Behavioral/Cognitive

Dissociable Roles for the Basolateral Amygdala and Orbitofrontal Cortex in Decision-Making under Risk of Punishment

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Several neuropsychiatric disorders are associated with abnormal decision-making involving risk of punishment, but the neural basis of this association remains poorly understood. Altered activity in brain systems including the basolateral amygdala (BLA) and orbitofrontal cortex (OFC) can accompany these same disorders, and these structures are implicated in some forms of decision-making. The current study investigated the role of the BLA and OFC in decision-making under risk of explicit punishment. Rats were trained in the risky decision-making task (RDT), in which they chose between two levers, one that delivered a small safe reward, and the other that delivered a large reward accompanied by varying risks of footshock punishment. Following training, they received sham or neurotoxic lesions of BLA or OFC, followed by RDT retesting. BLA lesions increased choice of the large risky reward (greater risk-taking) compared to both prelesion performance and sham controls. When reward magnitudes were equated, both BLA lesion and control groups shifted their choice to the safe (no shock) reward lever, indicating that the lesions did not impair punishment sensitivity. In contrast to BLA lesions, OFC lesions significantly decreased risk-taking compared with sham controls, but did not impair discrimination between different reward magnitudes or alter baseline levels of anxiety. Finally, neither lesion significantly affected food-motivated lever pressing under various fixed ratio schedules, indicating that lesion-induced alterations in risk-taking were not secondary to changes in appetitive motivation. Together, these findings indicate distinct roles for the BLA and OFC in decision-making under risk of explicit punishment.

Key words: **addiction; amygdala; decision-making; orbitofrontal cortex; punishment; risk**

Introduction

Individuals are regularly faced with decisions in which they have to weigh the consequences of multiple options before selecting the most beneficial. Oftentimes, the choice of a highly valuable option is accompanied by a risk of adverse or negative consequences. Most people are able to successfully navigate these decisions in an adaptive manner; however, many neuropsychiatric diseases are characterized by maladaptive decision-making in which risk-taking is either pathologically increased (e.g., addiction; Gowin et al., 2013) or decreased (e.g., schizophrenia, anorexia nervosa; Kaye et al., 2013; Reddy et al., 2014). Preclinical and clinical evidence shows that both the basolateral amygdala (BLA) and orbitofrontal cortex (OFC) are involved in risk-based decision-making (Floresco et al., 2008; Gowin et al., 2013). For example, patients with either BLA or OFC damage make more risky choices in laboratory tasks that simulate real-life decision-making involving uncertainty of reward gain or loss (Bechara et al., 1999, 2001; Rogers et al., 1999). In rodent models of risky decision-making, others have shown that BLA or OFC damage causes rats to make more disadvantageous choices (Pais-Vieira et al., 2007; Ghods-Sharifi et al., 2009; Zeeb and Winstanley, 2011). Together, these data suggest that dysfunction within these structures may underlie the pathological alterations in risk-taking that can accompany psychiatric disorders.

Despite data showing comparable effects of BLA and OFC damage in some tasks, the BLA and OFC have dissociable roles in other decision-making contexts, such as intertemporal choice (Winstanley et al., 2004). Moreover, most animal models of risk-taking to date have used designs in which the risk associated with the large reward or net gain is that of reward omission or a time-out period during which rewards are unavailable (Cardinal and Howes, 2005; Jentsch et al., 2010; Zeeb and Winstanley, 2011; Anselme, 2012). This distinction is important, as many real-world decisions involve not only failures to gain, but also (or instead) involve risks of explicit punishment which cannot readily be valued on the same scale as the gains (Shimp et al., 2014). In addition, the psychopharmacology of decisions involving risks of explicit punishment differs from that of decisions involving risks of reward omission, suggesting at least partially distinct neural mechanisms (St Onge and Floresco, 2009; Simon et al., 2011). Hence, it is important to consider the unique contributions of the

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BLA and OFC across a range of risk-based decision-making contexts.

To begin to address this issue, we used a rat model of risk-taking (the Risky Decision-making Task; RDT), which incorporates both rewards and risks of explicitly adverse consequences (delivery of a mild footshock; Simon et al., 2009; Mitchell et al., 2011; Simon and Setlow, 2012). The experiments herein used a lesion approach to test the hypothesis that the BLA and OFC are integral for adaptive decision-making involving risk of punishment.

Materials and Methods

Subjects

Male Long–Evans rats (P70; Charles River Laboratories) were individually housed and kept on a 12 h light/dark cycle with *ad libitum* access to food and water except as noted. During behavioral testing, rats were maintained at 85% of their free-feeding weight, with their target weights adjusted upward by 5 g/week to account for growth. Animal procedures were conducted in accordance with the University of Florida Institutional Animal Care and Use Committee and followed guidelines of the National Institutes of Health.

Apparatus

Behavioral testing was conducted in eight computer-controlled behavioral test chambers (Coulbourn Instruments). The chambers were housed in sound-attenuating cabinets and were outfitted with a recessed food-pellet delivery trough with a photobeam to detect nose pokes and a 1.12 W lamp to illuminate the food trough. The trough was located 2 cm above the floor in the center of the front wall of the chamber. Two retractable levers were located to the left and right of the trough and were positioned 11 cm above the floor of the chamber. A 1.12 W house light was mounted on the rear wall of the sound-attenuating cabinet. The floor of the chamber consisted of stainless steel rods coupled to a shock generator (Coulbourn Instruments) that delivered scrambled footshocks. An activity monitor was positioned on top of each test chamber to measure locomotor activity throughout each behavioral session. This monitor consisted of an array of infrared (body heat) detectors focused over the entire test chamber. Movement in the test chamber (in *x*, *y*, or *z* planes) was defined as a relative change in the infrared energy falling on the different detectors. The behavioral chambers were interfaced with a computer running Graphic State 3.0 software (Coulbourn Instruments), which controlled task event delivery and data collection.

Behavioral procedures

Overview of experimental design. In Experiment 1, rats were first trained in the RDT for 35 daily sessions. All rats then underwent stereotaxic surgery, in which they received either bilateral neurotoxic or sham lesions of the BLA. Upon recovery, rats were retrained on the RDT for 20 sessions. Subsequently, the effects on RDT performance of acute systemic amphetamine administration were evaluated. After allowing several days for drug washout, rats were tested on various fixed ratio schedules of food-reinforced lever pressing for five sessions (1 session/d). Finally, rats were retested on a modified version of the RDT in which reward magnitudes associated with each choice were equated such that rats chose between a small safe reward and a small risky reward. After all behavioral procedures, rats were killed and tissue was processed for lesion verification.

In Experiment 2, rats were trained in the RDT for 25 sessions. All rats then underwent stereotaxic surgery in which they received either bilateral neurotoxic or sham lesions of the OFC. Upon recovery, rats were retrained on the RDT for 12 sessions. Following the RDT, rats were tested in a reward discrimination task, in which they were given choices between the large and small reward in the absence of footshocks. Subsequently, rats were tested on various fixed ratio schedules of food-reinforced lever pressing for five sessions (1 session/d), and then tested in the elevated plus maze. After all behavioral testing, rats were killed and tissue was processed for lesion verification.

RDT. In the RDT, the term "risk" is used in the colloquial sense that refers to "the possibility that something unpleasant or unwelcome will happen" (*Oxford English Dictionary*, Oxford UP), rather than "uncertainty." Hence, "risky decision-making" as used here refers to decision-making under conditions in which there is a possibility of adverse consequences. Before training on the RDT, rats were shaped to perform the various task components (nose poking into the food trough and lever pressing for food delivery) as described previously (Simon et al., 2009, 2011; Mitchell et al., 2011). Each daily session in the RDT was 60 min in duration and consisted of five 18-trial blocks. Each 40 s trial began with illumination of the house light and the food trough light. A nose poke into the food trough extinguished the trough light and triggered extension of either a single lever (forced-choice trials) or both levers simultaneously (free-choice trials). If rats failed to nose poke within 10 s, both the house and trough lights were extinguished and the trial was scored as an omission. A press on one lever always resulted in a small food reward (1 pellet; small "safe" reward), whereas a press on the other lever always resulted in a large food reward (3 pellets; large "risky" reward), but was also accompanied by a possible footshock (1 s, 0.4–0.45 mA) contingent on a present probability specific to each trial block. The probability of the footshock was set at 0% in the first block and increased across successive blocks (25, 50, 75, and 100%, respectively). The large food reward was delivered upon every choice of the risky lever, regardless of shock delivery. Levers were counterbalanced across rats, but for each rat the identities of the safe and risky lever remained the same throughout the course of testing. Each block of trials began with eight forced-choice trials in which the punishment contingencies in effect for that block were established (4 presentations of each lever, randomly presented), and was followed by 10 free-choice trials. If rats failed to lever press within 10 s, the levers were retracted and the lights extinguished, and the trial was scored as an omission. Food delivery was accompanied by reillumination of the house light and food trough light, which were extinguished after collection of the food pellet or after 10 s, whichever occurred sooner. On the forced-choice trials (in which only one lever was present), the probability of shock following a press on the large reward lever was dependent across the four trials in each block. For example, in the 25% risk block, one and only one of those four forced-choice trials (randomly selected) always resulted in shock, and in the 75% risk block, three and only three of those four forced-choice trials always resulted in shock. In contrast, the probability of shock on the free-choice trials (in which both levers were present) was independent, such that the shock probability on each trial was the same, regardless of shock delivery on previous trials in that block.

Fixed-ratio schedule of reinforcement. To assess motivation to obtain food reward, rats were placed in the same chambers used for the RDT and given the opportunity to press a single lever for food delivery (1 pellet) under fixed-ratio (FR) schedules (FR1, FR3, FR10, FR20, and FR40; 1 schedule per session). Rats were given one 30 min FR session per day, and sessions were presented in ascending order. The lever used in the FR sessions corresponded to the small safe lever in the prior RDT sessions.

Elevated plus maze. To determine whether OFC lesions affected baseline anxiety levels, rats were tested in an elevated plus maze (open arms, 50 × 10 × 0.5 cm; closed arms, 50 × 10 × 45 cm; elevated 75 cm above the floor). Rats were placed in the center of the maze and were always positioned such that they faced the same open arm at the beginning of the session. Each 5 min session was recorded with an overhead camera connected to a Windows desktop computer. Using BioEPM3C software (v1.1.15; Bioseb In Vivo Research Instruments), six different measures were collected: (1) time spent in the center, (2) entries into the center, (3) time spent in the closed arms, (4) entries into the closed arms, (5) time spent in the open arms, and (6) entries into the open arms.

Drugs

To produce neurotoxic lesions, NMDA (Sigma-Aldrich; 15 mg/ml in 0.1 M PBS, pH 7.4) was bilaterally infused into the BLA or OFC. To assess the effects of acute amphetamine on risk-taking, D-amphetamine sulfate (0, 0.3, 1.0, and 1.5 mg/kg; Drug Supply Program at the National Institute on Drug Abuse) was administered systemically (i.p.) 10 min before the RDT every other day using a within-subjects, Latin square design as described previously (Mitchell et al., 2011; Simon et al., 2011).

Surgical procedures

Before surgery, rats were placed on a free feeding regimen for 1 week. On the day of surgery, rats were anesthetized with isofluorane gas (1–5% in O_2) and administered buprenorphine (0.05 mg/kg), Metacam (1 mg/kg), and sterile saline (10 ml) subcutaneously. After being placed into a stereotaxic apparatus (David Kopf), the scalp was incised and retracted. The skull was then leveled to ensure that bregma and lambda were in the same horizontal plane. Small burr holes were drilled in the skull to allow an injection needle (30 gauge; World Precision Instruments) to be lowered into the BLA (Experiment 1; AP: −2.8; ML: ±5.0; DV: −8.0 and −8.7 from skull surface) or OFC (Experiment 2; AP: +3.7, ML: ±3.6, DV: −4.4; AP: +3.7, ML: ±2.1, DV: −4.5; AP: 3.0, ML: ±3.9, DV: −5.2; AP: +3.0, ML: ±2.6, DV: −5.0). The injection needle was attached to polyethylene tubing, which was connected to a Hamilton syringe mounted on a syringe pump (Harvard Apparatus). For lesions of the BLA, NMDA was bilaterally infused at a rate of 0.1 μl/min (0.2 μl at the ventral coordinate and 0.1 μl at the dorsal coordinate). For lesions of the OFC, NMDA was bilaterally infused at a rate of 0.6 μl/min (0.18 μl total volume at each of the eight sites). After each injection, the needle was left in place for 5 min to allow for diffusion of the drug. Each burr hole was filled with bone wax, after which the overlying skin was stapled together and antibiotic ointment applied. Procedures for sham control surgeries were identical to those used in lesion surgeries with the exception that NMDA was not infused. After surgery, rats were given additional saline (10 ml, s.c.) and placed on a heating pad to recover before being returned to their home cage. Rats were allowed to recover for 1 week before being food restricted in preparation for retesting in the RDT.

Histology

After all behavioral testing, rats were overdosed with euthasol and perfused transcardially with 0.1 M PBS followed by 4% paraformaldehyde. Brains were extracted and postfixed in 4% paraformaldehyde for 24 h, followed by a 20% sucrose in 0.1 M PBS solution. Brains were sectioned (40 μm) on a cryostat maintained at −19°. Tissue was wet-mounted on electrostatic slides (Fisherbrand) with 70% ethanol and stained with 0.25% thionin to verify lesion placement and extent.

Data analysis

Choice performance on the RDT was measured as the percentage of free-choice trials on which a rat chose the large reward in each trial block. To determine stable behavioral performance on the RDT, a repeated-measures ANOVA (RDT session × trial block) was conducted on free-choice trials from five consecutive sessions. Stable performance was defined as the absence of either a main effect of session or interaction between session and trial block in this analysis (Winstanley et al., 2004; Simon and Setlow, 2012). Effects of lesion condition on free-choice trials (averaged across 5 consecutive sessions of stable performance) were determined using a repeated-measures ANOVA with time point (presurgery vs postsurgery) and trial block as within-subjects factors and lesion condition (lesion vs sham) as a between-subjects factor. Additional *post hoc* analyses were conducted on the first session and average of the first five sessions of postsurgical RDT performance, as well as performance on the control choice tasks. Choice response latencies were measured as the interval between lever extension and lever press, excluding trials on which rats failed to press the lever altogether (omissions). Forced-choice trials were used to assess response latencies because of insufficient data from free-choice trials (i.e., some rats chose one lever exclusively on some blocks of free-choice trials). In addition, latencies on the forced-choice trials provided a measure of incentive motivation for the rewards that was relatively uncontaminated by comparative reward values or decision processes (Giertler et al., 2003; Schoenbaum et al., 2003a; Shimp et al., 2014). Effects of lesion condition on forced-choice trial latencies were analyzed using a repeated-measures ANOVA (lesion condition × trial block × lever identity). Baseline locomotor activity was measured by averaging activity (in arbitrary units) across all intertrial intervals (in which no lights or levers were present). Shock reactivity was defined as activity during the 1 s shock periods, averaged across the test session. The effects of amphetamine on risk-taking were analyzed using a repeated-measures ANOVA (lesion condition × drug dose × trial block). Lever pressing in the FR schedules of reinforcement was analyzed using a repeated-measures ANOVA (lesion condition × FR schedule), with *post hoc* comparisons conducted on each FR schedule. For the analyses of performance in the elevated plus maze, independent *t* tests were used to compare lesion and sham groups on time spent in and entries into each compartment of the maze.

Results

Experiment 1: effects of lesions of the basolateral amygdala on risk-taking

Lesion placements

Of the initial 40 rats, seven were excluded due to inaccurate lesion placement or insufficient lesion size. Specifically, rats were excluded if the lesions extended dorsally into the striatum and/or medially into the adjacent central nucleus of the amygdala. Furthermore, the lesions had to encompass at least two-thirds of the BLA to be considered a "hit" and be included in the analyses. The placement and extent of the lesions in the remaining rats in the study ($n = 18$ lesion; $n = 15$ sham) are depicted in Figure 1.

Lesions of the basolateral amygdala cause an increase in risk-taking

Rats were trained in the RDT for 35 sessions (until stable performance emerged). Before surgery, there were no differences in RDT performance between rats assigned to the lesion or sham group (significant effect of block, $F_{(4,124)} = 34.57$, $p < 0.001$; no effect of group, $F_{(1,31)} = 0.18$, $p = 0.68$; no block × group interaction, $F_{(4,124)} = 0.93$, $p = 0.45$; Figure 2*A*). In all subsequent analyses, the main effect of trial block was statistically significant (at least $p < 0.01$) and will not be reported further. Following recovery from surgery, rats were retested in the RDT for 20 sessions. Final RDT performance was assessed during the last five sessions, at which point choice performance had restabilized. As depicted in Figure 2*B*, the lesion group chose the large risky reward considerably more than the sham group after surgery. This was confirmed with a three-factor repeated-measures ANOVA [time (presurgery vs postsurgery) × block × group]. Though there was not a significant effect of time ($F_{(1,31)} = 4.10$, $p = 0.05$) or group ($F_{(1,31)} = 4.06$, $p = 0.05$), there were significant interactions between time and group ($F_{(1,31)} = 11.19$, $p < 0.01$), between block and group ($F_{(1,124)} = 3.06$, $p = 0.02$), and between time, block and group ($F_{(4,124)} = 5.54$, $p < 0.001$). A two-factor repeated-measures ANOVA was then used to compare differences between the lesion and sham groups specifically during the postsurgery period. In contrast to the absence of group effects during presurgery performance (Fig. 2*A*), analysis of postsurgery performance revealed that the lesion group chose the large risky reward significantly more than the sham group. This lesion effect began to emerge on the first postsurgery test day (main effect of group: $F_{(1,31)} = 2.76$, $p = 0.12$; block × group: $F_{(4,124)} = 0.82$, $p = 0.52$), was robust as early as the first five sessions (block × group interaction: $F_{(4,124)} = 3.36$, $p = 0.01$; group: $F_{(1,31)} = 8.31$, $p = 0.01$) suggesting that minimal learning was required for expression of the lesion effect, and persisted through the final five sessions, at which point choice behavior reached stability (main effect of group: $F_{(1,31)} = 12.38$, $p < 0.01$; block × group interaction: $F_{(4,124)} = 7.22$, $p < 0.01$; Fig. 2*B*). Additional two-factor ANOVAs comparing stable performance of each group before and after surgery confirmed that the sham group did not shift its choice performance after surgery ($F_{(4,68)} = 0.89$, $p = 0.47$), whereas the lesion group significantly increased its choice of the large risky reward after surgery ($F_{(4,56)} = 5.15$, $p < 0.01$). Collectively, these results show that lesions of the BLA cause an increase

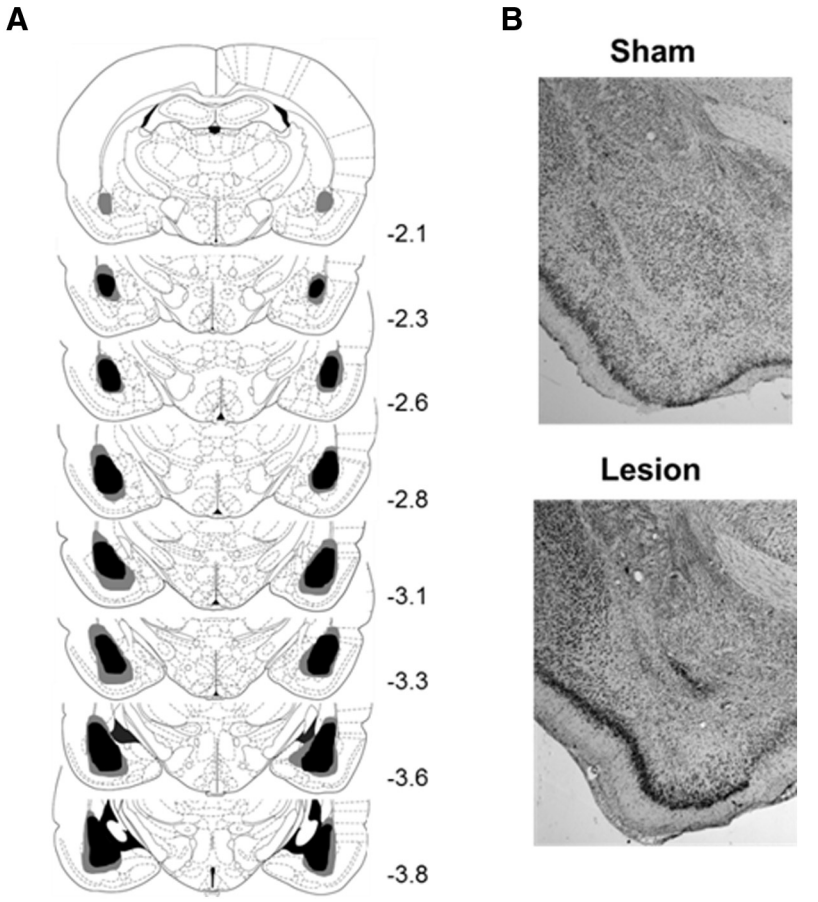

Figure 1. Representative histology from rats that received either neurotoxic or sham lesions of the basolateral amygdala. ***A***, The extent of neurotoxic lesions of the basolateral amygdala is depicted schematically, with the maximum lesion extent (gray) and minimum lesion extent (black) indicated in both hemispheres. ***B***, Representative thionin-stained coronal images of the basolateral amygdala in a lesioned rat and a sham rat are displayed. Images were taken using a 250× objective lens.

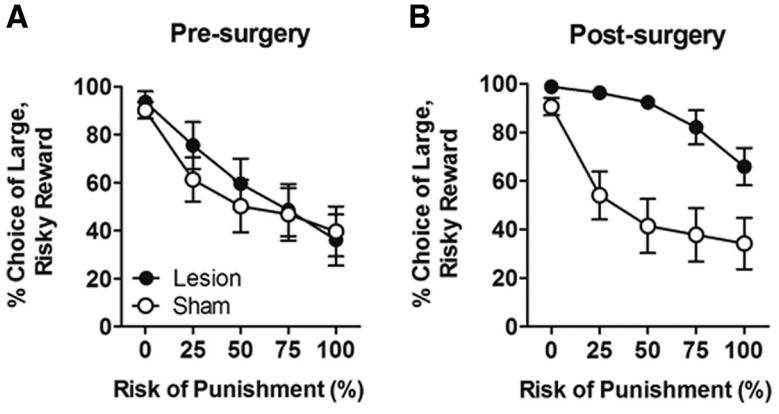

Figure 2. Choice performance on the risky decision-making task before and after basolateral amygdala lesions. ***A***, Before surgery, there was no difference in choice of the large risky reward between the groups that would go on to receive lesion or sham surgeries. ***B***, After surgery, the lesion group ($n = 18$) showed a significant increase in choice of the large risky reward compared with both presurgery baseline and postsurgery sham controls ($n = 15$). Data (mean ± SEM percentage choice of the large risky reward) are averaged over the final five sessions (both before and after surgery), at which point rats displayed stable performance.

in preference for large risky over small safe rewards, suggestive of an increase in risk-taking.

One caveat to the interpretation that BLA lesions cause an increase in risk-taking arises from the fact that the lesion group chose the large reward to a greater extent than controls in the final block of trials (100% probability of shock). Because there is technically no risk of shock during this block (shock delivery accompanying the large reward is guaranteed), it could be argued that the effects of the BLA lesions are unlikely to be on risk-taking *per se*. It is probable, however, that the lesion group's elevated choice of the large reward in the 100% block reflects some degree of "carryover" from previous blocks of trials. In support of this interpretation, similar patterns of apparently suboptimal choice behavior are observed in intact rats in a probabilistic discounting task, which is structured identically to the RDT but in which the "cost" associated with the large reward is reward omission rather than shock (Cardinal and Howes, 2005; St Onge and Floresco, 2009). In this task, we have found that under conditions of increasing probability of reward omission (0–100% across 5 blocks of trials), many intact rats continue to choose the large reward lever at a high rate (20–25%) even when there is 100% probability of reward omission (Simon et al., 2009; Mendez et al., 2010; Gilbert et al., 2012). These data suggest that although in both tasks the free-choice trials in each block are preceded by forced-choice trials to remind rats of the probabilities in effect for that block, rats likely compute probabilities using information from previous blocks of trials as well, resulting in the computed probability of punishment in a given block being less than the actual probability. By this view, the effects of BLA lesions in the current experiment in the 100% block may reflect such broad probability computation, resulting in "riskier" choice behavior even in the absence of actual risk.

To further evaluate the effects of BLA lesions on performance in the RDT, latency to respond at each lever during forced-choice trials was analyzed. A repeated-measures ANOVA was used to analyze differences in latencies between groups before surgery and after surgery, with group as the between-subjects factor and size (small vs large lever) and block as the within-subjects factors. An additional repeated-measures ANOVA was conducted to determine whether latencies to choose the small and large lever changed after surgery [time (presurgery vs postsurgery) and block as the within-subjects factors and group as the between-subjects factor]. Before surgery, there were no differences between lesion and sham groups in their latency to choose the lever associated with either the small ($F_{(4,124)} = 0.35$, $p = 0.85$) or large reward ($F_{(4,104)} = 0.03$, $p = 1.00$; Fig. 3*A*). In addition, as reported previously (Shimp et al., 2014), all rats had significantly longer latencies to press the large reward lever than the small reward lever [size (large vs small): $F_{(1,26)} = 8.11$, $p =$

0.01; size × block: $F_{(4,104)} = 17.01$, $p < 0.01$]. This difference in latencies to choose the large versus small lever did not differ between lesion and sham groups ($F_{(1,104)} < 0.05$, $p = 1.0$) before surgery. After surgery, however, latencies to press each lever shifted (Fig. 3*B*). Although there were no differences between groups in latency to press the lever associated with the small reward (block × group: $F_{(4,124)} = 1.59$, $p = 0.18$; group: $F_{(1,127)} = 1.63$, $p = 0.21$), there were significant group differences in latencies to press the large risky lever. Rats with BLA lesions had significantly shorter latencies to press the large risky lever compared with sham rats (group × block: $F_{(4,108)} = 3.78$, $p = 0.01$; group: $F_{(1,27)} = 7.61$, $p = 0.01$). To determine whether there were differences between presurgery latencies and postsurgery latencies, we conducted a three-factor repeated-measures ANOVA. This analysis revealed no differences in presurgery and postsurgery latencies in sham rats for the small (time: $F_{(1,17)} = 0.52$, $p = 0.48$]; time × block: $F_{(4,68)} = 0.52$, $p = 0.72$] or large reward lever [time (presurgery vs postsurgery): $F_{(1,12)} = 1.49$, $p = 0.25$; time × block: $F_{(4,48)} = 1.40$, $p = 0.25$; Fig. 3*B*). However, the latencies to press the small and large reward lever significantly changed after surgery in BLA lesion rats. Rats with BLA lesions had significantly shorter latencies to choose the large risky lever after surgery (time: $F_{(1,11)} = 7.83$, $p = 0.02$; time × block: $F_{(4,44)} = 4.46$, $p < 0.01$). Interestingly, after BLA lesions, there was also a significant increase in latencies to choose the small safe lever as risk of punishment increased within a session (time × block: $F_{(4,56)} = 5.03$, $p < 0.01$). To summarize, BLA lesions altered latencies to respond on each lever, such that responses were faster for the large risky reward, but slower for the small safe reward. These data suggest that, in contrast to sham controls, reward magnitude was the primary driver of choice behavior in BLA lesion rats. In combination with the choice data described above, these results indicate that BLA lesions impaired rats' ability to integrate information concerning risk of punishment to guide reward-related decision-making.

Damage to the amygdala can cause impairments in expression of fear, commonly assessed in rats as freezing behavior (lack of movement except that required for breathing; Blanchard and Blanchard, 1972). As such, it is possible that the increase in choice of the large risky reward in the lesion group was due to impaired expression of such fear-related behaviors. To determine whether BLA lesions affected rats' responses to the footshock, we analyzed locomotor activity during shock delivery (Table 1), as well as during intertrial intervals in sham and lesioned rats (Table 1). There were no differences in either intertrial interval locomotor activity ($t_{(31)} = 1.33$, $p = 0.19$) or shock reactivity ($t_{(28)} = 0.02$, $p = 0.98$), suggesting that lesion-induced alterations in freezing or related behaviors did not account for the choice differences. An additional independent *t* test was used to determine whether BLA lesions caused an increase in trial omissions, as this may also suggest alterations in processing of punishment associated with the large reward (Cooper et al., 2014). However, there were no differences in trial omissions between the lesion and sham groups ($t_{(31)} = 0.45$, $p = 0.66$; Table 1).

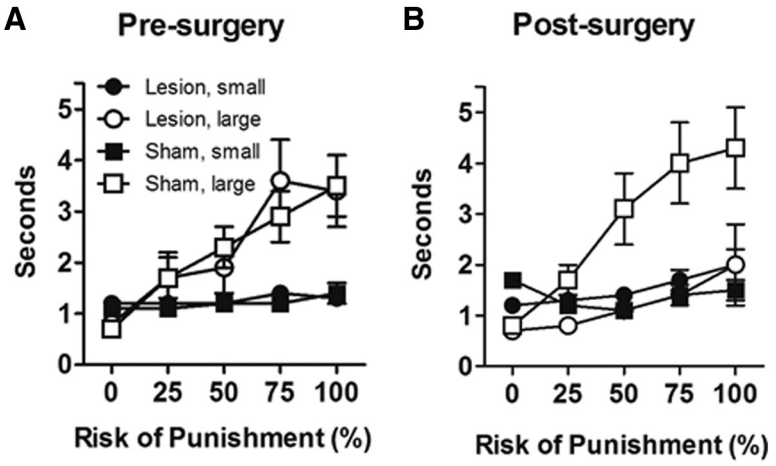

Figure 3. Response latencies in the risky decision-making task before and after basolateral amygdala lesions. ***A***, Before surgery, both groups had significantly longer latencies to respond on the lever associated with the large risky reward compared with the lever associated with the small safe reward. ***B***, After surgery, the lesion group ($n = 18$) had significantly longer latencies to respond on the small reward lever compared to their pre-surgical performance, but significantly shorter latencies to respond on the large reward lever compared to both pre-surgical performance and sham controls ($n = 15$). Data (mean ± SEM seconds) are averaged over the final five sessions (both before and after surgery), at which point rats displayed stable performance.

Table 1. Effects of BLA lesions and acute amphetamine administration on trial omissions, locomotor activity, and shock reactivity in the RDT

Session	Omitted trials, %	Locomotion (locomotor units/ITI)	Shock reactivity (locomotor units/shock)
Postsurgery			
Sham	3.49 (1.54)	9.01 (0.84)	3.13 (0.29)
Lesion	4.37 (1.11)	7.55 (0.66)	3.12 (0.14)
Amphetamine			
Vehicle			
Sham	4.40 (2.12)	14.97 (2.03)	3.56 (0.33)
Lesion	4.40 (1.83)	13.20 (1.09)	3.0 (0.16)
0.3 mg/kg			
Sham	3.40 (1.81)	16.73 (1.54)	3.55 (0.40)
Lesion	7.33 (2.17)	17.43 (1.80)	3.10 (0.24)
1.0 mg/kg			
Sham	10.20 (3.10)	21.05 (1.75)	3.50 (0.44)
Lesion	13.33 (2.72)	23.10 (1.86)	3.10 (0.13)
1.5 mg/kg			
Sham	17.4 (4.96)	20.7 (1.62)	3.04 (0.20)
Lesion	38.4 (7.69)*	23.13 (1.96)	2.98 (0.30)

Asterisks indicate a significant difference between lesion and sham groups. At the highest dose of amphetamine, there were significantly more omissions in BLA lesion than in sham rats. SEM is shown in parentheses.

Lesions of the basolateral amygdala do not affect shock avoidance

One possible interpretation of the BLA lesion-induced increase in risk-taking is that the lesions rendered rats insensitive to the response-suppressive effects of the shock or otherwise unable to discriminate between the shock (risky) and nonshock (safe) levers. To evaluate this possibility, rats were tested on a modified version of the RDT, in which the reward magnitude associated with each lever was equated such that choice of either lever yielded a small (1 pellet) reward, but the safe and risky properties of the levers remained unchanged. If the lesion-induced increase in risk-taking were due to impaired shock avoidance, then lesioned rats would be expected to continue to choose the risky lever to a greater degree than sham controls. A subset of rats ($n = 4$ sham and $n = 11$ lesion) was trained on this modified task for 14 sessions (note that the effects of BLA lesions on RDT performance in this subset were comparable to those in the entire cohort; main effect of group at postsurgical time point: $F_{(1,13)} =$

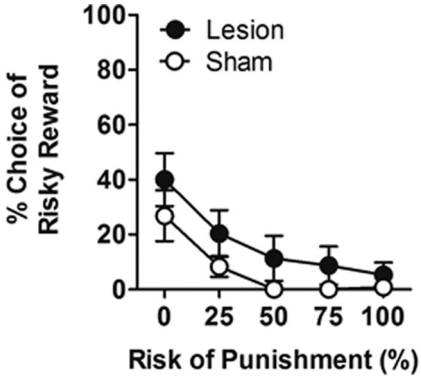

Figure 4. Performance in the risky decision-making task when reward magnitudes were equated. Both lesion ($n = 11$) and sham ($n = 4$) groups shifted their choices to the nonshock-associated (safe) lever to the same degree. Data (mean ± SEM percentage choice of the risky reward) are from the final five sessions of testing, at which point rats displayed stable performance.

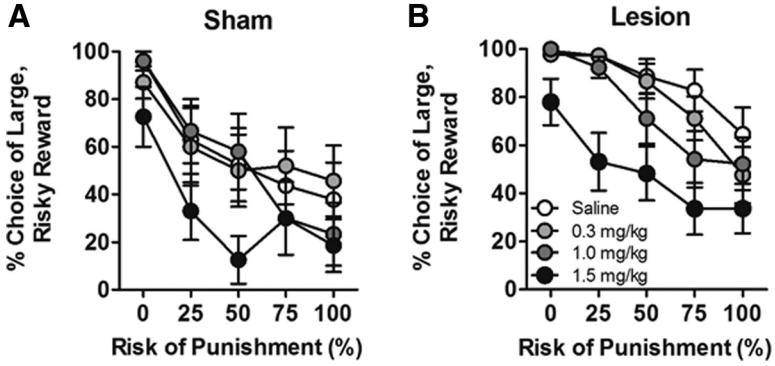

Figure 5. Effects of amphetamine on the risky decision-making task in rats with basolateral amygdala or sham lesions. ***A***, Amphetamine decreased risk-taking in the sham group, with the largest effect occurring at the highest dose. ***B***, Amphetamine decreased risk-taking in the lesion ($n = 15$) group to a similar extent as in shams ($n = 10$). Data are represented as the mean ± SEM percentage choice of the large risky reward.

5.91, $p = 0.03$; data not shown). Final performance on this modified RDT was assessed during the last five sessions, at which point performance was stable (Fig. 4). There was neither a main effect of group ($F_{(1,13)} = 1.01, p = 0.33$) nor a significant group × block interaction ($F_{(4,52)} = 0.34, p = 0.85$). Thus, when reward magnitudes were equated, the BLA lesion rats shifted their choices to the safe lever to the same degree as the sham rats, indicating that BLA lesions did not affect their sensitivity to punishment. Importantly, *post hoc* analyses showed that this shift to the safe lever (and the absence of a group difference) when rewards were equated was evident as early as the first session of training on the modified RDT (block × group: $F_{(4,52)} = 0.35, p = 0.84$; group: $F_{(1,13)} = 0.58, p = 0.46$), as well as across the first five sessions, (block × group: $F_{(4,52)} = 0.17, p = 0.95$; group: $F_{(1,13)} = 1.12, p = 0.31$), suggesting that the increased risk-taking observed in BLA lesioned rats on the RDT was unlikely to be attributable to impairments specifically in reversal learning and more generally in behavioral flexibility (i.e., both groups rapidly shifted their choices to the safe lever). Note that upon close inspection of Figure 4, it could appear as though the lesion group chose the risky lever slightly more than the sham group; however, this difference was driven by a single rat in the lesion group that showed anomalously high levels of preference for the risky lever (>2 SDs above the group mean). When this rat's data were excluded, the lines in Figure 4 overlapped almost entirely. Importantly, this exclusion had no impact on the effects of BLA lesions on the RDT, as there was still a significant time × block × group interaction in the data shown in Figure 2 ($F_{(4,120)} = 5.85, p < 0.01$).

Similar to the analyses in the RDT described above, latencies to select the small safe or small risky lever during forced-choice trials were also analyzed in the modified RDT using a three-factor ANOVA. There were no differences between lesion and sham groups in the latencies to choose the small safe lever (block × group: $F_{(4,52)} = 1.00, p = 0.42$; group: $F_{(1,13)} = 1.92, p = 0.19$) or in the latencies to press the small risky lever (block × group: $F_{(4,28)} = 0.75, p = 0.57$; group: $F(1, 70 = 2.50, p = 0.16$). Furthermore, although there was a main effect of lever (safe vs risky: $F_{(1,7)} = 29.00, p < 0.001$), this lever difference was equivalent in lesioned and sham rats (lever × group: $F_{(1,7)} = 1.44, p = 0.27$; lever × group × block: $F_{(4,28)} = 1.01, p = 0.42$; data not shown). Together with the choice data shown in Figure 4, these data are consistent with previous work showing that although BLA lesions impair freezing to a context previously paired with footshock, shock avoidance is spared, indicating that rats with BLA lesions can still learn associations between actions and aversive outcomes (Parent et al., 1995; Vazdarjanova and McGaugh, 1998). Furthermore, they argue against the possibility that the lesion-induced increase in risk-taking was due to impairment in devaluation of the large reward by the shock. If BLA lesions caused impairment in shock-induced reward devaluation, lesioned rats would have continued to select the small risky reward rather than shifting their preference to the small safe reward in the modified RDT. In addition, others have shown that BLA lesions only impair devaluation when made before, but not after, training (Pickens et al., 2003). Because BLA lesions occurred after training in the RDT and still affected choice performance, it is unlikely that impaired devaluation accounts for the increased risk-taking. Rather, it seems more likely that the lesions prevented the integration of reward-related information with risk of explicit punishment to guide adaptive behavior.

Systemic amphetamine administration decreases risk-taking

We have previously shown that systemic administration of amphetamine dose-dependently decreases risk-taking behavior, likely by potentiating the salience of the risk of shock and causing rats to become more risk-averse (Simon et al., 2009; Mitchell et al., 2011). Given that the BLA is important for processing aversive information (Pape and Pare, 2010; Orsini and Maren, 2012), it is possible that BLA lesions may attenuate this amphetamine-induced decrease in risk-taking. To test this possibility, a subset of rats ($n = 10$ sham; $n = 15$ lesion) received acute systemic injections of amphetamine (0, 0.3, 1.0, and 1.5 mg/kg) before testing in the RDT, using a within-subjects (Latin square) design. There was a significant main effect of amphetamine on choice of the large risky reward ($F_{(3,69)} = 22.43, p < 0.01$) and a near-significant dose × block interaction ($F_{(12,276)} = 1.78, p = 0.05$), such that rats chose the large risky reward less often (became more risk-averse) with higher doses of amphetamine (Fig. 5). Importantly, there was neither a significant dose × group interaction ($F_{(3,69)} = 0.71, p = 0.55$) nor a significant dose × group × block interaction, ($F_{(12,276)} = 1.29, p = 0.22$), indicating that

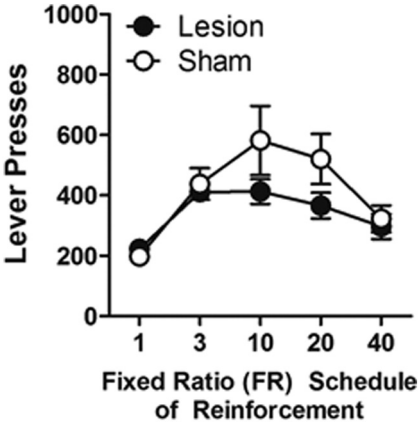

Figure 6. Effects of basolateral amygdala lesions on instrumental responding for food reward. Rats were tested under a series of fixed ratio schedules of lever pressing for food reward (FR1, FR3, FR10, FR20, and FR40), one schedule/d for 5 d. There was no difference between the lesion ($n = 15$) and sham ($n = 10$) groups on any of the individual FR schedules.

amphetamine had similar effects in the sham and lesion groups. Amphetamine also dose-dependently increased locomotor activity in the task ($F_{(3,69)} = 22.13$, $p < 0.01$), but there was neither a significant dose × group interaction ($F_{(3,69)} = 1.31$, $p = 0.28$) nor a main effect of group ($F_{(1,23)} = 0.17$, $p = 0.69$), indicating that this increased activity was not modulated by BLA lesions (Table 1). Importantly, amphetamine had no effect on shock reactivity (dose: $F_{(3,42)} = 1.75$, $p = 0.17$; Table 1). This was further confirmed by a lack of a main effect of group,($F_{(1,14)} = 0.93$, $p = 0.35$) and dose × group interaction ($F_{(3,42)} = 0.81$, $p = 0.49$). Finally, amphetamine dose-dependently increased trial omissions in the RDT ($F_{(3,69)} = 15.13$, $p < 0.01$). Although there was a significant dose × group interaction ($F_{(3,69)} = 2.88$, $p = 0.04$), there was no main effect of group, ($F_{(1,23)} = 4.15$, $p = 0.05$). This increase in omissions in BLA lesion rats relative to sham rats predominantly occurred with the highest dose, suggesting that BLA lesions may render rats more sensitive to the performance-disruptive and/or anorectic effects of amphetamine (a previous study showed that increasing satiety caused an increase in trial omissions without altering choice behavior; Simon et al., 2009). Collectively, however, these data demonstrate that amphetamine causes a reduction in risk-taking regardless of lesion condition, and support the contention that some aspects of aversive processing are spared by BLA lesions.

Lesions of the basolateral amygdala do not increase motivation to obtain food reward

The greater risk-taking in BLA lesion rats could conceivably reflect an increase in motivation to obtain food. To test this possibility, a subset of rats ($n = 10$ sham; $n = 15$ lesion) was assessed on a series of ascending fixed ratio schedules of instrumental responding for food reward (FR1, 3, 10, 20, 40), which we have shown previously to be sensitive to increases in incentive motivation (Mendez et al., 2009). A two-factor repeated-measures ANOVA revealed a significant interaction between FR schedule and group ($F_{(4,92)} = 3.27$, $p = 0.02$) such that lesioned rats pressed significantly less than sham rats across the five sessions of testing on the FR schedules. When assessing effects of lesion condition on each FR schedule, however, there was no effect of group on any of the schedules ($ps > 0.05$; Fig. 6). These results show that the lesion-induced increase in choice of the large risky reward in the RDT was not due to heightened motivation to obtain food.

Experiment 2: effects of orbitofrontal cortex lesions on risk-taking

Lesion placement

Of the 16 rats that initially began the study, only one was excluded due to inaccurate lesion placement. For a lesion to be considered a hit, the damage had to be restricted to the OFC without impinging on the overlying white matter. The lesion also had to encompass at least two-thirds of the OFC to ensure that the majority of the structure was damaged. Representative histological images for both sham ($n = 8$) and lesion ($n = 7$) groups are displayed in Figure 7.

Lesions of the orbitofrontal cortex cause a decrease in risk-taking

All rats were trained on the RDT for 25 sessions before surgery, at which point stable performance was reached. The last five sessions of performance were used for analyses. Although there was a significant effect of block ($F_{(4,52)} = 15.91$, $p < 0.001$), there were no differences in performance between the lesion and sham groups before surgery(group: $F_{(1,13)} = 0.12$, $p = 0.73$; group × block interaction: $F_{(4,52)} = 0.33$, $p = 0.86$; Fig. 8*A*). As in Experiment 1, all subsequent analyses shared a significant main effect of trial block ($p < 0.05$), and these effects will not be reported further. After surgery, rats were retrained on the RDT for 12 sessions, and final RDT performance was assessed during the last five sessions, at which point behavior had restabilized. As depicted in Figure 8*B*, rats with OFC lesions chose the large risky reward significantly less than sham rats after surgery. A three-factor ANOVA (time × block × group) revealed neither a significant time × group interaction ($F_{(1,13)} = 3.62$, $p = 0.08$) nor a block × group interaction ($F_{(4,52)} = 1.90$, $p = 0.1$), but there was a significant main effect of time ($F_{(1,13)} = 9.26$, $p = 0.01$), as well as a significant time × group × block interaction ($F_{(4,52)} = 3.34$, $p = 0.02$). This latter result indicates that OFC lesions caused a decrease in choice of the large risky reward across trial blocks relative to sham controls. A separate repeated-measures ANOVA was then conducted on RDT performance postsurgery. The sham and OFC lesion groups did not differ in their choice of the large risky reward during either the first postsurgery session (block × group: $F_{(4,52)} = 0.33$, $p = 0.86$; group: $F_{(1,13)} = 0.22$, $p = 0.65$) or across the initial five postsurgery sessions, (block × group: $F_{(4,52)} = 1.91$, $p = 0.12$; group: $F_{(1,13)} = 0.34$, $p = 0.57$), possibly indicating that, in contrast to the effects of BLA lesions, the value of the large risky reward had to be relearned following OFC lesions. In contrast, during the final five sessions, rats with OFC lesions chose the large risky reward significantly less than sham controls (block × group: $F_{(4,52)} = 5.02$, $p < 0.01$). Finally, a repeated-measures ANOVA comparing stable presurgery versus postsurgery performance in each group revealed no change in the sham group (time: $F_{(1,7)} = 1.28$, $p = 0.30$; time × block interaction: $F_{(4,28)} = 2.21$, $p = 0.09$), but a significant decrease in choice of the large risky reward in the lesion group (main effect of time: $F_{(1,6)} = 7.49$, $p = 0.03$; significant time × block interaction: $F_{(4,24)} = 4.07$, $p = 0.01$). Together, these results suggest that lesions of the OFC cause a decrease in risk-taking behavior. Interestingly, given that OFC lesion rats appeared to adapt their choice behavior in response to the changing shock probabilities within a session more rapidly than controls, the data appear to contrast with those from studies showing that OFC damage can impair reversal learning (Dias et al., 1996; McAlonan and Brown, 2003; Schoenbaum et al., 2003b) suggesting that some aspects of reversal learning-like behavior are spared by OFC lesions.

As in Experiment 1, latencies to respond on each lever during forced-choice trials were analyzed before and after surgery. A

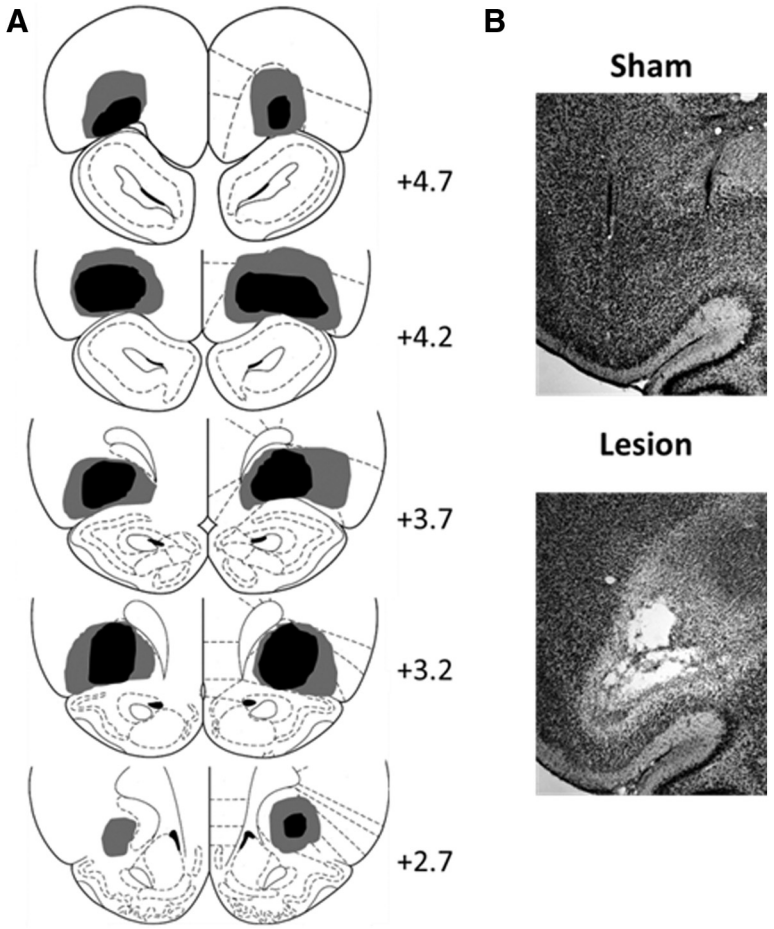

Figure 7. Representative histology from rats that received either neurotoxic or sham lesions of the orbitofrontal cortex. ***A***, The extent of neurotoxic lesions of the orbitofrontal cortex is depicted schematically, with the maximum lesion extent (gray) and minimum lesion extent (black) indicated in both hemispheres. ***B***, Representative thionin-stained coronal images of the orbitofrontal cortex in a lesioned rat and a sham rat are displayed. Images were taken using a 250× objective lens.

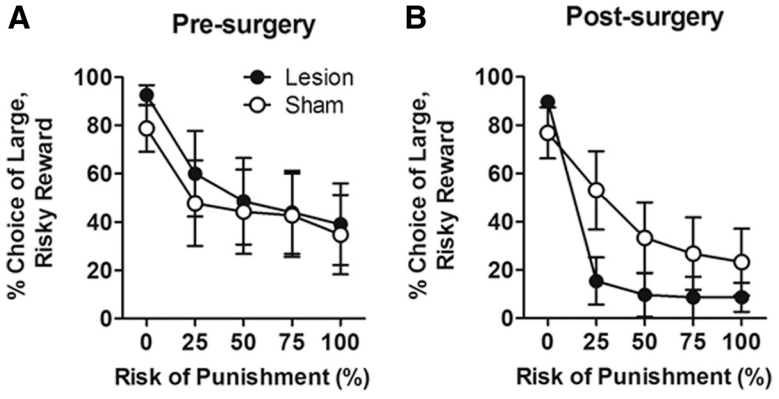

Figure 8. Performance in the risky decision-making task before and after orbitofrontal cortex lesions. ***A***, Before surgery, there was no difference in choice of the large risky reward between groups that would go on to receive lesion or sham surgeries. ***B***, After surgery, the lesion group ($n = 7$) showed a significant decrease in choice of the large risky reward compared with both presurgery baseline and postsurgery sham controls ($n = 8$). Data (mean ± SEM percentage choice of the large risky reward) are averaged over the final five sessions (both before and after surgery), at which point rats displayed stable performance.

repeated-measures ANOVA was used to analyze differences in latencies between groups before and after surgery, with group as the between-subjects factor and size (small vs large lever) and block as the within-subjects factors. An additional repeated-measures ANOVA was conducted to determine whether latencies to press the small and large lever changed after surgery [time (presurgery vs postsurgery) and block as the within-subjects factors and group as the between-subjects factor]. Before surgery, there were no differences between OFC lesion and sham rats in their latencies to respond on the lever associated with either the small safe ($F_{(4,52)} = 0.83$, $p = 0.52$) or large risky reward ($F_{(4,36)} = 0.28$, $p = 0.89$; Fig. 9*A*). All rats took significantly longer to respond on the large risky lever than the small safe lever (size × block:, $F_{(4,36)} = 6.48$, $p < 0.01$). This difference in latencies to choose the large versus small lever did not differ between lesion and sham groups (size × block × group: $F_{(4,36)} = 0.48$, $p = 0.76$). After surgery, however, latencies to press each lever shifted (Fig. 9*B*). Although there were no differences between groups in the latency to press the lever associated with the small reward ($F_{(1,13)} = 0.47$, $p = 0.50$), there were significant group differences in latencies to press the large risky reward lever ($F_{(1,8)} = 6.53$, $p = 0.03$), such that lesioned rats took significantly longer to respond than shams. To determine whether there were differences between presurgery latencies and postsurgery latencies, we conducted a three-factor repeated-measures ANOVA. This analysis revealed that there were no differences in presurgery and postsurgery latencies in sham rats for the small (time: $F_{(1,4)} = 0.13$, $p = 0.73$; time × block: $F_{(4,28)} = 0.39$, $p = 0.82$) or large lever (time: $F_{(1,4)} = 0.88$, $p = 0.40$; time × block: $F_{(4,16)} = 0.94$, $p = 0.47$; Fig. 9*B*). In OFC lesion rats, a trend toward significance emerged when latencies to respond on the small lever (time, $F_{(1,5)} = 0.24$, $p = 0.65$; time × block:, $F_{(4,20)} = 2.54$, $p = 0.07$) or large lever (time: $F_{(1,3)} = 6.80$, $p = 0.08$; time × block interaction: $F_{(4,12)} = 2.64$, $p = 0.09$) were analyzed. Together with the significant group differences in latencies to press the large reward lever after surgery, this indicates that OFC lesions caused rats to deliberate longer when forced to press the large risky lever. This increase in latency to respond on the large risky lever on forced-choice trials in lesioned rats is consistent with the decrease in choice of the large risky reward on free-choice trials. Together, these data demonstrate that lesions of the OFC induce a risk-averse pattern of behavior.

To determine whether the lesion-induced risk aversion was due to changes in shock sensitivity, we examined locomotor activity during both shock delivery and intertrial intervals (Table 2). An independent *t* test revealed no differences between lesion and sham groups in shock reactivity ($t_{(6)} = 0.28$, $p = 0.79$). This suggests that lesions of the OFC did

not affect rats' reactivity to footshock. Using a similar analysis, there were no differences between groups in locomotor activity during intertrial intervals ($t_{(13)} = -0.87$, $p = 0.40$) or in trial omissions ($t_{(13)} = 1.12$, $p = 0.29$).

Lesions of the orbitofrontal cortex do not disrupt reward discrimination

One possible account of the effects of OFC lesions on risk-taking is that they impaired rats' ability to discriminate between rewards of different magnitudes, leaving the presence or absence of shock as the most salient difference between the two choices. To investigate this possibility, the rats were tested in a reward discrimination task, in which they chose between a small food reward (1 pellet) and a large food reward (3 pellets). The parameters of the task were identical to those used in the RDT with the exception that no footshock was delivered. Rats were trained in this task for 10 sessions, at which point their behavior reached stability. An analysis of performance on the first session of testing in the reward discrimination task revealed a significant block × group interaction ($F_{(4,52)} = 3.24$, $p = 0.02$) but no main effect of group, ($F_{(1,13)} = 0.43$, $p = 0.52$), similar to the pattern of postlesion performance on the RDT (Fig. 8*B*). A similar analysis of performance averaged across the first five sessions, however, revealed no differences between the two groups (block × group: $F_{(4,52)} = 0.40$, $p = 0.81$; group: $F_{(1,13)} = 0.03$, $p = 0.87$). The absence of group differences was maintained during the final five sessions (after performance had reached stability; group: $F_{(1,13)} = 0.80$, $p = 0.39$; block × group: $F_{(4,52)} = 0.54$, $p = 0.71$), at which point both groups demonstrated a strong and equivalent preference for the large reward (Fig. 10*A*). These data show that the effects of OFC lesions on the RDT were not likely due to impaired reward discrimination abilities.

Finally, a repeated-measures ANOVA conducted on response latency data during forced-choice trials on this task showed that while there was a main effect of reward magnitude, such that latencies were shorter for the large than the small reward ($F_{(1,13)} = 49.31$, $p < 0.001$), there were no main effects or interactions involving lesion group (group: $F_{(1,13)} < 1.0$, $p = 0.99$; size × group: $F_{(1,13)} = 1.05$, $p = 0.33$; size × block × group: $F_{(4,52)} = 1.14$, $p = 0.35$; data not shown). These data provide further evidence that the OFC lesions did not impair perception of or motivation to obtain the large versus the small rewards.

Lesions of the orbitofrontal cortex do not affect motivation to obtain food reward

The risk-averse pattern of RDT choice performance in OFC lesion rats could be due to a lesion-induced decrease in food motivation. To test this possibility, rats were trained in the series of FR schedules as in Experiment 1. There was neither a main effect of group nor an interaction between FR schedule and group ($F_{(1,13)} = 1.85$, $p = 0.20$; $F_{(4,52)} = 1.33$, $p = 0.27$) across the 5 d of testing on the FR schedules (Fig. 10*B*), suggesting no lesion-induced deficits in food motivation.

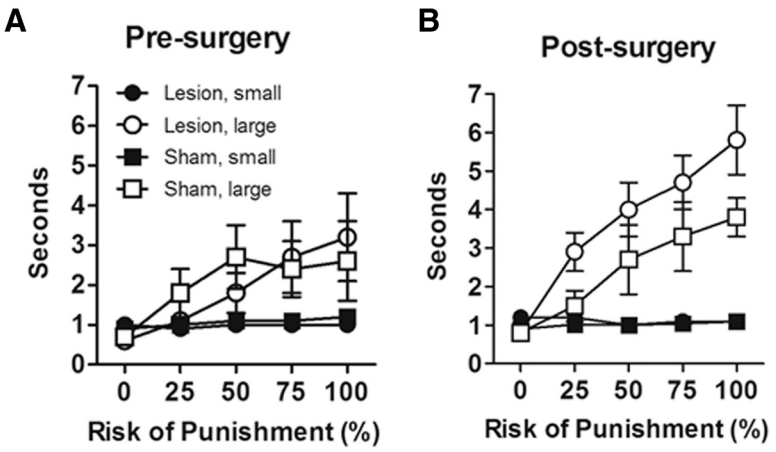

Figure 9. Response latencies in the risky decision-making task before and after orbitofrontal cortex lesions. ***A***, Before surgery, all rats had significantly longer latencies to respond on the lever associated with the large risky reward compared with the lever associated with the small safe reward. ***B***, After surgery, latencies to respond on the small reward lever were comparable between lesion and sham groups. However, the lesion group ($n = 7$) had significantly longer latencies than shams ($n = 8$) to respond on the large reward lever. Data (mean ± SEM seconds) are averaged over the final five sessions (both before and after surgery), at which point rats displayed stable performance.

Table 2. Effects of OFC lesions on trial omissions, locomotor activity, and shock reactivity in the RDT

Session	Omitted trials, %	Locomotion (locomotor units/ITI)	Shock reactivity (locomotor units/shock)
Postsurgery			
Sham	2.05 (0.79)	37.91 (4.94)	3.11 (0.44)
Lesion	3.83 (1.45)	29.95 (8.01)	3.30 (0.44)

There were no significant differences between OFC lesion and sham rats in trial omissions, locomotor activity, or shock reactivity in the RDT after surgery. SEM is shown in parentheses.

Lesions of the orbitofrontal cortex do not alter anxiety-like behavior

Though the reactivity to footshock was not altered, the decrease in risk-taking in OFC lesion rats could be a result of increased anxiety. To test this possibility, rats were tested in an elevated plus maze, and entries into and time spent in the open, closed, and center compartments were measured. Independent *t* tests showed no differences in any of these measures between sham and lesion groups ($p \geq 0.05$; Fig. 11). These data suggest that the lesion-induced risk aversion was not due to a general increase in anxiety.

Discussion

Despite a considerable body of evidence implicating the BLA and OFC in some forms of decision-making (Bechara et al., 1999; Mobini et al., 2002; Ghods-Sharifi et al., 2009; Zeeb and Winstanley, 2011), there is surprisingly little known regarding the roles of these structures in decisions involving risks of explicit punishment. The results of these experiments show that BLA lesions cause an increase in preference for large risky rewards, whereas OFC lesions have the opposite effect. These data demonstrate dissociable roles for the two structures in reward-related decision-making in which there is also the potential for harm.

BLA

The finding that BLA lesions cause an increase in risky choice is consistent with most extant work. For example, patients with amygdala damage make more risky choices than controls in laboratory gambling tasks (Bechara et al., 1999; Brand et al., 2007), and BLA lesions increase risky choice in a rodent gambling task (Zeeb and Winstanley, 2011). However, Ghods-Sharifi et al.

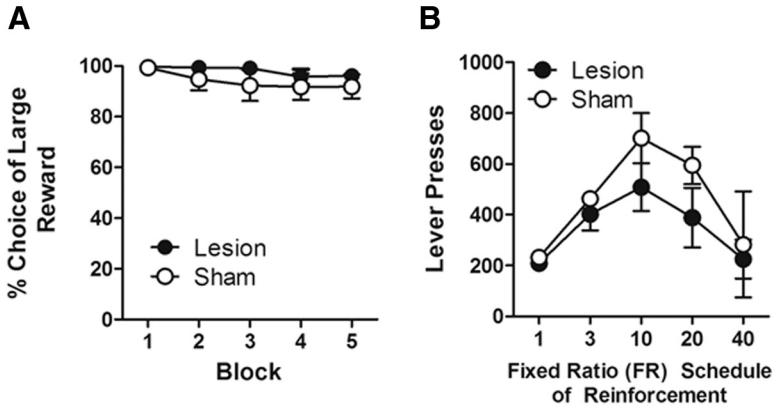

Figure 10. Effects of orbitofrontal cortex lesions on reward discrimination and instrumental responding for food reward. ***A***, When rats were given choices between the large and small rewards in the absence of shock, both groups showed a robust preference for the large reward. There were no differences between the lesion ($n = 7$) and sham ($n = 8$) groups. Data (mean ± SEM percentage choice of the large reward) are averaged across the final five sessions, during which rats displayed stable performance. ***B***, Rats were tested under a series of fixed ratio schedules of lever pressing for food reward (FR1, FR3, FR10, FR20, and FR40), one schedule/d for 5 d. There were no differences between the lesion and sham groups on any of the FR schedules. Data are represented as mean ± SEM.

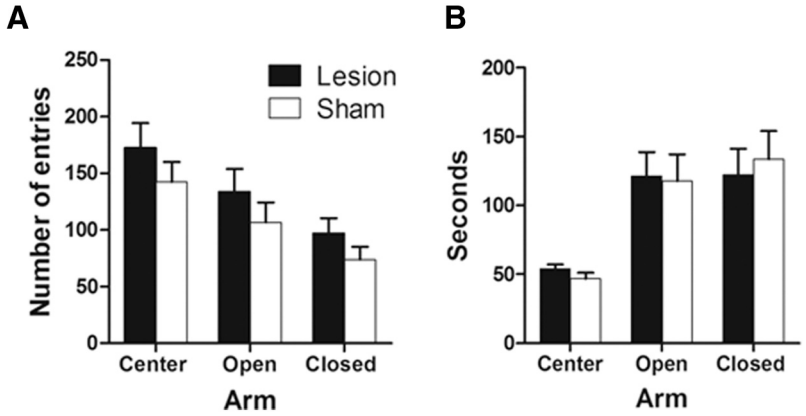

Figure 11. Effects of orbitofrontal cortex lesions on anxiety-like behavior. Rats were tested in the elevated plus maze during a 5 min session. ***A***, There were no significant differences between lesion ($n = 7$) and sham ($n = 8$) groups in the number of entries made into the open, closed or center compartments. ***B***, Similarly, there were no group differences in the amount of time spent in each of these compartments. Data for each measurement are represented as mean ± SEM.

(2009) reported that BLA inactivation decreases risky choice in a probability discounting task. The disparity between effects of BLA manipulations in the RDT and probability discounting task is likely due to differences in the types of risks associated with the large reward. In the latter task, the large reward is associated with risk of reward omission, whereas in the RDT, the large reward is associated with risk of footshock punishment. Furthermore, whereas reward magnitude is an important driver of choice behavior in probability discounting (Green and Myerson, 2004) and rat gambling tasks (van den Bos et al., 2006), the shock intensity is a primary driver of choice behavior in the RDT (Cooper et al., 2014; Shimp et al., 2014). Together, these data suggest that the use of distinct risk factors (punishment vs reward omission) may differentially engage neural mechanisms supporting decision-making.

Regardless of the type of risk, all of these studies, including the current one, indicate that the BLA is critical for making advantageous choices. In the probability discounting task, BLA inactivation induced risk aversion on trials when it was more profitable to choose the risky reward (Ghods-Sharifi et al., 2009). Similarly, BLA lesions resulted in fewer rewards earned in a rodent gambling task (Zeeb and Winstanley, 2011). In the current study, rats with BLA lesions preferred the large reward even at the highest probability of punishment. This inability to make advantageous choices in the RDT may be due to impairments in integrating reward-related information with the risk of explicit punishment associated with each choice. This was particularly evident when reward magnitudes in the RDT were equated. Under these conditions, both BLA lesion and sham groups showed equivalent choice of the safe reward (Fig. 4), indicating that the BLA is not necessary for discrimination or appropriate responding to risk of shock. Instead, its critical role appears to be in integrating this information with reward magnitude to guide choice behavior. This integrative process may occur via convergence of multiple streams of behaviorally relevant information within the BLA. Specifically, reward magnitude, punishment probability, and punishment magnitude, as well the incentive properties of their predictive cues, are conveyed to the BLA from upstream structures, such as the prefrontal cortex (Orsini et al., 2011; St Onge et al., 2012a). These signals may be integrated in the BLA and subsequently generate motor output through monosynaptic connections with the nucleus accumbens (NAc). Thus, the BLA may function as a "hub" that regulates choice behavior as risk of punishment changes.

Somewhat surprisingly, upon systemic administration of amphetamine, both BLA lesion and sham rats decreased their choice of the large risky reward. This suggests that amphetamine can rescue the decision-making impairment in BLA lesion rats, by increasing dopamine availability. Dopamine signaling in the NAc has been shown to mediate risky decision-making (Day et al., 2011; Simon et al., 2011; St Onge et al., 2012b) and BLA afferents can directly stimulate DA release in the NAc as well as in the prefrontal cortex (Floresco et al., 1998; Jackson and Moghaddam, 2001; Jones et al., 2010). Hence, the lesion-induced increase in risk-taking in the current study could be due in part to attenuated NAc dopamine release, which was reversed by amphetamine. This hypothesis is consistent with data showing that microinjection of a D2 dopamine receptor agonist into NAc reduces risk-taking in the RDT (Mitchell et al., 2014).

OFC

The finding that OFC lesions decreased risk-taking even with a small (25%) risk of punishment was surprising, as it runs counter to much preclinical and clinical data on this subject. For example, patients with OFC damage make riskier choices in laboratory gambling tasks (Rogers et al., 1999; Bechara et al., 2001), and OFC lesions increase risky choice in rodent gambling and probability discounting tasks (Pais-Vieira et al., 2007; Stopper et al., 2014). One other study has shown that pretraining OFC lesions (in contrast to post-training lesions used in the current study) decrease

preference for large uncertain rewards (Mobini et al., 2002). There are several possible explanations for these discrepancies. First, as discussed above, the risk involved in the previous studies has been that of reward omission (or loss of points in a token economy in human studies), whereas in the current study, the risk is that of explicit punishment. Thus, the OFC may be differentially engaged depending on the valence of the risky outcome. Second, lesions in the current study predominantly encompassed the lateral OFC (lOFC), leaving the medial OFC (mOFC) largely intact. Previous work shows that the lOFC and mOFC have dissociable roles in decision-making and in their responses to rewards and punishments (O'Doherty et al., 2001). Thus, different effects on risk-taking might have emerged had lesions in the current study been localized to the mOFC. Indeed, others have shown that mOFC (Stopper et al., 2014) but not lOFC (St Onge and Floresco, 2010) inactivation increases risk-taking in a probability discounting task (Mar et al., 2011).

A parsimonious account of the distinct effects of BLA and OFC lesions on RDT performance is that the BLA encodes the task costs (risks of punishment) and the OFC encodes the benefits (differential reward magnitudes); however, there is ample evidence that both structures process both rewarding and aversive information (Maren, 1999; Schoenbaum et al., 1999; Belova et al., 2007; Morrison and Salzman, 2011). An alternative interpretation is that OFC lesions rendered performance dependent upon "model-free" cognitive mechanisms. In intact subjects, the OFC is thought to facilitate "model-based" behavior, whereby potential outcomes of actions (i.e., rewards and punishment) are internally simulated ("modeled") so as to guide future behavior (Lucantonio et al., 2012; McDannald et al., 2012). When behavior is executed, the expected outcomes of an action are compared with the actual outcomes and the model is updated accordingly, allowing for adaptive decision-making. Previous work has shown that lesions or inactivation of the OFC impair tasks that require model-based information processing, resulting in inflexible ("model-free") behavior (Schoenbaum et al., 2003b; Pickens et al., 2005; McDannald et al., 2011, 2012, 2014). Importantly, this model-free system relies solely on immediate past reinforcement history to guide behavior. Within this framework, RDT performance in intact rats could be governed by a model-based system whereby rats rely on an internally generated model of the task and are able to calculate punishment probabilities to guide choice behavior. However, OFC lesion rats would be unable to rely on model-based representations to make appropriate choices, and thus their actions would be based on their immediate reinforcement history and the salience of each option. Such model-free cognition might preclude accurate calculation of the punishment probabilities associated with the large reward. This could account for the observation that as soon as the shock was introduced in the second block of trials, lesioned rats chose the small reward despite the low probability of punishment.

Interactions between BLA and OFC

The current study does not address how communication between the OFC and BLA contributes to risk-taking; however, others have shown that interactions between these structures play a critical role in choice behavior. For instance, Schoenbaum et al. (2003a) showed that BLA damage results in impaired encoding of outcome values in the OFC. Similarly, OFC lesions decrease BLA neural encoding of cue–outcome associations (Saddoris et al., 2005). Thus, bidirectional communication between these structures is required for normal encoding of task contingencies to guide behavior. By this view, it is possible that the effects of BLA and OFC lesions on risk-taking are partially due to the loss of critical input from the other structure. Future experiments will test the importance of this interaction in decision-making under risk of punishment.

This study represents the first evidence to date that damage to BLA and OFC has distinct and reciprocal effects on risk-taking. Importantly, these effects were observed in a task that mimics many forms of real-life risk-taking in that it involves weighing the risk of explicit punishment against net gain. Because altered risk-taking is a hallmark of several psychiatric disorders (Klein et al., 2012; Gowin et al., 2013; Kaye et al., 2013), a better understanding of how these structures contribute to normal risk-taking may ultimately offer insight into treatment strategies for those suffering from these disorders.

References

Anselme P (2012) Loss in risk-taking: absence of optimal gain or reduction in one's own resources? Behav Brain Res 229:443–446. CrossRef Medline

Bechara A, Damasio H, Damasio AR, Lee GP (1999) Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. J Neurosci 19:5473–5481. Medline

Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE (2001) Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 39:376–389. CrossRef Medline

Belova MA, Paton JJ, Morrison SE, Salzman CD (2007) Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. Neuron 55:970–984. CrossRef Medline

Blanchard DC, Blanchard RJ (1972) Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol 81:281–290. CrossRef Medline

Brand M, Grabenhorst F, Starcke K, Vandekerckhove MM, Markowitsch HJ (2007) Role of the amygdala in decisions under ambiguity and decisions under risk: evidence from patients with Urbach-Wiethe disease. Neuropsychologia 45:1305–1317. CrossRef Medline

Cardinal RN, Howes NJ (2005) Effects of lesions of the nucleus accumbens core on choice between small certain rewards and large uncertain rewards in rats. BMC Neurosci 6:37. CrossRef Medline

Cooper SE, Goings SP, Kim JY, Wood RI (2014) Testosterone enhances risk tolerance without altering motor impulsivity in male rats. Psychoneuroendocrinology 40:201–212. CrossRef Medline

Day JJ, Jones JL, Carelli RM (2011) Nucleus accumbens neurons encode predicted and ongoing reward costs in rats. Eur J Neurosci 33:308–321. CrossRef Medline

Dias R, Robbins TW, Roberts AC (1996) Dissociation in prefrontal cortex of affective and attentional shifts. Nature 380:69–72. CrossRef Medline

Floresco SB, Yang CR, Phillips AG, Blaha CD (1998) Basolateral amygdala stimulation evokes glutamate receptor-dependent dopamine efflux in the nucleus accumbens of the anaesthetized rat. Eur J Neurosci 10:1241–1251. CrossRef Medline

Floresco SB, St Onge JR, Ghods-Sharifi S, Winstanley CA (2008) Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. Cogn Affect Behav Neurosci 8:375–389. CrossRef Medline

Ghods-Sharifi S, St Onge JR, Floresco SB (2009) Fundamental contribution by the basolateral amygdala to different forms of decision making. J Neurosci 29:5251–5259. CrossRef Medline

Giertler C, Bohn I, Hauber W (2003) The rat nucleus accumbens is involved in guiding of instrumental responses by stimuli predicting reward magnitude. Eur J Neurosci 18:1993–1996. CrossRef Medline

Gilbert RJ, Mitchell MR, Simon NW, Bañuelos C, Setlow B, Bizon JL (2012) Risk, reward, and decision-making in a rodent model of cognitive aging. Front Neurosci 5:144. CrossRef Medline

Gowin JL, Mackey S, Paulus MP (2013) Altered risk-related processing in substance users: imbalance of pain and gain. Drug Alcohol Depend 132:13–21. CrossRef Medline

Green L, Myerson J (2004) A discounting framework for choice with delayed and probabilistic rewards. Psychol Bull 130:769–792. CrossRef Medline

Jackson ME, Moghaddam B (2001) Amygdala regulation of nucleus accumbens dopamine output is governed by the prefrontal cortex. J Neurosci 21:676–681. Medline

Jentsch JD, Woods JA, Groman SM, Seu E (2010) Behavioral characteristics

and neural mechanisms mediating performance in a rodent version of the Balloon Analog Risk Task. Neuropsychopharmacology 35:1797–1806. CrossRef Medline

Jones JL, Day JJ, Aragona BJ, Wheeler RA, Wightman RM, Carelli RM (2010) Basolateral amygdala modulates terminal dopamine release in the nucleus accumbens and conditioned responding. Biol Psychiatry 67:737–744. CrossRef Medline

Kaye WH, Wierenga CE, Bailer UF, Simmons AN, Bischoff-Grethe A (2013) Nothing tastes as good as skinny feels: the neurobiology of anorexia nervosa. Trends Neurosci 36:110–120. CrossRef Medline

Klein RG, Mannuzza S, Olazagasti MA, Roizen E, Hutchison JA, Lashua EC, Castellanos FX (2012) Clinical and functional outcome of childhood attention-deficit/hyperactivity disorder 33 years later. Arch Gen Psychiatry 69:1295–1303. CrossRef Medline

Lucantonio F, Stalnaker TA, Shaham Y, Niv Y, Schoenbaum G (2012) The impact of orbitofrontal dysfunction on cocaine addiction. Nat Neurosci 15:358–366. CrossRef Medline

Mar AC, Walker AL, Theobald DE, Eagle DM, Robbins TW (2011) Dissociable effects of lesions to orbitofrontal cortex subregions on impulsive choice in the rat. J Neurosci 31:6398–6404. CrossRef Medline

Maren S (1999) Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. Trends Neurosci 22:561–567. CrossRef Medline

McAlonan K, Brown VJ (2003) Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behav Brain Res 146:97–103. CrossRef Medline

McDannald MA, Lucantonio F, Burke KA, Niv Y, Schoenbaum G (2011) Ventral striatum and orbitofrontal cortex are both required for model-based, but not model-free, reinforcement learning. J Neurosci 31:2700–2705. CrossRef Medline

McDannald MA, Takahashi YK, Lopatina N, Pietras BW, Jones JL, Schoenbaum G (2012) Model-based learning and the contribution of the orbitofrontal cortex to the model-free world. Eur J Neurosci 35:991–996. CrossRef Medline

McDannald MA, Jones JL, Takahashi YK, Schoenbaum G (2014) Learning theory: a driving force in understanding orbitofrontal function. Neurobiol Learn Mem 108:22–27. CrossRef Medline

Mendez IA, Williams MT, Bhavsar A, Lu AP, Bizon JL, Setlow B (2009) Long-lasting sensitization of reward-directed behavior by amphetamine. Behav Brain Res 201:74–79. CrossRef Medline

Mendez IA, Simon NW, Hart N, Mitchell MR, Nation JR, Wellman PJ, Setlow B (2010) Self-administered cocaine causes long-lasting increases in impulsive choice in a delay discounting task. Behav Neurosci 124:470–477. CrossRef Medline

Mitchell MR, Vokes CM, Blankenship AL, Simon NW, Setlow B (2011) Effects of acute administration of nicotine, amphetamine, diazepam, morphine, and ethanol on risky decision-making in rats. Psychopharmacology 218:703–712. CrossRef Medline

Mitchell MR, Weiss VG, Beas BS, Morgan D, Bizon JL, Setlow B (2014) Adolescent risk taking, cocaine self-administration, and striatal dopamine signaling. Neuropsychopharmacology 39:955–962. CrossRef Medline

Mobini S, Body S, Ho MY, Bradshaw CM, Szabadi E, Deakin JF, Anderson IM (2002) Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology 160:290–298. CrossRef Medline

Morrison SE, Salzman CD (2011) Representations of appetitive and aversive information in the primate orbitofrontal cortex. Ann N Y Acad Sci 1239:59–70. CrossRef Medline

O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C (2001) Abstract reward and punishment representations in the human orbitofrontal cortex. Nat Neurosci 4:95–102. CrossRef Medline

Orsini CA, Maren S (2012) Neural and cellular mechanisms of fear and extinction memory formation. Neurosci Biobehav Rev 36:1773–1802. CrossRef Medline

Orsini CA, Kim JH, Knapska E, Maren S (2011) Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. J Neurosci 31:17269–17277. CrossRef Medline

Pais-Vieira M, Lima D, Galhardo V (2007) Orbitofrontal cortex lesions disrupt risk assessment in a novel serial decision-making task for rats. Neuroscience 145:225–231. CrossRef Medline

Pape HC, Pare D (2010) Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev 90:419–463. CrossRef Medline

Parent MB, Quirarte GL, Cahill L, McGaugh JL (1995) Spared retention of inhibitory avoidance learning after post-training amygdala lesions. Behav Neurosci 109:803–807. CrossRef Medline

Pickens CL, Saddoris MP, Setlow B, Gallagher M, Holland PC, Schoenbaum G (2003) Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. J Neurosci 23:11078–11084. Medline

Pickens CL, Saddoris MP, Gallagher M, Holland PC (2005) Orbitofrontal lesions impair use of cue-outcome associations in a devaluation task. Behav Neurosci 119:317–322. CrossRef Medline

Reddy LF, Lee J, Davis MC, Altshuler L, Glahn DC, Miklowitz DJ, Green MF (2014) Impulsivity and risk taking in bipolar disorder and schizophrenia. Neuropsychopharmacology 39:456–463. CrossRef Medline

Rogers RD, Everitt BJ, Baldacchino A, Blackshaw AJ, Swainson R, Wynne K, Baker NB, Hunter J, Carthy T, Booker E, London M, Deakin JF, Sahakian BJ, Robbins TW (1999) Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. Neuropsychopharmacology 20:322–339. CrossRef Medline

Saddoris MP, Gallagher M, Schoenbaum G (2005) Rapid associative encoding in basolateral amygdala depends on connections with orbitofrontal cortex. Neuron 46:321–331. CrossRef Medline

Schoenbaum G, Chiba AA, Gallagher M (1999) Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. J Neurosci 19:1876–1884. Medline

Schoenbaum G, Setlow B, Saddoris MP, Gallagher M (2003a) Encoding predicted outcome and acquired value in orbitofrontal cortex during cue sampling depends upon input from basolateral amygdala. Neuron 39:855–867. CrossRef Medline

Schoenbaum G, Setlow B, Nugent SL, Saddoris MP, Gallagher M (2003b) Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals. Learn Mem 10:129–140. CrossRef Medline

Shimp KG, Mitchell MR, Beas BS, Bizon JL, Setlow B (2014) Affective and cognitive mechanisms of risky decision making. Neurobiol Learn Memory. Advance online publication. Retrieved July 1, 2014. CrossRef Medline

Simon NW, Setlow B (2012) Modeling risky decision making in rodents. Methods Mol Biol 829:165–175. CrossRef Medline

Simon NW, Gilbert RJ, Mayse JD, Bizon JL, Setlow B (2009) Balancing risk and reward: a rat model of risky decision making. Neuropsychopharmacology 34:2208–2217. CrossRef Medline

Simon NW, Montgomery KS, Beas BS, Mitchell MR, LaSarge CL, Mendez IA, Bañuelos C, Vokes CM, Taylor AB, Haberman RP, Bizon JL, Setlow B (2011) Dopaminergic modulation of risky decision-making. J Neurosci 31:17460–17470. CrossRef Medline

St Onge JR, Floresco SB (2009) Dopaminergic modulation of risk-based decision making. Neuropsychopharmacology 34:681–697. CrossRef Medline

St Onge JR, Floresco SB (2010) Prefrontal cortical contribution to risk-based decision making. Cereb Cortex 20:1816–1828. CrossRef Medline

St Onge JR, Stopper CM, Zahm DS, Floresco SB (2012a) Separate prefrontal-subcortical circuits mediate different components of risk-based decision making. J Neurosci 32:2886–2899. CrossRef Medline

St Onge JR, Ahn S, Phillips AG, Floresco SB (2012b) Dynamic fluctuations in dopamine efflux in the prefrontal cortex and nucleus accumbens during risk-based decision making. J Neurosci 32:16880–16891. CrossRef Medline

Stopper CM, Green EB, Floresco SB (2014) Selective involvement by the medial orbitofrontal cortex in biasing risky, but not impulsive, choice. Cereb Cortex 24:154–162. CrossRef Medline

van den Bos R, Houx BB, Spruijt BM (2006) The effect of reward magnitude differences on choosing disadvantageous decks in the Iowa gambling task. Biol Psychol 71:155–161. CrossRef Medline

Vazdarjanova A, McGaugh JL (1998) Basolateral amygdala is not critical for cognitive memory of contextual fear conditioning. Proc Natl Acad Sci U S A 95:15003–15007. CrossRef Medline

Winstanley CA, Theobald DE, Cardinal RN, Robbins TW (2004) Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. J Neurosci 24:4718–4722. CrossRef Medline

Zeeb FD, Winstanley CA (2011) Lesions of the basolateral amygdala and orbitofrontal cortex differentially affect acquisition and performance of a rodent gambling task. J Neurosci 31:2197–2204. CrossRef Medline