ($F_{9,198}=2.2$, $p=0.02$), and, as in Phase A1, a significant lesion group by session interaction

($F_{18,198}=2.4$, $p<0.01$). Instead of work aversion, which BLA-lesioned rats showed recovery from, this group showed enhanced choice of the HR arm across sessions, relative to OFC and SHAM groups, Figure 6D.

Discussion

We found that OFC-lesioned rats displayed work aversion only in No Cue trials and in the presence of the effortful barrier (for both Phase A1 and Phase A2). We interpret this as a failure to recover from the food reward unavailability in the effortful arm (signaled in the Cue trials). The pattern of choices observed in Phase B for the OFC group provides support for this: when the barrier is removed and rats are required to discriminate between Cue and No Cue trials, they use the cue to guide their choices and are able to maximize rewards, like the SHAM rats. OFC rats are once again impaired only when the barrier is reintroduced (Phase A2).

Electrophysiological studies in both rats and monkeys have shown that OFC neurons fire in response to rewards as well as to the cues that predict them, (Furuyashiki, Holland, & Gallagher, 2008; Simmons & Richmond, 2008) providing what many view as the neural basis for expected outcome. From this perspective, the reward in the effortful arm in the present study was most difficult to predict: 50% of the trials were rewarded with a large reward and 50% were unrewarded. If rats had difficulty tracking the cue predicting the reward magnitude in this arm, choosing this arm represented a greater risk of nonreward. Thus, OFC-lesioned rats opted to secure a small, certain reward rather than invest the energy to procure the larger, uncertain one. OFC-lesioned rats were not similarly impaired in making choices involving an uncertain reward in the absence of the barrier, when the effort requirement was waived (Phase B). SHAM rats, conversely, were able to vary their responses to secure the most rewards in all phases of the experiment. These data are consistent with the idea that OFC may be recruited more heavily when task demands require reducing interference of reward-irrelevant stimuli (Kim & Ragozzino, 2005) and that this ability is necessary in task switching and in changing the strategy to obtain rewards. Our findings are also consistent with a recent study (Burke et al., 2009) providing evidence for OFC as a primarily associative neural substrate, critical for the integration of various cues in the environment to guide responses.

In line with results by Burke et al. (2009), OFC-lesioned rats were able to inhibit responding to an unrewarded option, but were unable to maintain responding when it was later advantageous to do so. Specifically on our task, the OFC group rarely chose the effortful arm in the Cue trials, when the arm resulted in no reward, but then also rarely chose the effortful arm in the No Cue trials, when the incentive was high. This response pattern is different than the hallmark perseverative tendency observed in OFC-lesioned animals on reversal learning and instrumental extinction tasks (Izquierdo et al. 2004; Izquierdo & Murray, 2005). These results corroborate the idea that OFC is critical in the associative learning of cues to generate predictions about reward, not simply in mediating behavioral flexibility or inhibition of response.

Though OFC lesions are known to produce impairments on reversal learning in rodents and monkeys (Chudasama & Robbins, 2003; Izquierdo et al., 2004), lesions of the amygdala do not affect reversal learning (Izquierdo and Murray, 2007; Stalnaker, Franz, Singh, & Schoenbaum, 2007) and even result in facilitated instrumental extinction learning (Izquierdo & Murray, 2007). In No Cue trials of Phase A1 of the present study, climbing the effortful arm resulted in non-reward. The work aversion in the BLA group could be interpreted as facilitated extinction learning since rats were less likely to climb over the barrier for non-reward. Quicker extinction learning does not likely explain this impairment: BLA-lesioned rats chose optimally in Phase A2. Across Phase A1 and Phase A2, the BLA group displayed

responses in the same direction as the SHAMs, illustrating enhanced discrimination of the cue. Conversely, the OFC-lesioned rats performed worse, not better, upon retest in Phase A2.

Our results may have differed if cued trials had signaled a less dramatic decrease in the magnitude of reward in the effortful arm, equivalent, for example, to the low reward or, if we had instead manipulated palatability of the reward. Results might also have differed if we had presented the cue pseudorandomly instead of in blocks (though initial pilot studies in our lab showed poor learning even in unoperated rats using this design). Ongoing studies are aimed at determining how quantity and quality of the reward as well as how and when presentation of the cue affect effortful choices. Related to the latter, when the two experiments are considered together, one limitation in our study is the inability to account for order effects on reward choices because experiment order was not counterbalanced. The rats had experience with increasing barrier heights in Experiment 1 that may have affected their performance on Experiment 2. However, previous studies have also only introduced effort in an easy-to-difficult order (Walton et al., 2002; Floresco & Ghods-Sharifi, 2007; Ghods-Sharifi et al., 2009; Salamone et al., 2009) and use the same animals in multiple experiments manipulating effort (Walton et al. 2002). The foregoing experiments, as well as the present study, involve repeated trial presentations and the possibility of reward procurement on each trial. Consequently these trials likely involve instrumental learning processes. Though the neural substrates of reward choice and reward learning may be highly overlapping, they are difficult to disentangle in these designs. Future experiments should address this limitation.

In Experiment 1, BLA-lesioned animals were initially impaired and showed gradual increases in their high reward choices. Similarly in Experiment 2, the BLA-lesioned rats showed recovery of function with repeated training (from Phase A1 to Phase A2). Comparing both Experiments, the recovery in this group appeared somewhat gradual and involved repeated experience with the task reward values and contingencies. As evidence, the BLA group chose the HR less than the other two groups for the 15 cm barrier condition (Test 2) and similarly tended to choose the HR less than the other two groups on the 30 cm barrier at the end of Test 2 (two BLA-lesioned rats did not reach the 85% HR choice criterion). The rats that learned to climb over the 30 cm barrier at the end of Test 2 were impaired anew in Experiment 2, with new learning requirements. Thus, it appears that there was a tendency to revert back to work aversion in the absence of continuous training. As discussed in Experiment 1, the BLA may be most important in the initial learning of the incentive value of reward (Ramirez & Savage, 2007) but then becomes less required once expectancies and reward predictions solidify with increased training. The new learning requirements could explain why BLA-lesioned rats were impaired anew on Experiment 2 after having acquired work tolerance at the end of Experiment 1. Our findings are also consistent with a recent report showing that BLA may exert an overarching role in maintaining optimal reward choices (which would be consistent with the need for continuous training in rats with BLA lesions in the present experiment) and a more circumscribed role for OFC in learning about the rules that govern reward delivery (Zeeb & Winstanley, 2011), presumably by mediating the associative learning of rewards with the cues that predict them.

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Figure 1.

The effortful t-maze (A) was equipped with one start arm and two goal arms, each baited with either a high reward, HR (4 1/2 foot loops) or a low reward, LR (1/2 foot loop). Wooden blocks (B) of varying heights 15, 20, 25, and 30 cm were used to increase the effort required to access the high reward arm in Experiment 1.

Figure 2.
Order of surgery and behavioral testing for Experiments 1 and 2.

Figure 3. Representative lesion plots in rats with OFC (n=6) and BLA (n=6) lesions. Darker shading represents greater overlap in lesion location across rats. Numerals indicate distance from bregma. From *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), Figures 5, 7, 8, 25, 29, 31 by G. Paxinos & C. Watson, 1997, New York, NY: Academic Press. Copyright 1997 by Elsevier Academic Press. Adapted with permission.

Figure 4.

Effortful choices before barriers and on Test 1 and Test 2, Experiment 1. (A) Mean $\pm$ SEM percent high reward (HR) choices per lesion group on the last session of free-choice discrimination trials, before barrier training on Test 1 (B) Mean $\pm$ SEM percent high reward (HR) choices per lesion group for Test 1 as a function of increasing barrier heights 15, 20, 25, and 30 cm. Lower scores indicate greater work aversion. BLA-lesioned rats displayed work aversion. (C) Mean $\pm$ SEM percent high reward (HR) choices per lesion group for Test 2 as a function of increasing barrier heights 15, 20, 25, and 30 cm. The BLA group recovered upon retest (Test 2).

Figure 5.

Cue-guided effort, Phase A1, Experiment 2. **(A)** Mean $\pm$ SEM percent high reward (HR) arm choices per lesion group during no cue presentation across sessions. Reward availability was unchanged from Experiment 1 (HR = 4 $\frac{1}{2}$ foot loops, LR = $\frac{1}{2}$ foot loop) and only the 30 cm barrier was used to impede the HR arm. Both OFC and BLA groups showed work aversion relative to SHAM rats in this phase. **(B)** Mean $\pm$ SEM high reward (HR) arm choices per lesion group during the cue presentation across sessions. Reward availability was different from Experiment 1 (HR = 0 foot loops, LR = $\frac{1}{2}$ foot loop) and the 30 cm barrier impeded the HR arm. Compared to OFC and SHAM, the BLA group showed the most work aversion initially but chose the unrewarded HR arm more frequently on later sessions. Post hoc Fisher's PLSD $*p \leq 0.05$ BLA < SHAM, $**p \leq 0.01$ BLA < SHAM, $^+p \leq 0.05$ BLA < OFC, $^{++}p \leq 0.01$ BLA < OFC, $^{\gamma}p \leq 0.05$ OFC < SHAM, $^{\gamma\gamma}p \leq 0.01$ OFC < SHAM, $^{\#}p \leq 0.05$ SHAM < BLA, $^{\sigma}p \leq 0.01$ OFC < BLA.

Figure 6.

Cue-guided choice, Phase B (Top, A and B) and Cue-guided effort retest, Phase A2 (Bottom, C and D), Experiment 2. (A) Mean $\pm$ SEM percent high reward (HR) arm choices per lesion group during no cue presentation across sessions. Reward availability was the same as in Experiment 1 (HR = 4 $\frac{1}{2}$ froot loops, LR = $\frac{1}{2}$ froot loop) and no barrier was used to impede the HR arm. When the choice involved no effort, all groups chose the HR at a high rate across sessions. (B) Mean $\pm$ SEM high reward (HR) arm choices per lesion group during the cue presentation across sessions. Reward availability was different from Experiment 1 (HR = 0 froot loops, LR = $\frac{1}{2}$ froot loop) and no barrier was used to impede the HR arm. All groups showed the ability to discriminate and use the cue to guide arm selection when the choice involved no effort. (C) Mean $\pm$ SEM percent high reward (HR) arm choices per lesion group during no cue presentation across sessions. Reward availability was the same as in Experiment 1 (HR = 4 $\frac{1}{2}$ froot loops, LR = $\frac{1}{2}$ froot loop) with reintroduction of the 30 cm barrier impeding access to the HR arm. In the absence of the cue, only the OFC showed work aversion. (D) Mean $\pm$ SEM percent high reward (HR) arm choices per lesion group during cue presentation across sessions. Reward availability was different from Experiment 1 (HR = 0 froot loops, LR = $\frac{1}{2}$ froot loop) with reintroduction of the 30 cm barrier impeding access to the HR arm. As in Phase A1, Compared to OFC and SHAM rats, the BLA group tended to choose the unrewarded HR arm more frequently on later sessions. Post hoc Fisher's PLSD $\#p \leq 0.05$ SHAM < BLA, $\#\#p \leq 0.01$ SHAM < BLA, $\gamma p \leq 0.06$ OFC < SHAM, $\sigma p \leq 0.05$ OFC < BLA, $\sigma\sigma p \leq 0.01$ OFC < BLA.