bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1 **Abbreviated title:** Optogenetic BLA inhibition and risky decision making

2

3

4

5

6 Optogenetic inhibition reveals distinct roles for basolateral amygdala activity at 7 discrete timepoints during risky decision making

8

9 Caitlin A. Orsini[1], Caesar M. Hernandez[2], Sarthak Singhal[3], Kyle B. Kelly[3], Charles J. Frazier[3],
10 Jennifer L. Bizon[1,2,4], Barry Setlow[1,2,4,5*]

11

12 [1]Department of Psychiatry; [2]Department of Neuroscience, [3]Department of Pharmacodynamics,
13 [4]Center for Addiction Research Education, [5]Department of Psychology, University of Florida,
14 Gainesville, FL, 32610
15

16 *Corresponding author. Correspondence should be addressed to Dr. Caitlin Orsini or Dr. Barry
17 Setlow, Department of Psychiatry, University of Florida College of Medicine, P.O. Box 100256,
18 Gainesville, FL 32610-0256; email: orsini@ufl.edu; setlow@ufl.edu; Tel: 352-294-5203.

19
20 Number of figures: 7
21 Number of tables: 1
22 Number of pages: 35
23 Number of words for abstract: 237
24 Number of words for Significance Statement: 119
25 Number of words for Introduction: 661
26 Number of words for Discussion: 1,547
27

28 **Acknowledgements:** We thank Mr. Matthew Bruner and Mrs. Shannon Wall for their technical
29 assistance. We also wish to acknowledge the McKnight Brain Research Foundation for their
30 support of this project. The work was further supported by a Thomas H. Maren Fellowship and
31 K99DA041493 (CAO), a McKnight Predoctoral Fellowship and the Pat Tillman Foundation (CMH),
32 and R01DA036534 (BS).
33

34 **Conflicts of interest:** The authors declare no competing financial interests.

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

35 **Abstract**

36 Decision making is a multifaceted process, consisting of several distinct phases that likely
37 require different cognitive operations. Previous work showed that the basolateral amygdala (BLA)
38 is a critical substrate for decision making involving risk of punishment; however, it is unclear how
39 the BLA is recruited at different stages of the decision process. To this end, the current study
40 used optogenetics to inhibit the BLA during specific task phases in a model of risky decision
41 making (Risky Decision-making Task; RDT) in which rats choose between a small, "safe" reward
42 and a large reward accompanied by varying probabilities of footshock punishment. Rats received
43 intra-BLA microinjections of viral vectors carrying either halorhodopsin (eNpHR3.0-mCherry) or
44 mCherry alone (control) followed by optic fiber implants and were trained in the RDT. Laser
45 stimulation during the task occurred during either intertrial interval, deliberation, or reward
46 outcome phases, the latter of which was further divided into the three possible outcomes (small,
47 safe; large, unpunished; large, punished). Inhibition of the BLA selectively during the deliberation
48 phase decreased choice of the large, risky outcome (decreased risky choice). In contrast, BLA
49 inhibition selectively during delivery of the large, punished outcome increased risky choice.
50 Inhibition had no effect during the other phases, nor did it affect performance in control rats.
51 Collectively, these data indicate that the BLA can either inhibit or promote choice of risky options,
52 depending on the phase of the decision process in which it is active.

53

54

55

56

57

58

59

60

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

61 **Significance Statement**

62 To date, most behavioral neuroscience research on neural mechanisms of decision
63 making has employed techniques that preclude assessment of distinct phases of the decision
64 process. Here we show that optogenetic inhibition of the basolateral amygdala (BLA) has opposite
65 effects on choice behavior in a rat model of risky decision making depending on the phase in
66 which inhibition occurs. BLA inhibition during a period of deliberation between small, safe and
67 large, risky outcomes decreased risky choice. In contrast, BLA inhibition during receipt of the
68 large, punished outcome increased risky choice. These findings highlight the importance of
69 temporally targeted approaches to understand neural substrates underlying complex cognitive
70 processes. More importantly, they reveal novel information about dynamic BLA modulation of
71 risky choice.

72
73
74
75
76
77
78
79
80
81
82
83
84
85
86

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

87 **Introduction**

88 The ability to make adaptive choices requires multiple cognitive operations that work in
89 concert to guide efficient and optimal behavior (Rangel et al., 2008). For example, an organism
90 must calculate the objective and subjective value of the available options, which entails evaluating
91 the relative value of both the benefits and costs associated with each option. This information
92 must be acquired from past experience, such as the contingencies of previous actions and their
93 outcomes, as well as other motivational (e.g., hunger) and environmental (e.g. presence of salient
94 predictive cues) factors. Finally, the organism must determine the value of the actual outcome of
95 its choice, and use this information as feedback to guide future choices. Together, these
96 processes allow an organism to execute or inhibit its choice behavior as appropriate to its past,
97 current, and anticipated future conditions. While the majority of individuals are able to effectively
98 engage these processes and make adaptive decisions, individuals with psychiatric diseases such
99 as substance use disorder, anorexia nervosa, and post-traumatic stress disorder exhibit impaired
100 decision making (Bechara and Damasio, 2002; Crowley et al., 2010; Najavits et al., 2011;
101 Schneider et al., 2012; Kaye et al., 2013; Gonzalez et al., 2015; Dekkers et al., 2016), often
102 resulting in maladaptive choices. The specific components of the decision making process that
103 are perturbed in these pathological conditions, however, are unclear.

104 Decision making is mediated by interconnected brain structures within the
105 mesocorticolimbic circuit (Orsini et al., 2015b). One such structure within this circuit that has
106 received considerable attention in cost/benefit decision making is the basolateral amygdala (BLA)
107 (Winstanley and Floresco, 2016). Using well-validated rodent models of risk-based decision
108 making, previous work has shown that lesions or pharmacological inactivation of the BLA result
109 in disadvantageous choices (Winstanley et al., 2004; Ghods-Sharifi et al., 2009; Zeeb and
110 Winstanley, 2011; Hosking et al., 2014; Tremblay et al., 2014). This is consistent with
111 neuroimaging data showing that the amygdala is activated during assessment of risky choices
112 (De Martino et al., 2006; Roiser et al., 2009) and is hypoactive in individuals with impaired risky

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

113 decision making (Crowley et al., 2010; Gowin et al., 2013). More recently, the BLA has been
114 implicated in decision making involving risk of explicit punishment (Orsini et al., 2015a). In well-
115 trained rats, BLA lesions increased choice of a large reward associated with risk of footshock
116 punishment. These data suggested that the BLA is critical for the integration of reward- and
117 punishment-related information to guide optimal behavior. Importantly, however, it is unclear how
118 and at what point in the decision-making process this integration occurs.

119 *In vivo* electrophysiological studies show that BLA neurons do not respond uniformly to
120 salient stimuli but instead mediate different aspects of motivated behavior. For example, different
121 populations of BLA neurons respond differentially to rewarding and aversive outcomes
122 (Schoenbaum et al., 1998, 1999; Paton et al., 2006; Belova et al., 2007; Belova et al., 2008;
123 Shabel and Janak, 2009; Sangha et al., 2013; Gore et al., 2015), and are organized into
124 intrinsically (Zhang et al., 2013) and extrinsically distinct circuits (Namburi et al., 2015; Beyeler et
125 al., 2016). In addition, amygdala neurons differentially contribute to generation of prospective
126 plans to obtain immediate rewards (Grabenhorst et al., 2012) as well as rewards in the distant
127 future (Hernadi et al., 2015). This functional heterogeneity within the BLA supports the hypothesis
128 that the BLA is differentially engaged during decision making involving rewarding and aversive
129 outcomes. How the BLA is recruited, however, may depend on the specific cognitive components
130 of the decision-making process. In other words, how the BLA contributes to the deliberative
131 process of decision making may be distinct from how it contributes to processing the outcomes
132 of past choices.

133 The advent of optogenetics affords the ability to test this hypothesis by examining BLA
134 involvement in decision making during distinct components of the decision process. Hence, the
135 experiments herein examined the effects of BLA inhibition during the deliberation and outcome
136 phases of a risky decision making task involving risk of explicit punishment.

137

138 **Materials and Methods:**

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

139 ***Subjects***

140 Male Long-Evans rats (weighing 250-275 g upon arrival; Charles River Laboratories,
141 Raleigh, NC) were individually housed and kept on a 12 h light/dark cycle with free access to food
142 and water except as indicated below. Upon arrival, rats were handled daily for one week prior to
143 undergoing surgery. During behavioral testing, rats were maintained at 90% of their free-feeding
144 weight, with their target weights adjusted upward by 5 g/week to account for growth. Animal
145 procedures were conducted in accordance with the University of Florida Institutional Animal Care
146 and Use Committee and followed guidelines of the National Institutes of Health.

147 ***Apparatus***

148 Behavioral testing was conducted in three computer-controlled operant test chambers
149 (Coulbourn Instruments), each of which was contained in a sound-attenuating cabinet. Chambers
150 were equipped with a centrally located food trough (TAMIC Instruments) that projected 3 cm into
151 the chamber and contained a photobeam to detect trough entries. The trough was connected to
152 a feeder, from which 45 mg food pellets (Test Diet, AIN-76A, 5TUL) were delivered into the trough.
153 A nosepoke hole was located above the food trough and two retractable levers were positioned
154 to the left and right of the trough, 11 cm above the floor of the chamber. A 1.12 W lamp was
155 positioned on the back wall of the sound-attenuating cabinet, and served as a houselight. The
156 floor of the test chamber was comprised of stainless steel rods connected to a shock generator
157 that delivered scrambled footshocks. Each operant test chamber was interfaced with a computer
158 running Graphic State 4.0 software (Coulbourn Instruments), which controlled chamber hardware
159 (e.g., lever insertion, nosepoke illumination, food pellet delivery) and recorded task events.

160 ***Laser stimulation***

161 During behavioral test sessions, laser light (560 nm, 8-10 mW output, Shanghai Laser &
162 Optics Century Co., Ltd.) was delivered bilaterally into the BLA in rats expressing halorhodopsin

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

163 (eNpHR3.0 group) or mCherry (control group) in the BLA. To reach the brain, light was passed
164 from the laser through a patch cord (200 µm core, Thor Labs), a rotary joint (1 X 2, 200 µm core,
165 Doric Lenses) located above the operant chamber, 2 additional patch cords (200 µm core, 0.22
166 NA, Thor Labs) and bilateral optic fibers (200 µm core, 0.22 NA, 8.3 mm in length; Precision Fiber
167 Products) implanted in the BLA. The laser was interfaced with the computer running Graphic State
168 4.0 software to allow for precise timing of light delivery during different task phases.

169 ***Surgical procedures***

170 Rats were anesthetized with isoflurane gas (1-5% in O_2) and received subcutaneous
171 injections of meloxicam (2 mg/kg), buprenorphine (0.05 mg/kg), and sterile saline (10 mL). Rats
172 were placed into a stereotaxic apparatus (David Kopf) and the scalp was cleaned with a
173 chlorohexidine/isopropyl alcohol swab. A sterile adhesive surgical drape was subsequently
174 placed over the body.

175 For rats used in *in vitro* electrophysiology experiments, the scalp was incised and retracted
176 and the skull was leveled to ensure that bregma and lambda were in the same horizontal plane.
177 Two burr holes were drilled for bilateral virus injections into the BLA (AP: -3.2, ML: ±4.9, DV: -8.5,
178 -8.1 mm from skull surface). At each site, an injection needle was lowered to the target depth and
179 AAV5-CAMKIIα-eHpNR3.0-mCherry (University of North Carolina Vector Core) was infused into
180 the BLA (0.4 µl at the ventral DV coordinate and 0.2 µl at the dorsal DV coordinate, at a rate of
181 0.5 µl/min). The injection needle was attached to polyethylene tubing, which was connected to a
182 10 µl Hamilton syringe mounted on a syringe pump (Harvard Apparatus). After each injection, the
183 needle was left in place for an additional 5 minutes to allow for diffusion of the virus. The incision
184 was then sutured and rats were given an additional 10 mL of saline before being placed on a
185 heating pad to recover from surgery.

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

186 For rats used in behavioral experiments, the scalp was incised and retracted and six small
187 burr holes were drilled into the skull for placement of jeweler's screws. Two screws were placed
188 anterior to bregma, two between bregma and lambda and two posterior to lambda. This
189 configuration was used to ensure that the headcap was secured evenly across the skull surface.
190 After leveling the skull to ensure that bregma and lambda were in the same horizontal plane, two
191 additional burr holes were drilled for bilateral implantation of guide cannulae (22 gauge; Plastics
192 One) above the BLA (AP: -3.3, ML: ±4.9, DV: -7.3 from skull surface). Dental cement was used
193 to anchor the cannulae in place. Once the dental cement was set, an injection needle was lowered
194 into each cannula (the tip of the injection needle extended 1.5 mm beyond the end of the cannula)
195 and AAV5-CAMKIIα-eHpNR3.0-mCherry or AAV5-CAMKIIα-mCherry (University of North
196 Carolina Vector Core) was infused into the BLA (0.6 μl at a rate of 0.5 μl/min). A sterile stylet was
197 inserted into each cannula at the completion of the injections. Rats were given an additional 10
198 mL of saline and were placed on a heating pad to recover from surgery. Rats were allowed to
199 recover for one week before being food restricted in preparation for behavioral testing.

200 ***In vitro electrophysiology***

201 Rats (n = 4) were anesthetized with an intraperitoneal injection of a 75-100 mg/kg
202 ketamine and 5-10 mg/kg xylazine solution and were decapitated using a small animal guillotine.
203 Their brains were rapidly extracted and coronal sections containing the BLA (300 μm thick) were
204 obtained using a Leica VT 1000s vibratome while submerged in ice cold sucrose laden
205 oxygenated artificial cerebrospinal fluid (aCSF) containing in (mM): 2 KCl, 1.25 NaH_2PO_4, 1
206 $MgSO_4$, 10 D-glucose, 1 $CaCl_2$, 206 sucrose, 25 $NaHCO_3$. Slices were then incubated for 30
207 minutes at 37˚C in aCSF which contained in (mM): 124 NaCl, 2.5 KCl, 1.23 NaH_2PO_4, 3 $MgSO_4$,
208 10 D-glucose, 1 $CaCl_2$, and 25 $NaHCO_3$. Following this incubation period slices were allowed to
209 equilibrate to room temperature for a minimum of 30 minutes prior to being used for experiments.
210 All solutions were saturated with 95 % O_2/ 5 % CO_2 to maintain a pH of 7.3. For whole cell patch

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

211 clamp recordings, slices were transferred to a slice chamber where they were continuously
212 perfused at a rate of 1.5-2ml/min with an aCSF bath solution that contained (in mM): 126 NaCl, 3
213 KCl, 1.2 NaH_2PO_4, 1.5 $MgSO_4$, 11 D-glucose, 2.4 $CaCl_2$ and 25 $NaHCO_3$. This solution was also
214 saturated with 95 % O_2/ 5 % CO_2 to maintain a pH of 7.3 and bath temperature was maintained
215 at 30-32˚C. Slices were visualized using infrared differential interference contrast (IR-DIC)
216 microscopy with an Olympus BX51WI upright stereomicroscope, a 12-bit IRC CCD camera
217 (QICAM Fast 1394, QImaging), and a 40x water immersion lens. Patch pipettes were prepared
218 with a Flaming/Brown type pipette puller (Sutter Instrument, P-97) from 1.5 mm/0.8 mm
219 borosilicate glass capillaries (Sutter Instrument) and pulled to a tip resistance of 4-7 MΩ. Whole
220 cell patch clamp recordings were performed using an Axon Mutliclamp 700B amplifier (Molecular
221 Devices, Sunnyvale, CA) and data were collected at 20 kHz, filtered at 2 KHz and recorded with
222 a Digidata 1322A using Clampex v. 9 or 10 (Molecular Devices, Sunnyvale, CA). BLA neurons
223 expressing mCherry were identified using an epifluoresence microscopy XF102-2 filter set
224 (Omega Optical, excitation: 540-580 nm, emission: 615-695 nm). The light source for
225 epifluoresence microscopy was an X-Cite Series 120Q (Lumen Dynamics). Whole cell patch
226 clamping was initiated under IR-DIC using a potassium-based internal solution that contained (in
227 mM): 130 K-gluconate, 10 KCl, 5 NaCl, 2 $MgCl_2$, 0.1 EGTA, 2 Na_2-ATP, 0.3 NaGTP, 10 HEPES
228 and 10 phosphocreatine, pH adjusted to 7.3 using KOH and volume adjusted to 285–300mOsm.
229 Halorhodopsin was activated using 1000 msec light pulses, delivered through the excitation filter
230 in the XF102-2 filter set. Experiments were performed in voltage clamp (at -70 mV), in current
231 clamp (at I=0), or in current clamp during 100-200 pA current injection that was sufficient to drive
232 action potentials. Data were analyzed using custom software written in OriginC (OriginLab,
233 Northampton, MA) by CJF.

234 ***Behavioral procedures***

235 *Risky Decision-Making Task*

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

236 Rats were initially shaped to perform the various components of the decision-making task
237 (e.g., lever pressing; nosepoking to initiate a trial) as described previously (Orsini et al., 2015a).
238 They then began training in the Risky Decision-Making task (RDT), which was comprised of three
239 28-trial blocks and lasted 56 min in duration [this task design was a modification of a similar design
240 used in our laboratory (Simon et al., 2009; Orsini et al., 2015a)]. Each 40 s trial (Figure 1A) began
241 with illumination of the nosepoke and houselight. Upon nosepoking, the nosepoke light was
242 extinguished and either a single lever (forced choice trials) or both levers (free choice trials)
243 extended into the chamber. If rats failed to nosepoke within 10 s, the trial was considered an
244 omission. A press on one lever (left or right; counterbalanced across rats) always yielded a small,
245 "safe" food reward (one food pellet) and a press on the other lever always yielded a large, "risky"
246 food reward (2 food pellets). Delivery of the large reward was accompanied by a variable
247 probability of punishment in the form of a mild footshock (0.25-0.6 mA). The probability of
248 punishment was contingent on a preset probability specific to each block of trials: the probability
249 in the first block was set to 0% and increased across successive blocks (25%, 75%, respectively).
250 The large food reward was delivered irrespective of punishment delivery. Although the levers
251 were counterbalanced across rats, the identities of the small, "safe" lever and large, "risky" lever
252 remained constant for each rat throughout testing. Each block of trials started with eight forced
253 choice trials in which a single lever was extended into the chamber. It is through these forced
254 choice trials that the punishment contingencies for that block were established (four presentations
255 of each lever, randomly presented). During forced choice trials, the probability of punishment
256 following a press for the large reward was dependent upon the outcomes of the other forced
257 choice trial lever presses in that block. For example, in the 25% block, one and only one of the
258 four forced choice trials (randomly selected) resulted in footshock. Similarly, in the 75% block,
259 three and only three of those forced choice trials resulted in footshock. The forced choice trials
260 were followed by 20 free choice trials in which both levers were extended. If rats failed to lever
261 press within 10 s, the house light was extinguished and the trial was counted as an omission. In

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

262 contrast to the forced choice trials, the probability of punishment in free choice trials was
263 independent, such that the shock probability on each trial was the same regardless of shock
264 delivery on previous trials in that block. During RDT training, shock intensities were adjusted
265 individually for each rat to ensure that there was sufficient parametric space to observe either
266 increases or decreases in risk taking during optogenetic inhibition of BLA.

267 Upon reaching stable baseline performance (see *Experimental design and statistical*
268 *analysis* section for description of stability), rats were lightly anesthetized and optic fibers were
269 inserted into the BLA cannulae such that they extended 1 mm beyond the tips of the cannulae.
270 The fibers were cemented into position and dust caps were placed on the fibers to keep them free
271 from debris. In each subsequent RDT session, spring-insulated patch cords fastened to the rotary
272 joint were attached to the implanted fibers in the rat. Rats were trained in this manner until their
273 performance returned to baseline levels (approximately 3 sessions). Upon reaching this criterion,
274 optogenetic manipulations during test sessions began (note that shock intensities were not
275 adjusted between baseline and laser stimulation sessions). Laser stimulation occurred during
276 three different free choice trial phases (Figure 1B): 1) deliberation 2) reward outcome and 3)
277 intertrial interval (ITI). The deliberation phase consisted of the time between the nosepoke to
278 trigger lever extension and a lever press, and thus captured the period in which rats were
279 presumably deciding between the two available options. Laser stimulation commenced 0.5 s prior
280 to nosepoke illumination and remained on until a lever press occurred or 5 s elapsed, whichever
281 occurred first. For the reward outcome phase, there were three different stimulation conditions:
282 1) delivery of the small safe reward 2) delivery of the large reward without punishment and 3)
283 delivery of the large reward with punishment. During each outcome condition, laser stimulation
284 began as soon as the rat pressed the lever to yield that outcome and lasted for 5 s. Finally, during
285 the ITI phase, laser stimulation (5 s) occurred 8-15 s after each reward delivery. A randomized,
286 within-subjects design was used such that each rat was tested across multiple stimulation phases.

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

287 Because of attrition due to detachment of headcaps, however, not all rats were tested for all
288 phases. In between each stimulation session, rats were tethered and tested in the RDT until their
289 performance in the task across two consecutive sessions was no different from their original
290 baseline prior to any stimulation. If choice performance shifted during these re-baselining
291 sessions, shock intensities were adjusted until performance was comparable to the original
292 baseline.

293 *Determination of Shock Intensity Threshold*

294 Upon completion of testing in the RDT, rats in the eNpHR3.0 group underwent test
295 sessions in which their shock reactivity was assessed under stimulation and non-stimulation
296 conditions. The procedures were based on those developed by Bonnet and Peterson (1975) to
297 determine the shock thresholds at which specific motor responses were elicited. These test
298 sessions occurred across two days, with each day consisting of two tests: one with laser
299 stimulation and the other without laser stimulation. The order of the test sessions on each day
300 was counterbalanced across the two days. Irrespective of stimulation condition, each test session
301 began with a 2 min baseline period followed by delivery of an unsignaled footshock (0.4 mA, 1 s),
302 which decreased spontaneous motor activity and facilitated detection of motor responses at
303 subsequent low shock intensities. The shock intensity was then set to 0.05 mA and a series of
304 five footshocks (1 s each), each separated by 10 s, was delivered. After each series of footshocks,
305 the shock intensity was increased by 0.025 mA. The increase in shock intensities continued until
306 all motor responses of interest were observed. The shock intensity threshold for a given motor
307 response was determined by the shock intensity at which the given response was elicited by three
308 out of the five footshocks in a series. The motor responses for which shock thresholds were
309 determined consisted of 1) flinch of a paw or a startle response 2) elevation of one or two paws
310 3) rapid movement of three or all paws. For test sessions with laser stimulation, light was delivered
311 bilaterally (560 nm, 8-10mW) using the same procedures and system used during decision-

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

312 making sessions. To mimic parameters used for laser stimulation during delivery of the large,
313 punished outcome, laser stimulation and footshock were delivered concomitantly, but the laser
314 remained on for an additional 4 s (total stimulation time of 5 s). Even though no light was delivered
315 during test sessions without laser stimulation, rats were still tethered for the duration of the test.

316 ***Histology and immunohistochemistry***

317 Upon completion of behavioral testing, rats were overdosed with Euthasol and
318 transcardially perfused with cold 0.1M phosphate-buffered saline (PBS) followed by cold 4%
319 paraformaldehyde. Brains were extracted and post-fixed in 4% paraformaldehyde for 24 h before
320 being transferred into a 20% sucrose in 0.1M PBS solution. Brains were sectioned on a cryostat
321 (35 μm) maintained at -20˚C. Coronal sections (30 μm) were collected in a 1-in-4 series and
322 placed in wells filled with 0.1M PBS.

323 Immunohistochemistry was performed on free-floating tissue sections and began with
324 three 10 min washes in 0.1M Tris-buffered saline (TBS). Tissue was then incubated in 3% normal
325 donkey serum (NDS) and 0.3% Triton-X-100 in 0.1M TBS for 1 h at room temperature. Tissue
326 was then immediately transferred into primary antibody [rabbit anti-mCherry at 1:1000 (ab167453,
327 Abcam solution) in 3% NDS and 0.3% Triton-X-100] for 72 hours at 4˚C. After primary antibody
328 incubation, tissue was washed three times in 0.1M TBS for 10 min and then incubated in
329 secondary antibody solution [donkey anti-rabbit conjugated to Alexa Fluor 488 at 1:300 (A-21206,
330 Invitrogen) in 3% NDS and 0.3% Triton-X-100) for 2 h at room temperature. Finally, tissue was
331 washed three times in 0.1M TBS for 10 min and then mounted onto electrostatic slides
332 (Fisherbrand) in 0.1M TBS. Slides were coverslipped with Prolong Gold Antifade Mountant
333 (P36941, Invitrogen) and sealed with clear nail polish.

334 ***Experimental design and statistical analyses***

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

335 Using pilot data collected from several eNpHR3.0 rats, a power analysis was conducted
336 with G*Power software. This analysis indicated that a sample size of at least 4 rats was required
337 to detect significant differences between baseline and stimulation conditions with effect sizes of
338 0.8 and above, assuming an alpha level of 0.05. To account for possible attrition over the course
339 of the experiment, group sizes were larger than that calculated from the power analysis. A total
340 of 35 male Long-Evans rats were used in these experiments. Twenty-six rats received intra-BLA
341 microinjections of the viral vector containing eNpHR3.0, four of which were used for *in vitro*
342 electrophysiology experiments. Nine rats received intra-BLA microinjections of the viral vector
343 containing mCherry. Within the eNpHR3.0 group, some rats did not undergo every stimulation
344 session due to illness or detachment of headcaps over the course of the experiment. In addition,
345 only a subset of rats (n=6) was used for shock threshold testing. In the control group, there was
346 attrition due to illness or detachment of headcaps, resulting in only four of the initial nine rats
347 completing the stimulation sessions. All 4 rats, however, completed all stimulation conditions.

348 Raw data files were analyzed using a customized analysis template written in Graphic
349 State 4.0 software. This template extracted data for specific task events of interest: numbers of
350 lever presses during forced and free choice trials, latencies to press levers, latencies to nosepoke,
351 and numbers of omissions during forced and free choice trials. The behavioral and statistical
352 procedures were conducted identically for the eNpHR3.0 and control groups. Choice performance
353 in each block of the RDT was measured as the percentage of free choice trials (each block
354 consisted of 20 free choice trials; excluding omissions) on which rats chose the large, risky
355 outcome. Each rat was trained in the RDT until it reached stable baseline performance. Stable
356 baseline was obtained when the coefficient of variation (CV) for choice of the large, risky outcome
357 was less than 20% in each block for at least two consecutive sessions. Once this criterion was
358 met, stimulation sessions commenced. In between each stimulation session, rats were re-trained
359 in the RDT until their behavior re-stabilized, which was determined using the same criterion. To

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

360 ensure that the baseline after stimulation was similar to the original baseline (before any
361 stimulation sessions took place), the CV of the means of each block between baseline sessions
362 had to fall below 20%. Upon reaching this criterion, rats were advanced to the next stimulation
363 session. Effects of stimulation (i.e., BLA inhibition) on choice performance were determined using
364 a two-factor repeated measures ANOVA with session condition (i.e., baseline vs. inhibition) and
365 trial block as within-subjects factors. In all analyses, a *p*-value of 0.05 or less was considered
366 statistically significant. Latencies to nosepoke to trigger lever extension were measured as the
367 interval between the illumination of the nosepoke light and a nosepoke response, excluding trials
368 on which the rat failed to nosepoke altogether (omissions). Using a repeated measures ANOVA,
369 nosepoke response latencies were specifically compared between baseline and deliberation
370 stimulation sessions to determine whether laser stimulation (which was initiated 0.5 sec before
371 nosepoke illumination) affected this aspect of behavior. Effects of BLA inhibition on omissions
372 during free and forced choice trials were analyzed using a paired *t*-test with session condition as
373 the within-subjects factor.

374 To better understand the effects of BLA inhibition during task phases in which inhibition
375 significantly affected choice behavior, additional analyses were conducted to determine whether
376 optogenetic manipulations altered the degree to which feedback from past trials influenced
377 subsequent choices. Specifically, this analysis provided a measure of how BLA inhibition affected
378 the likelihood of choosing the large, risky outcome upon receipt of the large reward in the absence
379 of punishment on the previous trial (win-stay performance) vs. the likelihood of choosing the large,
380 risky outcome upon receipt of the large reward accompanied by punishment on the previous trial
381 (lose-shift performance; Bari et al., 2011; St Onge et al., 2011). To perform this analysis, choices
382 were categorized according to the outcome of the previous trial (large, punished outcome vs.
383 large, unpunished outcome). Win-stay performance was calculated as the number of trials within
384 each free choice block in which a rat chose the large, risky lever after receipt of a large,

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

385 unpunished outcome (win), divided by the total number of free choice trials in which the rat
386 received a large, unpunished outcome. Similarly, lose-shift performance was calculated as the
387 number of trials within each free choice block in which a rat chose the small, safe lever after
388 receipt of a large, punished outcome (lose), divided by the total number of free choice trials in
389 which the rat received a large, punished outcome. Effects of BLA inhibition on the percentage of
390 win-stay and lose-shift trials were each analyzed using paired *t*-tests with session (baseline vs.
391 inhibition) as the within-subjects factor.

392 Shock threshold intensities for the laser stimulation or no laser stimulation sessions were
393 averaged across the two test days. Analysis of shock intensity thresholds was conducted using a
394 two-factor repeated measures ANOVA with stimulation condition (inhibition vs. no inhibition) and
395 motor response as the within-subjects factors. To eliminate the possibility that the order of the
396 test sessions on each day contributed to differences in shock reactivity thresholds, another
397 repeated measures ANOVA was conducted using the same within-subjects factors and also
398 included order of laser stimulation as a between-subjects factor. If either of these parent ANOVAs
399 resulted in main effects or significant interactions, additional repeated measures ANOVA or paired
400 *t*-tests were performed to determine the source of significance.

401

402 **Results**

403 ***In vitro electrophysiology***

404 In slices from rats injected with AAV5-CAMKIIα-eHpNR3.0-mCherry, BLA neurons
405 expressing mCherry were identified with epifluorescence microscopy and recorded from using
406 conventional whole-cell recording techniques (see *Methods* section). mCherry-positive BLA
407 neurons (n=11) had a mean whole cell capacitance of 149 ± 14.9 pF. A subset of these neurons
408 was filled with biocytin, immunolabeled with Alexa-594, and imaged with 2-photon meditated

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

409 epifluorescence microscopy. Cells examined in this manner were all multipolar and had dense
410 local dendritic branches within the BLA (Figure 2A). Collectively, these features are consistent
411 with effective transduction of glutamatergic BLA principal neurons. A 1 s activation of eHpNR3.0
412 in mCherry-positive BLA neurons voltage clamped at -70 mV (see *Methods* section) produced a
413 clear outward current which had a peak amplitude of 117 ± 29.6 pA, obtained within ~100 msec
414 of activation, and a mean amplitude of 80.0 ± 20.8 pA as observed during the last 200 msec of
415 activation (Figure 2B). Identical stimulation in current clamp (I=0) produced a maximum
416 hyperpolarization of -16 ± 3.1 mV (also obtained within ~100 msec of activation), and a mean
417 hyperpolarization of -9.0 ± 2.0 mV as observed during the last 200 msec of activation (Figure 2C).
418 This hyperpolarization was sufficient to completely silence 9 out of 11 cells tested when firing
419 under a 100-200 pA load (Fig. 1D). Firing rate was slowed, but not eliminated, in the other two
420 cells. Collectively, these results demonstrate that activation of eHpNR3.0 produces robust
421 functional inhibition of BLA principal neurons.
422
423 ***Histology***
424 Of the 22 rats that received the viral vector containing eNpHR3.0 for optogenetic
425 manipulations, one died during surgery and five were euthanized during training due to detached
426 headcaps. Of the remaining 16 rats, three were excluded due to off-target fiber placements (too
427 ventral; n = 1) or lack of eNpHR3.0 expression in one hemisphere (n = 2). Figure 3A displays the
428 maximum (light gray) and minimum (dark gray) spread of the virus, and Figure 3B depicts the
429 location of optic fiber tips of rats that were included in the final data analysis. A representative
430 placement of a fiber tip in the BLA with eNpHR3.0 expression is shown in Figure 3C.
431 Of the 9 rats that received the viral vector containing mCherry alone, one died during
432 surgery and four were euthanized during training due to detached headcaps, resulting in a final n
433 = 4. Figure 3D displays the maximum (light gray) and minimum (dark gray) spread of the virus

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

434 and Figure 3E shows the location of optic fiber tips of control rats that were included in the final
435 data analysis.
436
437 ***Optogenetic BLA inhibition during decision making in eNpHR3.0 rats***
438 *BLA inhibition during deliberation*
439 Optogenetic inhibition of the BLA during deliberation (n = 12) caused a significant
440 decrease in choice of the large, risky outcome [decreased risky choice; inhibition, F (1, 11) =
441 14.57, $p < 0.01$; inhibition X trial interaction [F (2, 22) = 10.29, $p < 0.01$; Figure 4A]. Importantly,
442 this effect was only observed in blocks of trials in which there was a risk of punishment: while
443 there was no effect of inhibition in block 1 [t (11) = -1.27, $p = 0.23$], BLA inhibition decreased
444 choice of the large, risky outcome in both block 2 [t (11) = 4.51, $p < 0.01$] and block 3 [t (11) =
445 2.16, $p = 0.05$].
446 Additional analyses were performed to determine whether BLA inhibition during
447 deliberation affected the percentage of win-stay or lose-shift trials (Figure 4B). There was no effect
448 of inhibition on the percentage of win-stay trials (t (8) = 1.61, $p = 0.15$), but there was a near
449 significant increase in the percentage of lose-shift trials (t (9) = -1.99, $p = 0.08$). Note that in the
450 win-stay analysis, three rats were excluded because they either never chose the large, risky
451 outcome or never encountered a trial in which they chose the large, risky outcome and received
452 the large reward without punishment. Similarly, in the lose-shift analysis, two rats were excluded
453 because they never selected the large, risky outcome. This slight increase in lose-shift trials
454 suggests that BLA inhibition slightly increased the likelihood for rats to shift their choice to the
455 small, safe outcome after receiving a large reward accompanied by punishment. Collectively,
456 these results show that BLA inhibition during the period in which rats deliberated between the two
457 available options caused an increase in risk aversion.
458 Finally, there was no effect of BLA inhibition during deliberation on omissions in either the
459 forced choice trials [t (11) = -0.87, $p = 0.44$] or the free choice trials [t (11) = -0.26, $p = 0.80$].

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

460 Additional analyses were conducted to determine whether inhibition affected rats' latency to
461 nosepoke to trigger lever extension. While there was no main effect of inhibition [F (1, 11) = 0.05,
462 p = 0.82], there was a trend toward a significant inhibition X block interaction [F (2, 22) = 3.10, p
463 = 0.07], with BLA inhibition causing a slight decrease in latency to nosepoke, particularly in block
464 3 [mean of 1.87 (±0.24) s for baseline; mean of 1.48 (± 0.15) s for stimulation]. Note, however,
465 that because light onset commenced 0.5 s before the nosepoke was illuminated to signal the
466 beginning of a trial, BLA inhibition should have been maximal prior to the start of the deliberation
467 period.

468

469 *BLA inhibition during delivery of the small, safe outcome*

470 Optogenetic inhibition of the BLA during delivery of the small, safe outcome (n = 10) had
471 no effect on choice of the large, risky outcome [inhibition, F (1, 9) = 0.09, p = 0.77; inhibition X
472 block, F (2, 18) = 1.73, p = 0.21; Figure 5A]. Additionally, inhibition had no effect on omissions
473 (Table 1) during forced choice trials [t (9) = -0.32, p = 0.76] or during free choice trials [t (9) = -
474 0.91, p = 0.39]. Hence, BLA inhibition during delivery of the small, safe outcome did not alter
475 choice behavior.

476

477 *BLA inhibition during delivery of the large, unpunished outcome*

478 Similarly, there was no effect of BLA inhibition during the large, unpunished outcome (n =
479 9) on choice behavior [inhibition, F (1, 8) = 0.45, p = 0.52; inhibition X block, F (2, 16) = 0.30, p
480 =0.74; Figure 5B]. There were also no effects of inhibition on omissions (Table 1) during forced
481 choice trials [t (8) = 0.50, p =0.63] or free choice trials [t (8) = -1.0, p = 0.35]. Collectively, these
482 findings indicate that BLA inhibition during delivery of the large, unpunished outcome did not affect
483 choice behavior.

484

485 *BLA inhibition during delivery of the large, punished outcome*

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

486 In contrast to BLA inhibition during delivery of the large, unpunished outcome, optogenetic
487 BLA inhibition during delivery of the large, punished outcome (n = 10) significantly increased
488 choice of the large, risky outcome [inhibition, $F(1, 9) = 82.75$, $p < 0.01$; inhibition X block, $F(1, 9)$
489 $= 39.22$, $p < 0.01$; Figure 6A]. It is important to note that this analysis only used choice behavior
490 in the 25% and 75% blocks from baseline and stimulation sessions, as they were the only blocks
491 in which BLA inhibition could occur.

492 Given the significant effects of BLA inhibition during this phase of the task, additional
493 analyses were performed to determine how this manipulation affected the percentage of win-stay
494 or lose-shift trials (Figure 6B). There was no effect of BLA inhibition on the percentage of win/stay
495 trials [$t(9) = -0.44$, $p = 0.67$]; however, there was a significant decrease in the percentage of
496 lose/shift trials [$t(9) = 3.02$, $p = 0.01$] compared to baseline. Thus, BLA inhibition during delivery
497 of the large, punished outcome caused rats to increase the likelihood of choosing the large, risky
498 outcome, despite having been punished for this choice on the preceding trial.

499 Lastly, there were no effects of BLA inhibition on omissions (Table 1) during free choice
500 trials [$t(9) = 0.09$, $p = 0.93$], although inhibition did cause a significant decrease in omissions
501 during forced choice trials compared to baseline conditions [$t(9) = 2.56$, $p = 0.03$].

502

503 *BLA inhibition during shock threshold testing*

504 Rather than affecting processes related to risk taking *per se*, the effects of BLA inhibition
505 during delivery of the large, punished outcome may have been due to an inhibition-induced
506 decrease in shock sensitivity. To address this, a subset of rats (n = 6) was tested in a behavioral
507 assay that evaluates the thresholds at which selective motor responses (as described in the
508 *Methods* section) are elicited by shock delivery. These thresholds were obtained under stimulation
509 and no stimulation (inhibition vs. no inhibition, respectively) conditions (Figure 6C). A two-factor
510 repeated measures ANOVA revealed neither a main effect of inhibition [$F(1, 5) = 4.00$, $p = 0.10$]
511 nor an inhibition X motor response interaction [$F(2, 10) = 0.04$, $p = 0.96$]. Thus, the increase in

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

512 risky choice during sessions in which BLA inhibition occurred during delivery of the large,
513 punished outcome cannot be accounted for by a decrease in footshock sensitivity.
514
515 *BLA inhibition during ITIs*
516 Optogenetic inhibition of the BLA during the ITI (n = 13) had no effect on choice of the
517 large, risky outcome [inhibition, $F(1, 12) = 0.01$, $p = 0.91$; inhibition X block, $F(2, 24) = 0.02$, $p =$
518 0.98; Figure 5C]. Similarly, BLA inhibition during ITIs did not affect omissions during forced choice
519 trials [$t(12) = 0.3$, $p = 0.77$], but caused a near significant increase in omissions during free choice
520 trials [$t(12) = -2.04$, $p = 0.06$].
521
522 ***Optogenetic BLA stimulation during decision making in control rats***
523 To ensure that the effects of BLA inhibition were not due to light delivery alone, another
524 group of rats received intra-BLA microinjections of a vector carrying mCherry alone and were then
525 trained in the RDT. Because BLA inhibition only altered choice behavior during deliberation and
526 delivery of the large, punished outcome in eNpHR3.0 rats, control rats only received stimulation
527 during these two phases (in separate sessions, in a randomized order across rats).
528
529 *BLA stimulation during deliberation*
530 BLA stimulation during deliberation (n = 4) had no effect on choice of the large, risky
531 outcome compared to baseline conditions [stimulation, $F(1, 3) = 1.00$, $p = 0.39$; stimulation X
532 block, $F(2, 6) = 1.00$, $p = 0.42$; Figure 7A]. There was no main effect of BLA stimulation on latency
533 to nosepoke to initiate lever extension [$F(1, 3) = 2.33$, $p = 0.23$; Table 1]; however, it appeared
534 that under stimulation conditions, latency to nosepoke did increase across the session [$F(2, 6) =$
535 7.70, $p = 0.02$]. While there was a trend toward a significant effect of stimulation on omissions
536 (Table 1) during forced choice trials [$t(3) = -2.82$, $p = 0.07$], this was due to the fact that there
537 were fewer omissions under stimulation compared to baseline conditions. There were no effects

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

538 of BLA stimulation on omissions during free choice trials [t (3) = 1.00, p = 0.39]. Collectively, these
539 results indicate that laser stimulation of BLA alone during deliberation did not affect risky decision
540 making in control rats.
541
542 *BLA stimulation during delivery of the large, punished outcome*
543 There was also no effect of BLA stimulation during delivery of the large, punished outcome
544 (n = 4) on choice of the large, risky outcome [stimulation, F (1, 3) = 1.00, p = 0.39; stimulation X
545 block, F (2, 6) = 1.0, p = 0.42; Figure 7B]. There was a trend toward a significant effect of
546 stimulation on omissions (Table 1) during forced choice trials [t (3) = -2.82, p = 0.07]; however,
547 this again appeared to be due to fewer omissions under stimulation compared to baseline
548 conditions. There were no differences in omissions during free choice trials between stimulation
549 and baseline conditions [t (3) = 1.67, p = 0.19]. Hence, laser stimulation of BLA alone during
550 delivery of the large, punished outcome had no effect on risky decision making in control rats.
551
552 **Discussion**
553 Decision making involves coordination of multiple cognitive functions to generate choice
554 behavior. While there is a growing body of literature delineating the neural substrates governing
555 decision making, less is known about how and when such brain regions are engaged during the
556 decision process. The current study demonstrates that the BLA plays distinct roles during different
557 components of risky decision making. Whereas optogenetic inhibition of the BLA during
558 deliberation resulted in a decrease in choice of the large, risky outcome (decreased risky choice),
559 BLA inhibition during delivery of the large, punished outcome had the opposite effect (increased
560 risky choice). These effects were specific to the task phase in which inhibition occurred because
561 BLA inhibition had no effect on choice behavior during delivery of the small, safe outcome, the
562 large, unpunished outcome, or the ITI. Further, there were no effects of light delivery into the BLA

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

563 during deliberation or delivery of the large, punished outcome in control rats (in the absence of
564 eNpHR3.0).

565 The overall finding that BLA manipulation alters choice performance during risky decision
566 making is consistent with previous studies implicating this region in cost/benefit decision making.
567 In a risky decision making task involving choices between a small, certain food reward and a
568 large, probabilistic food reward, pharmacological inactivation of BLA decreased choice of the
569 large, probabilistic reward, but only at probabilities at which it was more profitable to choose this
570 reward (Ghods-Sharifi et al., 2009). Consistent with this, BLA lesions induce a pattern of
571 disadvantageous choice behavior in another rodent model of risky decision making designed to
572 simulate the Iowa Gambling Task (Zeeb and Winstanley, 2011). More recently, we showed that
573 BLA lesions increase risky choice in the RDT (Orsini et al., 2015a), and control experiments
574 suggested that this increase was due to impaired integration of reward magnitude and
575 punishment-related information. Given the complexity of the decision-making process, however,
576 the use of lesions and pharmacological inactivation, while informative, may obscure a complete
577 understanding of how the BLA is engaged during the course of individual decisions.

578 To circumvent this issue, the current study employed optogenetics to selectively inhibit the
579 BLA during distinct phases of the decision-making process. In contrast to effects of permanent
580 BLA lesions (Orsini et al. 2015a), optogenetic inhibition caused both an increase *and* decrease in
581 risky choice depending on the timepoint at which inhibition occurred. These results suggest that
582 the contribution of the BLA to risky choice is not uniform, but instead that it may function in different
583 capacities even over the course of a few seconds of a decision-making trial. During deliberation,
584 various sources of information must be assimilated to bias behavior toward a specific choice. In
585 particular, information about the anticipated rewarding aspects of each potential outcome must
586 be integrated and weighed against the negative/adverse aspects of those outcomes. BLA
587 inhibition during this period interfered with this integrative process such that choices were more
588 strongly biased by punishment-related information. One possibility is that this is due to a loss of

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

589 reward magnitude information, although this seems unlikely given that choice behavior was intact
590 in the first block of trials (in which there was no risk of punishment). Alternatively, and consistent
591 with the slight increase in lose-shift trials, BLA inhibition may have augmented the salience of the
592 punishment associated with the large reward. This also seems unlikely, however, given that
593 lesions and pharmacological inactivation of the BLA reduce fear expression in other contexts
594 (Helmstetter and Bellgowan, 1994; Maren et al., 1996). A final, and more likely possibility is that
595 BLA inhibition during deliberation may have attenuated the incentive salience of anticipated
596 outcomes and, consequently, the ability to bias action selection toward more salient rewards.
597 Hence, the BLA may be important for tagging available outcomes based on their incentive
598 salience (i.e., to favor larger, albeit risker, outcomes). In the absence of an intact BLA, the
599 punishment history and/or aversive properties of these outcomes prevail and drive choice
600 behavior.

601 In contrast, the increase in risk-taking following BLA inhibition during delivery of the large,
602 punished outcome suggests that the BLA is engaged in a manner different from that during
603 deliberation. Incorporating feedback about outcomes of past choices to guide future choice is a
604 critical aspect of adaptive decision making. The BLA has long been implicated in encoding and
605 representing aversive properties of stimuli in Pavlovian and instrumental learning tasks (Wassum
606 and Izquierdo, 2015). Thus, inhibition during delivery of the large, punished outcome may have
607 prevented the BLA from encoding the punishing aspects of this outcome and therefore impaired
608 the ability to use this information as feedback to adjust future choice behavior. This would result
609 in choice performance being driven by rewarding properties of this outcome, irrespective of
610 whether its delivery was accompanied by footshock. This is supported by the significant decrease
611 in lose-shift trials such that rats continued to choose the large, risky outcome despite having been
612 punished on the preceding trial. Importantly, the effects of BLA inhibition during this phase were
613 not due to alterations in shock sensitivity, as there were no changes in thresholds at which shock-
614 induced motor responses were elicited. This is consistent with previous work showing that BLA

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

615 lesions do not affect discrimination between punished and unpunished rewards of the same
616 magnitude (Orsini et al., 2015a) and when considered together, demonstrates that the BLA is not
617 necessary for encoding shock alone. Collectively, these data suggest that when a rewarding
618 outcome is accompanied by an adverse consequence, the BLA may be responsible for encoding
619 the negative aspects of that outcome that can then be used as feedback during future deliberation.
620 The idea that the BLA functions in a heterogeneous manner during risky decision making
621 is consistent with previous work showing that BLA neurons that encode outcomes of different
622 valences are segregated into distinct populations (Schoenbaum et al., 1998; Paton et al., 2006;
623 Belova et al., 2007; Belova et al., 2008; Shabel and Janak, 2009; Sangha et al., 2013; Zhang et
624 al., 2013; Namburi et al., 2015; Beyeler et al., 2016). Aversive and appetitive outcomes are
625 predominantly represented by separate BLA cell populations (Namburi et al., 2015; Beyeler et al.,
626 2016), suggesting that the functional heterogeneity of the BLA during risky decision making could
627 arise from distinct neuronal populations representing incentive salience (positive-value neurons)
628 vs. aversive properties (negative-value neurons) of choice outcomes. The current data further
629 suggest that these separate populations are differentially engaged depending on the phase of the
630 decision process. Thus, positive-value neurons may be important during the deliberative process
631 for signaling the incentive salience of possible outcomes, whereas negative-value neurons may
632 be critical for sensitivity to negative feedback. It is not clear, however, whether these separate
633 populations of neurons interact with one another and if so, when and where this interaction occurs.
634 If, in fact, these distinct neuronal populations are differentially engaged during decision
635 making, how do they ultimately affect choice behavior? One possibility is that the positive- and
636 negative-value neurons have divergent and non-overlapping downstream targets. Indeed, BLA
637 neurons that project to the nucleus accumbens (NAc) selectively support reward conditioning
638 whereas BLA neurons that project to the central nucleus of the amygdala (CeA) selectively
639 support fear conditioning (Namburi et al., 2015; Beyeler et al., 2016). While the BLA-NAc
640 projection is implicated in risky decision making (St Onge et al., 2012), the contribution of the

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

641 BLA-CeA circuit is unknown. It is also possible that putative positive- and negative-value BLA
642 neurons modulate risky choice through divergent projections to the core and shell subregions of
643 the NAc, respectively. This hypothesis is consistent with the canonical theory that the NAc core
644 (NAcC) is important for facilitating approach behavior whereas the NAc shell (NAcSh) is required
645 for suppressing ongoing behavior (Floresco, 2014). This functional dichotomy extends to
646 instrumental tasks involving conflict or punishment: NAcSh inactivation increases punished
647 responding (Piantadosi et al., 2017) and decreases avoidance responses (Fernando et al., 2014),
648 whereas NAcC inactivation decreases overall reward-seeking, irrespective of accompanying
649 punishment (Piantadosi et al., 2017). Further evidence indicates that these distinct functions are
650 modulated by BLA input. For example, activation of the BLA-NAcC pathway drives reward-
651 seeking behavior (Ambroggi et al., 2008; Stuber et al., 2011; Namburi et al., 2015) and interruption
652 of this circuit impairs reward conditioning and decision making (Ambroggi et al., 2008; Stuber et
653 al., 2011; St Onge et al., 2012). In contrast, the BLA-NAcSh, but not the BLA-NAcC, pathway
654 supports active avoidance behavior (Ramirez et al., 2015). Thus, positive-encoding BLA neurons
655 may contribute to the deliberative process via their downstream connections with the NAcC and
656 negative-encoding BLA neurons may provide negative feedback information through their
657 interactions with the NAcSh. Interestingly, it has been proposed that networks of inhibitory BLA
658 interneurons may play a permissive role in determining which neuronal circuits are engaged
659 during motivated behavior (Janak and Tye, 2015), which could allow flexible shifts in choice
660 behavior as reward or punishment contingencies change.

661 To our knowledge, this study is the first to demonstrate multiple roles for the BLA in
662 decision making depending on the phase of the decision process engaged. These results highlight
663 the need to use more temporally targeted manipulations to understand the neural circuitry
664 supporting complex cognitive operations. More importantly, these findings provide a more refined
665 understanding of how the BLA contributes to risk-based decision making, and a foundation for
666 future work on development of novel approaches for remediating maladaptive choice behavior.

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

667 **Figure Captions:**

668 ***Figure 1:*** **Design of the Risky Decision-Making Task. A.** Each block consists of 8 forced choice
669 trials and 20 free choice trials. Each free choice trial consists of a deliberation and outcome phase.
670 Rats must nosepoke for the extension of either one lever (forced choice trial) or both levers (free
671 choice trial). A press on one lever yields a small safe reward and a press on the other yields a
672 large reward accompanied by variable probabilities of footshock punishment. **B.** Optogenetic
673 stimulation occurred at one of five possible phases during each free choice trial (with stimulation
674 during each test session taking place in only one of the five phases). Green bars indicate periods
675 of laser stimulation.

676

677 ***Figure 2.*** **Functional validation of eNpHR3.0 in the BLA. A.** Two-photon z-series projection of
678 a mCherry-positive BLA neuron filled with biocytin and immunolabeled with Alexa-594. **B.**
679 mCherry-positive cells (n=11) voltage clamped at -70 mV show an increase in holding current
680 upon light stimulation (yellow line) and return to baseline holding current upon light termination.
681 Black line indicates the mean response and shaded area indicates SEM. **C.** mCherry-positive
682 cells (n=11) current clamped at 0 pA show hyperpolarization upon light stimulation and return to
683 resting membrane potential upon light termination. Black line indicates the mean response and
684 shaded area indicates SEM. **D.** A representative mCherry-positive cell that was current clamped
685 at 0 pA shows an increase in firing rate upon injection of a +150 pA current pulse, which is
686 effectively suppressed during light delivery.

687

688 ***Figure 3.*** **eNpHR3.0 expression and optic fiber placement in the BLA. A.** Schematic depicting
689 the maximum (light gray) and minimum (dark gray) spread of eNpHR3.0 expression in the BLA.
690 **B.** Optic fiber placements in the BLA. Black circles represent the tips of the optic fibers. **C.**
691 Representative micrograph depicting eNpHR3.0 expression and the tip of the optic fiber in the
692 BLA. Dashed white line represents the borders of the BLA. **D.** Schematic depicting the maximum

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

693 (light gray) and minimum (dark gray) spread of mCherry expression in the BLA of control rats. **E.**
694 Optic fiber placements in the BLA in control rats. Black circles represent the tips of the optic fibers.
695

696 ***Figure 4.*** **BLA inhibition during deliberation decreases risky choice. A.** BLA inhibition
697 decreases choice of the large, risky outcome. **B.** There were no effects of BLA inhibition on win-
698 stay trials. In contrast, there was a near-significant increase in lose-shift trials upon BLA inhibition.
699 Data are represented as mean ± standard error of mean (SEM). An asterisk indicates a significant
700 difference and a cross indicates a trend (p = 0.08) toward a significant difference between
701 inhibition and baseline (no laser) conditions.
702

703 ***Figure 5.*** **BLA inhibition has no effect on risky choice during other task phases. A.** BLA
704 inhibition during delivery of the small, safe outcome did not affect choice of the large, risky
705 outcome. **B.** BLA inhibition during delivery of the large, unpunished outcome had no effect on
706 choice of the large, risky outcome. C. BLA inhibition during the ITI had no effect on choice of the
707 large, risky outcome. Data are represented as mean ± SEM.
708

709 ***Figure 6.*** **BLA inhibition during delivery of the large, punished outcome increases risky**
710 **choice. A.** BLA inhibition increased choice of the large, risky outcome. **B.** There was no effect of
711 BLA inhibition on win-stay performance. In contrast, BLA inhibition decreased lose-shift
712 performance. **C.** BLA inhibition did not alter the intensity thresholds at which shock elicited a flinch,
713 elevation of 1-2 paws, or rapid movement of 3 or all paws. Data are represented as mean ± SEM.
714 Asterisks indicate a significant difference between inhibition and baseline (no laser) conditions.
715

716 ***Figure 7.*** **Laser stimulation of the BLA has no effect on risky choice in control rats. A.** In
717 rats injected with vectors carrying mCherry alone, BLA stimulation during deliberation did not
718 affect choice of the large, risky outcome. **B**. BLA stimulation during delivery of the large, punished

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

719 outcome had no effect on choice of the large, risky outcome. Data are represented as mean ±
720 SEM.
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

745 **References**

746 Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate
747 reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648-661.
748 Bari A, Mar AC, Theobald DE, Elands SA, Oganya KC, Eagle DM, Robbins TW (2011)
749 Prefrontal and monoaminergic contributions to stop-signal task performance in rats. The
750 Journal of neuroscience : the official journal of the Society for Neuroscience 31:9254-
751 9263.
752 Bechara A, Damasio H (2002) Decision-making and addiction (part I): impaired activation of
753 somatic states in substance dependent individuals when pondering decisions with
754 negative future consequences. Neuropsychologia 40:1675-1689.
755 Belova MA, Paton JJ, Salzman CD (2008) Moment-to-moment tracking of state value in the
756 amygdala. The Journal of neuroscience : the official journal of the Society for
757 Neuroscience 28:10023-10030.
758 Belova MA, Paton JJ, Morrison SE, Salzman CD (2007) Expectation modulates neural
759 responses to pleasant and aversive stimuli in primate amygdala. Neuron 55:970-984.
760 Beyeler A, Namburi P, Glober GF, Simonnet C, Calhoon GG, Conyers GF, Luck R, Wildes CP,
761 Tye KM (2016) Divergent Routing of Positive and Negative Information from the
762 Amygdala during Memory Retrieval. Neuron 90:348-361.
763 Crowley TJ, Dalwani MS, Mikulich-Gilbertson SK, Du YP, Lejuez CW, Raymond KM, Banich MT
764 (2010) Risky decisions and their consequences: neural processing by boys with
765 Antisocial Substance Disorder. PloS one 5:e12835.
766 De Martino B, Kumaran D, Seymour B, Dolan RJ (2006) Frames, biases, and rational decision-
767 making in the human brain. Science 313:684-687.
768 Dekkers TJ, Popma A, Agelink van Rentergem JA, Bexkens A, Huizenga HM (2016) Risky
769 decision making in Attention-Deficit/Hyperactivity Disorder: A meta-regression analysis.
770 Clin Psychol Rev 45:1-16.
771 Fernando AB, Urcelay GP, Mar AC, Dickinson TA, Robbins TW (2014) The role of the nucleus
772 accumbens shell in the mediation of the reinforcing properties of a safety signal in free-
773 operant avoidance: dopamine-dependent inhibitory effects of d-amphetamine.
774 Neuropsychopharmacology : official publication of the American College of
775 Neuropsychopharmacology 39:1420-1430.
776 Floresco SB (2014) The Nucleus Accumbens: An Interface Between Cognition, Emotion, and
777 Action. Annual review of psychology.
778 Ghods-Sharifi S, St Onge JR, Floresco SB (2009) Fundamental contribution by the basolateral
779 amygdala to different forms of decision making. The Journal of neuroscience : the official
780 journal of the Society for Neuroscience 29:5251-5259.
781 Gonzalez R, Schuster RM, Mermelstein RM, Diviak KR (2015) The role of decision-making in
782 cannabis-related problems among young adults. Drug and alcohol dependence 154:214-
783 221.
784 Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E, Russo MJ, Gordon JA,
785 Salzman CD, Axel R (2015) Neural Representations of Unconditioned Stimuli in
786 Basolateral Amygdala Mediate Innate and Learned Responses. Cell 162:134-145.
787 Gowin JL, Mackey S, Paulus MP (2013) Altered risk-related processing in substance users:
788 Imbalance of pain and gain. Drug and alcohol dependence 132:13-21.
789 Grabenhorst F, Hernadi I, Schultz W (2012) Prediction of economic choice by primate amygdala
790 neurons. Proceedings of the National Academy of Sciences of the United States of
791 America 109:18950-18955.

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

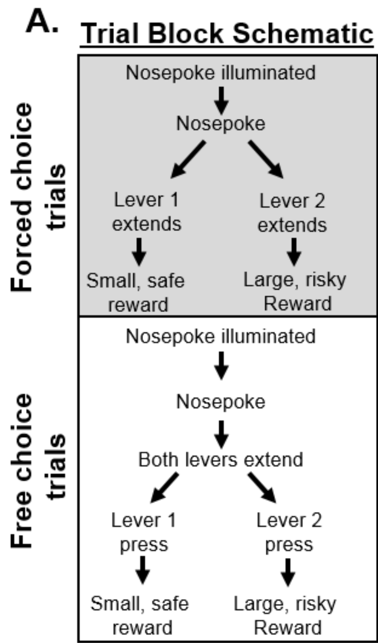
792 Helmstetter FJ, Bellgowan PS (1994) Effects of muscimol applied to the basolateral amygdala
793 on acquisition and expression of contextual fear conditioning in rats. Behavioral
794 neuroscience 108:1005-1009.
795 Hernadi I, Grabenhorst F, Schultz W (2015) Planning activity for internally generated reward
796 goals in monkey amygdala neurons. Nature neuroscience 18:461-469.
797 Hosking JG, Cocker PJ, Winstanley CA (2014) Dissociable contributions of anterior cingulate
798 cortex and basolateral amygdala on a rodent cost/benefit decision-making task of
799 cognitive effort. Neuropsychopharmacology : official publication of the American College
800 of Neuropsychopharmacology 39:1558-1567.
801 Janak PH, Tye KM (2015) From circuits to behaviour in the amygdala. Nature 517:284-292.
802 Kaye WH, Wierenga CE, Bailer UF, Simmons AN, Bischoff-Grethe A (2013) Nothing tastes as
803 good as skinny feels: the neurobiology of anorexia nervosa. Trends in neurosciences
804 36:110-120.
805 Maren S, Aharonov G, Fanselow MS (1996) Retrograde abolition of conditional fear after
806 excitotoxic lesions in the basolateral amygdala of rats: absence of a temporal gradient.
807 Behavioral neuroscience 110:718-726.
808 Najavits LM, Meyer T, Johnson KM, Korn D (2011) Pathological gambling and posttraumatic
809 stress disorder: a study of the co-morbidity versus each alone. J Gambl Stud 27:663-
810 683.
811 Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens
812 KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, Tye KM (2015) A circuit
813 mechanism for differentiating positive and negative associations. Nature 520:675-678.
814 Orsini CA, Trotta RT, Bizon JL, Setlow B (2015a) Dissociable Roles for the Basolateral
815 Amygdala and Orbitofrontal Cortex in Decision-Making under Risk of Punishment. The
816 Journal of neuroscience : the official journal of the Society for Neuroscience 35:1368-
817 1379.
818 Orsini CA, Moorman DE, Young JW, Setlow B, Floresco SB (2015b) Neural mechanisms
819 regulating different forms of risk-related decision-making: Insights from animal models.
820 Neuroscience and biobehavioral reviews.
821 Paton JJ, Belova MA, Morrison SE, Salzman CD (2006) The primate amygdala represents the
822 positive and negative value of visual stimuli during learning. Nature 439:865-870.
823 Piantadosi PT, Yeates DC, Wilkins M, Floresco SB (2017) Contributions of basolateral
824 amygdala and nucleus accumbens subregions to mediating motivational conflict during
825 punished reward-seeking. Neurobiology of learning and memory 140:92-105.
826 Ramirez F, Moscarello JM, LeDoux JE, Sears RM (2015) Active avoidance requires a serial
827 basal amygdala to nucleus accumbens shell circuit. The Journal of neuroscience : the
828 official journal of the Society for Neuroscience 35:3470-3477.
829 Rangel A, Camerer C, Montague PR (2008) A framework for studying the neurobiology of value-
830 based decision making. Nature reviews Neuroscience 9:545-556.
831 Roiser JP, de Martino B, Tan GC, Kumaran D, Seymour B, Wood NW, Dolan RJ (2009) A
832 genetically mediated bias in decision making driven by failure of amygdala control. The
833 Journal of neuroscience : the official journal of the Society for Neuroscience 29:5985-
834 5991.
835 Sangha S, Chadick JZ, Janak PH (2013) Safety encoding in the basal amygdala. The Journal of
836 neuroscience : the official journal of the Society for Neuroscience 33:3744-3751.
837 Schneider S et al. (2012) Risk taking and the adolescent reward system: a potential common
838 link to substance abuse. The American journal of psychiatry 169:39-46.
839 Schoenbaum G, Chiba AA, Gallagher M (1998) Orbitofrontal cortex and basolateral amygdala
840 encode expected outcomes during learning. Nature neuroscience 1:155-159.

bioRxiv preprint doi: https://doi.org/10.1101/180885; this version posted August 25, 2017. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

841 Schoenbaum G, Chiba AA, Gallagher M (1999) Neural encoding in orbitofrontal cortex and
842 basolateral amygdala during olfactory discrimination learning. The Journal of
843 neuroscience : the official journal of the Society for Neuroscience 19:1876-1884.
844 Shabel SJ, Janak PH (2009) Substantial similarity in amygdala neuronal activity during
845 conditioned appetitive and aversive emotional arousal. Proceedings of the National
846 Academy of Sciences of the United States of America 106:15031-15036.
847 Simon NW, Gilbert RJ, Mayse JD, Bizon JL, Setlow B (2009) Balancing risk and reward: a rat
848 model of risky decision making. Neuropsychopharmacology : official publication of the
849 American College of Neuropsychopharmacology 34:2208-2217.
850 St Onge JR, Abhari H, Floresco SB (2011) Dissociable contributions by prefrontal D1 and D2
851 receptors to risk-based decision making. The Journal of neuroscience : the official
852 journal of the Society for Neuroscience 31:8625-8633.
853 St Onge JR, Stopper CM, Zahm DS, Floresco SB (2012) Separate prefrontal-subcortical circuits
854 mediate different components of risk-based decision making. The Journal of
855 neuroscience : the official journal of the Society for Neuroscience 32:2886-2899.
856 Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM,
857 Kempadoo KA, Zhang F, Deisseroth K, Bonci A (2011) Excitatory transmission from the
858 amygdala to nucleus accumbens facilitates reward seeking. Nature 475:377-380.
859 Tremblay M, Cocker PJ, Hosking JG, Zeeb FD, Rogers RD, Winstanley CA (2014) Dissociable
860 effects of basolateral amygdala lesions on decision making biases in rats when loss or
861 gain is emphasized. Cogn Affect Behav Neurosci 14:1184-1195.
862 Wassum KM, Izquierdo A (2015) The basolateral amygdala in reward learning and addiction.
863 Neuroscience and biobehavioral reviews 57:271-283.
864 Winstanley CA, Floresco SB (2016) Deciphering Decision Making: Variation in Animal Models of
865 Effort- and Uncertainty-Based Choice Reveals Distinct Neural Circuitries Underlying
866 Core Cognitive Processes. The Journal of neuroscience : the official journal of the
867 Society for Neuroscience 36:12069-12079.
868 Winstanley CA, Theobald DE, Cardinal RN, Robbins TW (2004) Contrasting roles of basolateral
869 amygdala and orbitofrontal cortex in impulsive choice. The Journal of neuroscience : the
870 official journal of the Society for Neuroscience 24:4718-4722.
871 Zeeb FD, Winstanley CA (2011) Lesions of the basolateral amygdala and orbitofrontal cortex
872 differentially affect acquisition and performance of a rodent gambling task. The Journal
873 of neuroscience : the official journal of the Society for Neuroscience 31:2197-2204.
874 Zhang W, Schneider DM, Belova MA, Morrison SE, Paton JJ, Salzman CD (2013) Functional
875 circuits and anatomical distribution of response properties in the primate amygdala. The
876 Journal of neuroscience : the official journal of the Society for Neuroscience 33:722-733.

877

Figure 1



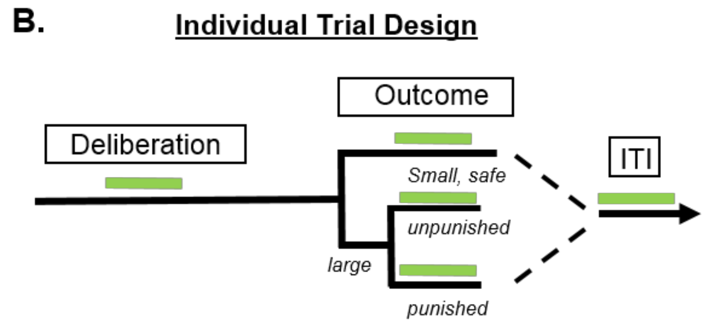
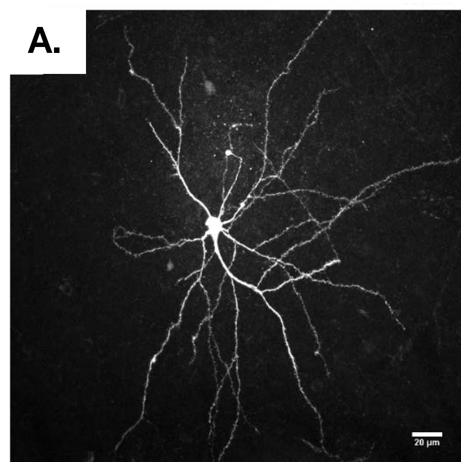


Figure 2



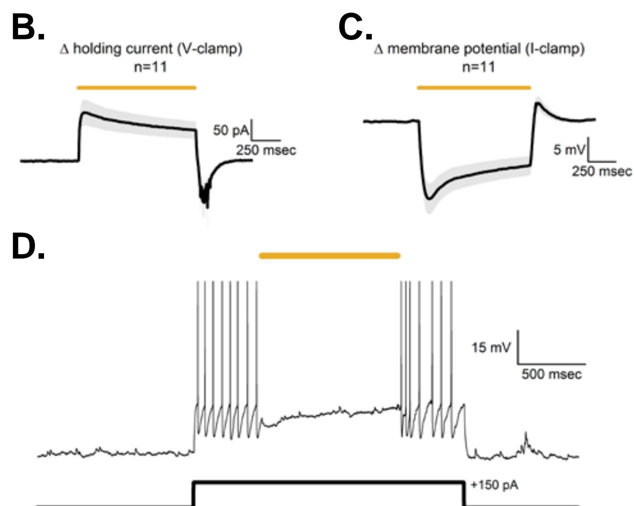


Figure 3

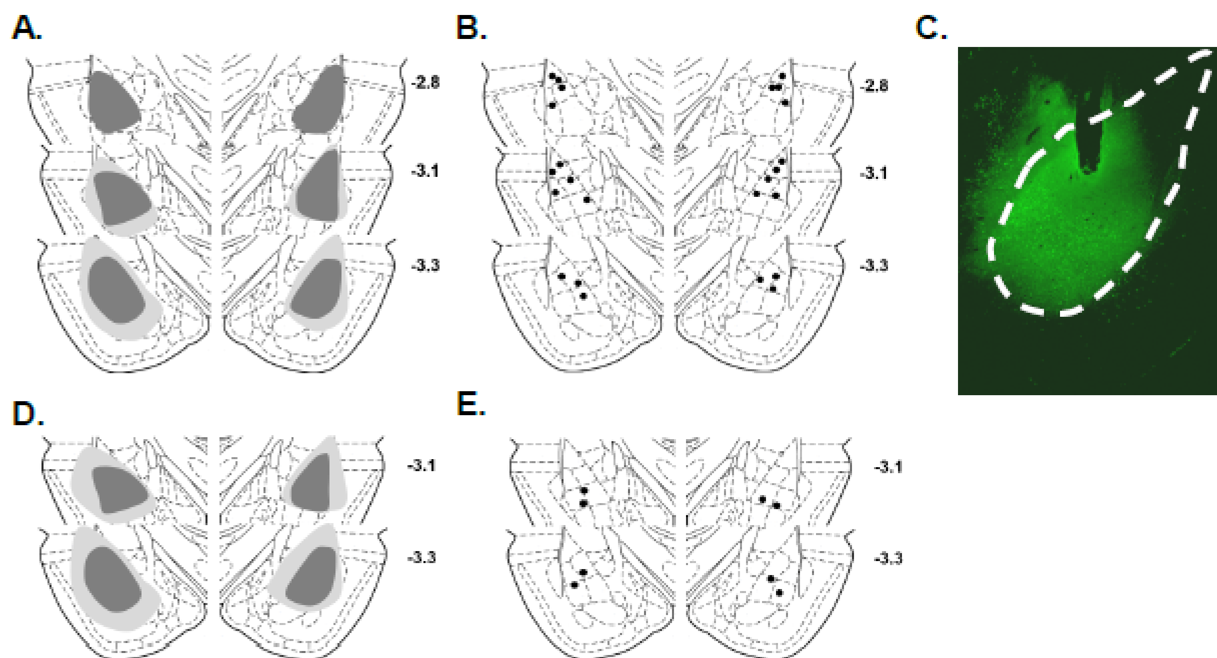
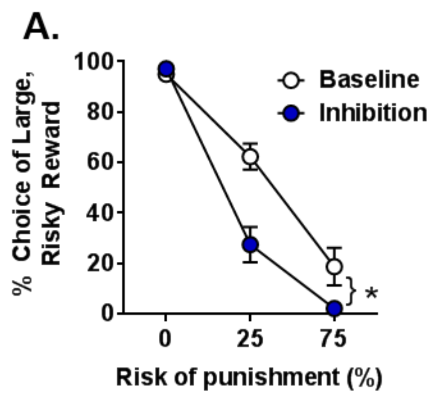


Figure 4



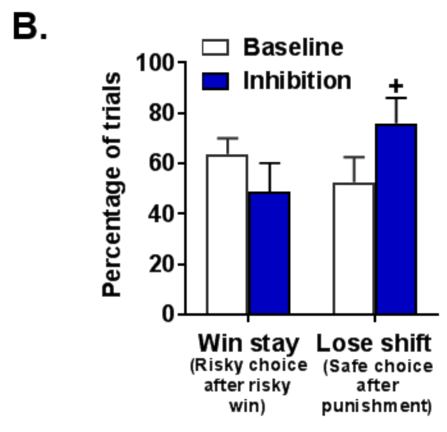
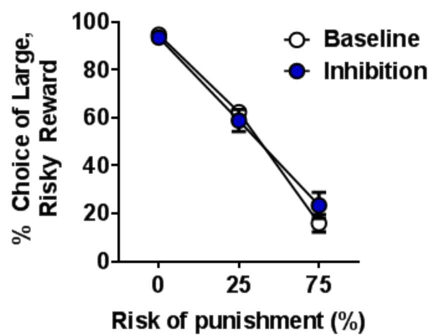
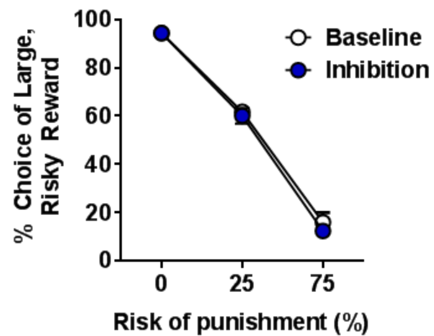


Figure 5





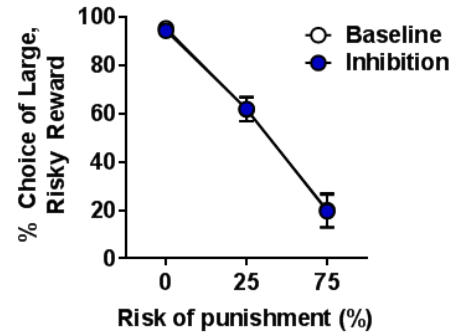
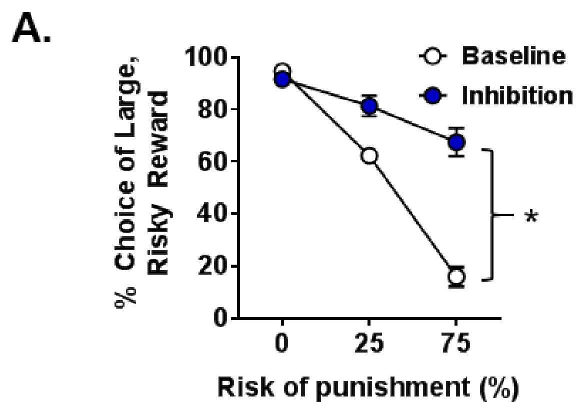
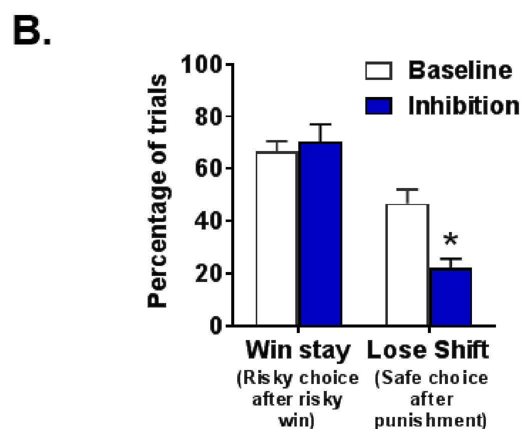


Figure 6





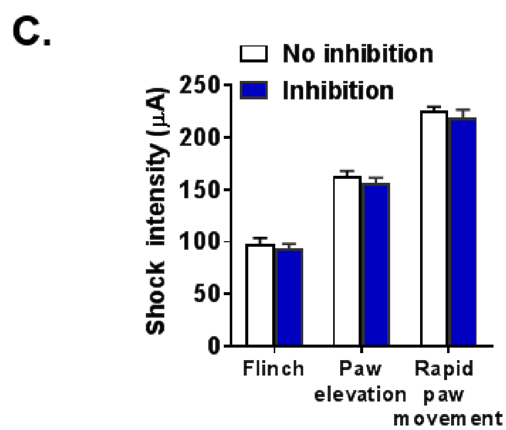


Figure 7