Enhanced preference for delayed rewards requires the basolateral amygdala and retrosplenial cortex

Merridee J. Lefner[1], Alexa P. Magnon[1], James M. Gutierrez[1], Matthew R. Lopez[1], and Matthew J. Wanat[1]

[1]Neurosciences Institute and Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249, USA

Corresponding Author:

Matthew J. Wanat

Neurosciences Institute

Department of Biology

University of Texas at San Antonio

One UTSA Circle

San Antonio, TX 78249

Matthew.wanat@utsa.edu

210.458.6684

Abstract:

'Sunk' or irrecoverable costs impact reward-based decisions across species. However, it is not known if these sunk costs elicit a sustained change in reward value. To address this, we developed behavioral task to examine how sunk temporal costs alter reward value and reward preference in rats. We first identified the relative preference between different flavored food pellets during a free-feeding test. Animals were then trained to experience the initial less preferred reward after long delays (high temporal cost) and the initial preferred reward after short delays (low temporal cost). This training regimen enhanced the consumption and preference for the initial less desirable food reward. We probed whether this change in preference required neural systems involved with reward valuation. Pharmacological manipulations and site-specific lesions were performed to examine the potential involvement of the dopamine system, the orbitofrontal cortex (OFC), the basolateral amygdala (BLA), and the retrosplenial cortex (RSC). The change in reward preference was unaffected by systemic dopamine receptor antagonism or OFC lesions. In contrast, lesions of the BLA or the RSC prevented the enhanced consumption and preference for the initial less desirable reward. These findings demonstrate that both the BLA and RSC participate in how sunk temporal costs alter reward value and reward preference.

Significance Statement:

From an economic standpoint, only future costs should factor into one's decisions. However, behavioral evidence across species illustrates that *past* costs can alter decisions. The goal of this study was to identify the neural systems responsible for past costs influencing subsequent actions. We demonstrate that delivering an initially less desirable reward after long delays (high temporal cost) subsequently increases the consumption and preference for that reward. Furthermore, we identified the basolateral amygdala and the retrosplenial cortex as essential nuclei for mediating this change in reward preference elicited by past temporal costs.

Introduction:

Reward-based decisions are driven by cost-benefit analyses[1,2]. A purely economic decision should be influenced solely by the future costs associated with earning a reward[3,4]. However, behavioral evidence across species demonstrate that *past* costs influence decision-making policies[5-12]. These 'sunk costs' are responsible for the enhanced preference for conditioned stimuli associated with rewards that follow substantial effort or delays[5-7]. Furthermore, sunk costs can result in fewer rewards earned, as subjects will persist in an action even though it is advantageous to disengage from their current behavior[11,12]. The effect of sunk costs on behavior has been primarily studied in tasks in which choosing one course of action comes at the expense of an alternative outcome[11]. However, this approach cannot determine if sunk costs increase the value of the chosen outcome and/or decrease the value of the alternative outcome.

To address this, we developed a rodent task to determine how sunk temporal costs prior to a reward delivery subsequently alters reward consumption and preference. We first identified the relative preference between different flavored food rewards in a free-feeding test. Rats then underwent training sessions in which the initial less preferred reward was delivered after long delays (high temporal cost), while the initial preferred reward was delivered after short delays (low temporal cost). The impact of these training sessions on food consumption and reward preference was then assessed in a second free-feeding test performed one day after the last training session. In this manner, we could test the hypothesis that high temporal costs imposed during training sessions subsequently increases the value (consumption) and relative preference for the initial less desirable reward.

By utilizing this behavioral task, we could identify brain regions responsible for changes in reward preference elicited by sunk temporal costs. Neural systems involved with reward valuation and learning are potential candidates for mediating changes in reward preference. These neurobiological processes are evident throughout the brain. For example, dopamine neurons convey the relative value between reward options and contribute to appetitive learning[13-18]. Pyramidal neurons in the orbitofrontal cortex (OFC) encode value-based parameters and participate in reward-learning and decision-making[19-25]. The basolateral

amygdala (BLA) is also a candidate due to its role in learning, reward valuation, and reward seeking[26-31]. Finally, the retrosplenial cortex (RSC) could be required for long-lasting changes in reward preference since RSC neurons contribute to memory formation and encode value-related signals[32-35]. Therefore, in this study we performed pharmacological manipulations and site-specific lesions to establish whether changes in preference elicited by incurred temporal costs require dopamine signaling, the OFC, the BLA, and/or the RSC.

Methods:

Subjects and surgery

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas at San Antonio. 300-350g male and female Sprague-Dawley rats (Charles River, MA) were pair-housed upon arrival and given ad libitum access to water and chow and maintained on a 12-hour light/dark cycle.

All surgeries were performed under isoflurane anesthesia and drug infusions were delivered at a rate of 0.1 μL/min. Surgical coordinates and injection volumes were based on prior research[25,31,36]. OFC-lesioned rats received injections of NMDA (12.5 μg/μL in saline vehicle; Tocris) at the following locations (relative to bregma): 3.0mm AP, ± 3.2mm ML, -5.2mm DV (0.05 μL); 3.0mm AP, ± 4.2mm ML, -5.2mm DV (0.1 μL); 4.0mm AP, ± 2.2mm ML, -3.8mm DV (0.1 μL); 4.0mm AP, ± 3.7mm ML, -3.8mm DV (0.1 μL). BLA-lesioned rats received injections of NMDA (20 μg/μL) at the following locations: -3.3mm AP, ± 4.6mm ML, -8.6mm DV (0.2 μL); -3.3mm AP, ± 4.6mm ML, -8.4mm DV (0.1 μL). RSC-lesioned rats received injections of NMDA (20 μg/μL) at the following locations: -1.6mm AP, ± 0.5mm ML, -1.3mm DV (0.26 μL); -2.8mm AP, ± 0.5mm ML, -1.3mm DV (0.26 μL); -4.0mm AP, ± 0.5mm ML, -1.3mm DV (0.26 μL injection); -5.3mm AP, ± 0.5mm ML, -2.0mm DV (0.26 μL injection). All sham surgeries involved lowering the injector to the respective injection sites[25,31,36]. Animals recovered for >1 week following surgery before beginning training.

Training

Rats were placed and maintained on mild food restriction (~15 g/day of standard lab chow) to target 90% free-feeding weight, allowing for an increase of 1.5% per week. Behavioral sessions were performed in chambers that had grid floors, a house light, and two food trays on a single wall. In free-feeding sessions, plastic barriers were placed over the food trays. Additionally, a plastic insert was placed over the grid floors that contained two fixed cups in which the food pellets were placed. Experimental 45mg sucrose pellets (chocolate flavor #F0025 and banana flavor #F0024; Bio-Serv) were placed in their home cages to minimize neophobia. Rats first underwent a free-feeding session (10mins) in which a single food pellet flavor was offered (6.5g total). On the following day, rats underwent a second free-feeding session in which the alternate flavor was offered (ordering counterbalanced between animals). For the free-feeding preference test, rats were allowed 10min to consume both chocolate and banana food pellets that were freely available in cups affixed to the floor. To ensure an ample supply of food, we provided 13g of each flavor, which was 3g higher than the maximal amount consumed in pilot studies. We identified which reward flavor was the Initial Preferred and the Initial Less Preferred based on the food consumed during this preference test.

Rats next underwent training sessions (1/day) in which one of the rewards was delivered non-contingently for a total of 50 pellets per session. The Initial Less Preferred reward was delivered after a 60 ± 5s inter-trial interval (ITI) in Long Delay sessions. The Initial Preferred reward was delivered after a 30 ± 5s ITI in Short Delay sessions. There were a total of 10 training sessions, which alternated between Long and Short Delay sessions with the first session counterbalanced between animals. Rats underwent a second free-feeding preference test following this training regimen (**Fig. 1A**). In a control experiment, rats were trained as described above except that a 45 ± 5s ITI was utilized for both the Initial Less Preferred and the Initial Preferred reward training sessions (see **Fig. 2A**). Injections of flupenthixol (225 μg/kg i.p., Tocris) or saline were administered 1 hour prior to training sessions.

Data analysis

The latency to respond was calculated as the length of time to make a head entry into the food port after a pellet was delivered. The latency measurement was capped at 25s (shortest

possible trial duration) to account for the rare occasion when a pellet was not retrieved before the subsequent trial. The preference ratio was calculated as the amount consumed of the Initial Less Preferred reward relative to the total food consumed during the free-feeding test. We performed statistical analyses using GraphPad Prism 8. The effect of training on behavioral outcomes were analyzed using a paired t-test or a mixed-effects model fit (restricted maximum likelihood method), repeated measures where appropriate, followed by a post-hoc Sidak's test. The Geisser-Greenhouse correction was applied to address unequal variances between groups. The significance level was set to $\alpha = 0.05$ for all tests. The full list of statistical analyses is in **Supplementary Data**.

Histology

Rats were intracardially perfused with 4% paraformaldehyde, and brains were removed and postfixed for at least 24 hours. Brains were subsequently placed in 15% and 30% sucrose solutions in phosphate−buffered saline. Brains were then flash frozen on dry ice, coronally sectioned, and stained with cresyl violet to verify the location and spread of the surgical lesions.

Results:

We developed a rodent task to examine how sunk temporal costs impact reward value and preference. Rats first underwent a free-feeding preference test between food rewards that had an identical nutritional profile but differed in flavor (chocolate or banana). We identified which was the Initial Preferred and the Initial Less Preferred flavor for each rat based on the food consumed during this free-feeding test. Rats then underwent training sessions in which one of the food rewards was delivered non-contingently for a total of 50 pellets per session. The Initial Less Preferred reward was delivered after a Long Delay (60 ± 5s ITI), while the Initial Preferred reward was delivered after a Short Delay (30 ± 5s ITI) in separate sessions. Rats underwent a total of 10 training sessions, which alternated between Long and Short Delay sessions, with the first session counterbalanced between animals. To assess the impact of this training regimen on food consumption and reward preference, rats underwent a second free-feeding preference test following training (different delay training; **Fig. 1A**).

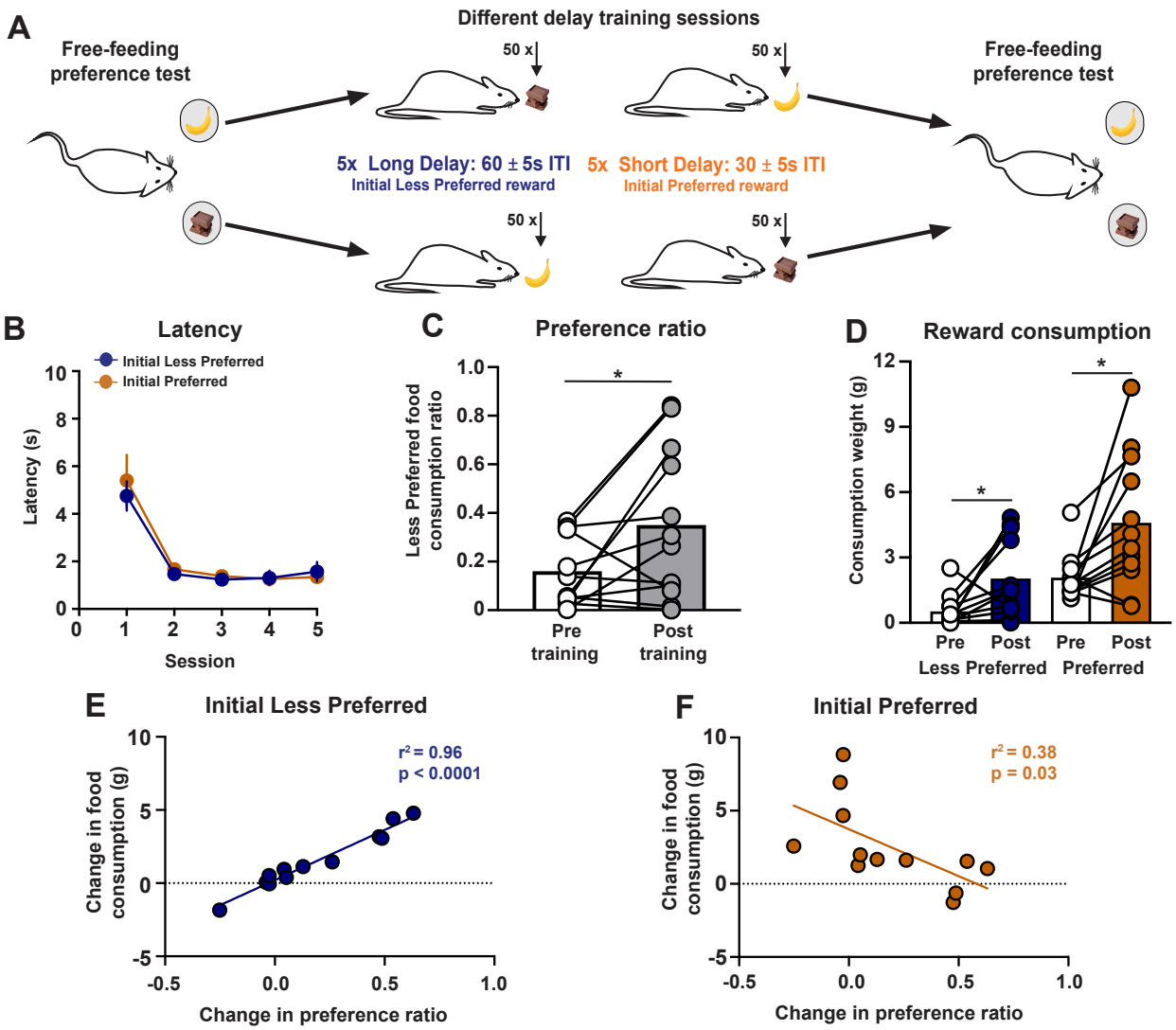

Figure 1. Increased preference for rewards delivered after high temporal costs. (A) Training schematic. (B) Latency to make a head entry into the food port after a reward is delivered for the Initial Less Preferred (Long Delay) and Initial Preferred (Short Delay) training sessions. (C) Preference ratio plotted as the amount of the Initial Less Preferred food consumed out of the total food consumed during preference tests. (D) Reward consumption for each flavor during the preference tests. (E,F) Linear regression relating the change in food consumption as a function of the change in the preference ratio. * $p < 0.05$.

Food pellets were delivered non-contingently and were not preceded by reward-predictive cues during training sessions. Rats decreased the post-reward latency into the food port across training, but there was no difference in the latency to respond between Long and Short Delay sessions (two-way mixed-effects analysis; session effect: $F_{(1.316,14.48)} = 54.17$, $p < 0.0001$; delay effect: $F_{(1,11)} = 0.16$, $p = 0.7002$; interaction effect: $F_{(1.30,12.43)} = 0.23$, $p = 0.6672$; n = 12 rats; **Fig. 1B**). This training regimen increased the rats' preference for the Initial Less Preferred reward (paired t-test: $t_{(11)} = 2.33$, $p = 0.0402$; **Fig. 1C**) due to increased consumption of

that reward option (post-hoc Sidak's test: $t_{(11)} = 2.65$, $p = 0.0451$; **Fig. 1D**). This cohort also exhibited an increased consumption of the Initial Preferred reward after training (post-hoc Sidak's test: $t_{(11)} = 2.98$, $p = 0.0251$; **Fig. 1D**). We next examined how the change in reward preference correlated with the change in food consumption across subjects. A stronger bias toward the Initial Less Preferred reward was positively related to the consumption of that reward option ($r^2 = 0.96$, $p < 0.0001$, **Fig. 1E**) and inversely related to the consumption of the Initial Preferred reward ($r^2 = 0.38$, $p = 0.03$, **Fig. 1F**).

The change in reward preference following the different delay training regimen could be due to the temporal costs imposed during training sessions or alternatively due to increased exposure with the Initial Less Preferred reward. To address this possibility, we trained a separate group of rats as described above except that a 45 ± 5s ITI was used for both the Initial Less Preferred and Initial Preferred reward training sessions (same delay training; **Fig. 2A**). There was no difference in the latency between sessions (two-way mixed-effects analysis; session effect: $F_{(1.341,8.049)} = 2.97$, $p = 0.1178$; delay effect: $F_{(1,6)} = 14.93$, $p = 0.2675$; interaction effect: $F_{(2.094,12.04)} = 0.62$, $p = 0.5636$; n = 7 rats; **Fig. 2B**). Rats undergoing the same delay training regimen did not alter their initial reward preference (paired t-test: $t_{(6)} = 0.91$, $p = 0.40$; **Fig. 2C**), as there was no change in consumption of the Initial Less Preferred reward (post-hoc Sidak's test: $t_{(6)} = 1.56$, $p = 0.3104$; **Fig. 2D**) and an increased consumption of the Initial Preferred reward (post-hoc Sidak's test: $t_{(6)} = 3.71$, $p = 0.0199$; **Fig. 2D**). Finally, there was no relationship between changes in preference and the consumption of the Initial Less Preferred reward ($r^2 = 0.16$, $p = 0.38$, **Fig. 2E**) or the Initial Preferred reward ($r^2 = 0.00$, $p = 0.97$, **Fig. 2F**) when the temporal cost was held constant across all training sessions. These experiments collectively demonstrate that the enhanced preference for the Initial Less Preferred reward following the different delay training regimen was due to differential temporal costs experienced during training sessions and not a result of increased exposure to the rewards over training.

We next sought to identify the neural systems responsible for sunk temporal costs altering reward preference. Dopamine neurons respond to rewards to convey the relative preference between options as well as changes in reward value[13-16,37]. Additionally, the dopamine system contributes to reward learning[17,18]. Therefore, the increased preference for rewards that follow

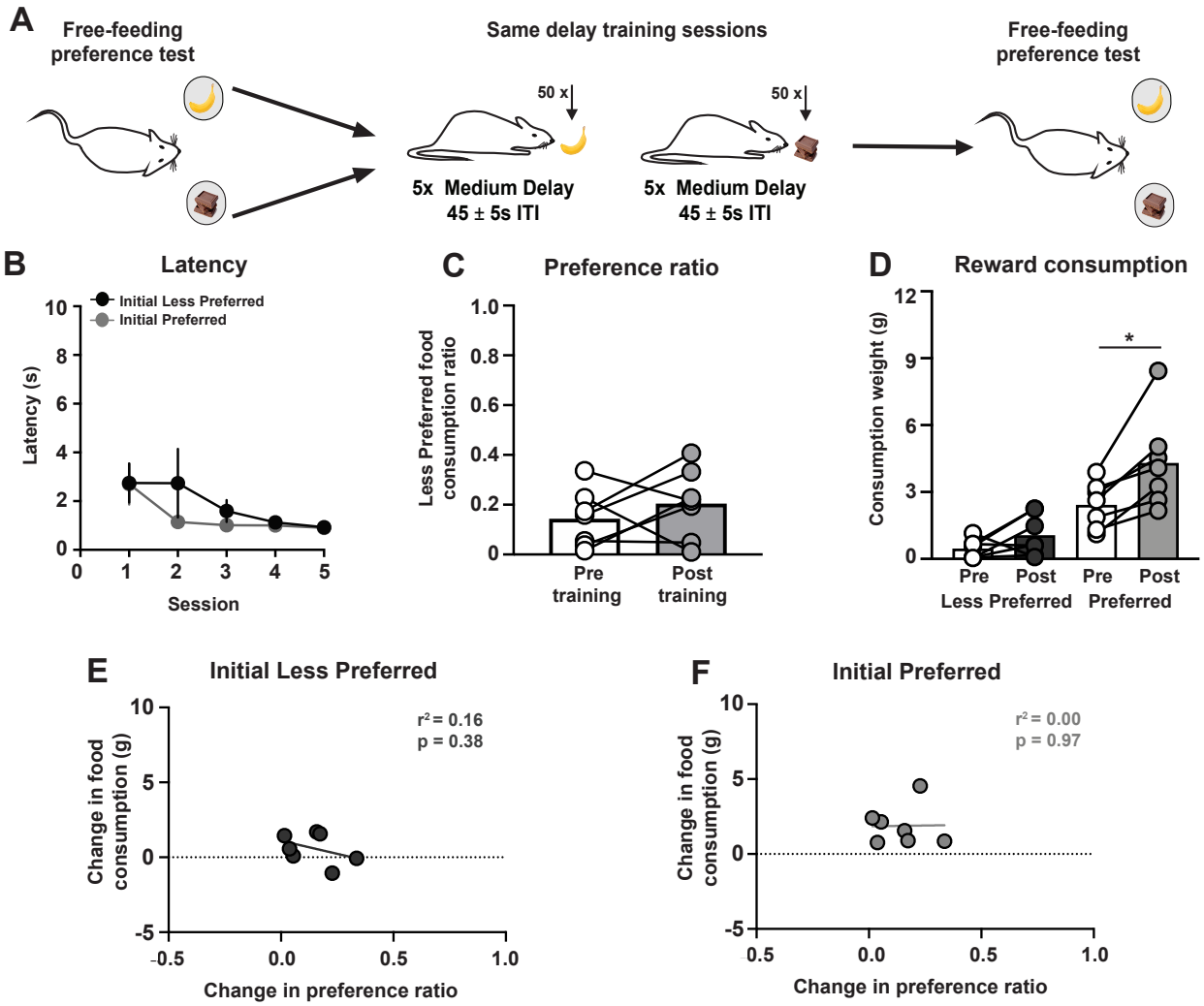

Figure 2. No change in reward preference following same delay training sessions. (A) Training schematic. (B) Latency to make a head entry into food port after a reward is delivered for the Initial Less Preferred and the Initial Preferred training sessions. (C) Preference ratio. (D) Reward consumption for each flavor during the preference tests. (E,F) Linear regression relating the change in food consumption as a function of the change in the preference ratio. * $p < 0.05$.

greater delays could be mediated by dopamine signaling. To address this possibility, we systemically administered flupenthixol prior to the different delay training sessions and examined the consequence on reward preference (**Fig. 3A**). Flupenthixol treatment slowed the time to retrieve the reward during training sessions (three-way mixed-effects analysis; session effect: $F_{(4,88)} = 43.04$, $p < 0.0001$; treatment effect: $F_{(1,22)} = 6.18$, $p = 0.016$; delay effect: $F_{(1,22)} = 0.02$, $p = 0.8840$; interaction effect: $F_{(4,88)} = 1.53$, $p = 0.2010$; n = 12 saline, n = 12 flupenthixol rats; **Fig. 3B**). However, antagonizing dopamine receptors during training sessions did not prevent the increased preference for the Initial Less Preferred reward (two-way mixed-effects

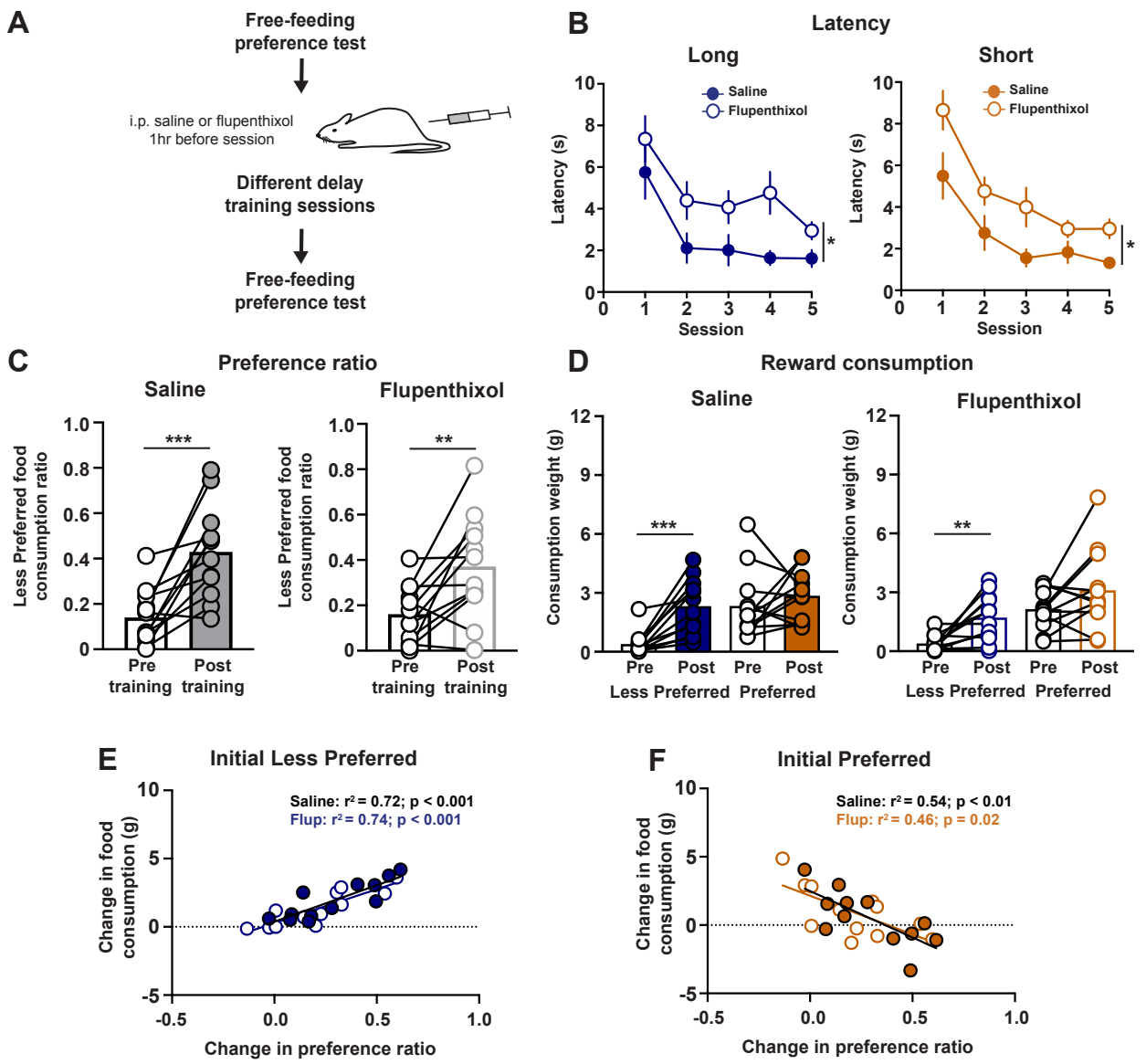

Figure 3. The change in reward preference does not involve dopamine signaling. (A) Training schematic. (B) Latency to make a head entry into the food port after a reward is delivered during training sessions in rats that received injections of saline or flupenthixol. (C) Preference ratio in rats receiving saline (left) or flupenthixol (right) injections. (D) Reward consumption for each flavor during the preference tests in rats receiving saline (left) or flupenthixol (right) injections. (E,F) Linear regression relating the change in food consumption as a function of the change in the preference ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

analysis; training effect: $F_{(1,22)} = 30.86$, $p < 0.0001$; treatment effect: $F_{(1,22)} = 0.11$, $p = 0.7384$; interaction effect: $F_{(1,22)} = 0.77$, $p = 0.3901$; post-hoc Sidak's test; saline: $t_{(22)} = 4.55$, $p = 0.0003$; flupenthixol: $t_{(22)} = 3.31$, $p = 0.0064$; **Fig. 3C**). Both saline- and flupenthixol-treated rats increased the consumption of the Initial Less Preferred reward (post-hoc Sidak's test; saline: $t_{(11)} = 4.92$, $p = 0.0009$; flupenthixol: $t_{(11)} = 3.54$, $p = 0.0092$; **Fig. 3D**) with no change in consumption of the Initial Preferred reward (post-hoc Sidak's test; saline: $t_{(11)} = 0.91$, $p = 0.6169$;

flupenthixol: $t_{(11)} = 1.77$, $p = 0.1984$; **Fig. 3D**). The change in reward preference was positively related to the consumption of the Initial Less Preferred reward (saline: $r^2 = 0.72$, $p < 0.001$, flupenthixol: $r^2 = 0.74$, $p < 0.001$; **Fig. 3E**) and inversely related to the consumption of the Initial Preferred reward in both saline- and flupenthixol-treated animals (saline: $r^2 = 0.54$, $p < 0.01$, flupenthixol: $r^2 = 0.46$, $p = 0.02$, **Fig. 3F**). These data collectively demonstrate that the dopamine system regulates the latency to respond during training sessions, but does not mediate the change in reward preference elicited by sunk temporal costs.

The OFC encodes value-based parameters and participates in reward-learning and decision-making[19-25], which suggests the OFC could mediate the change in reward preference. We performed excitotoxic lesions of the OFC or sham surgeries prior to the initial preference test (**Fig. 4A-B**). The latency to retrieve the food pellet did not differ between sham and OFC-lesioned rats across training sessions (three-way mixed-effect analysis; session effect: $F_{(4,72)} = 42.42$, $p < 0.0001$; treatment effect: $F_{(1,18)} = 0.099$, $p = 0.7556$; delay effect: $F_{(1,18)} = 0.05$, $p = 0.8211$; interaction effect: $F_{(4,72)} = 0.90$, $p = 0.4697$; n = 10 sham rats, n = 10 lesion rats; **Fig. 4C**). Lesioning the OFC did not prevent the change in preference following training (two-way mixed-effects analysis; training effect: $F_{(1,18)} = 28.64$, $p < 0.0001$; treatment effect: $F_{(1,18)} = 0.35$, $p = 0.5599$; interaction effect: $F_{(1,18)} = 0.0001$, $p = 0.99$; **Fig. 4D**). Training resulted in an enhanced preference for the Initial Less Preferred reward in sham surgery and OFC-lesioned rats (post-hoc Sidak's test; sham: $t_{(18)} = 3.78$, $p = 0.0028$; lesion: $t_{(18)} = 3.79$, $p = 0.0027$; **Fig. 4D**). Both groups exhibited a selective increase in the consumption of the Initial Less Preferred reward (post-hoc Sidak's test; sham: $t_{(9)} = 3.89$, $p = 0.0074$; lesion: $t_{(9)} = 3.26$, $p = 0.0195$; **Fig. 4E**) with no change in the consumption of the Initial Preferred reward (post-hoc Sidak's test; sham: $t_{(9)} = 0.47$, $p = 0.8773$; lesion: $t_{(9)} = 0.73$, $p = 0.7312$; **Fig. 4E**). The change in reward preference was positively related to the consumption of the Initial Less Preferred reward (sham: $r^2 = 0.95$, $p < 0.001$, lesion: $r^2 = 0.85$, $p < 0.001$, **Fig. 4F**) and inversely related to the consumption of the Initial Preferred reward in sham surgery and OFC-lesioned rats (sham: $r^2 = 0.68$, $p < 0.01$, lesion: $r^2 = 0.48$, $p = 0.03$, **Fig. 4G**). Therefore, the OFC is not involved with the change in reward preference following different delay training sessions.

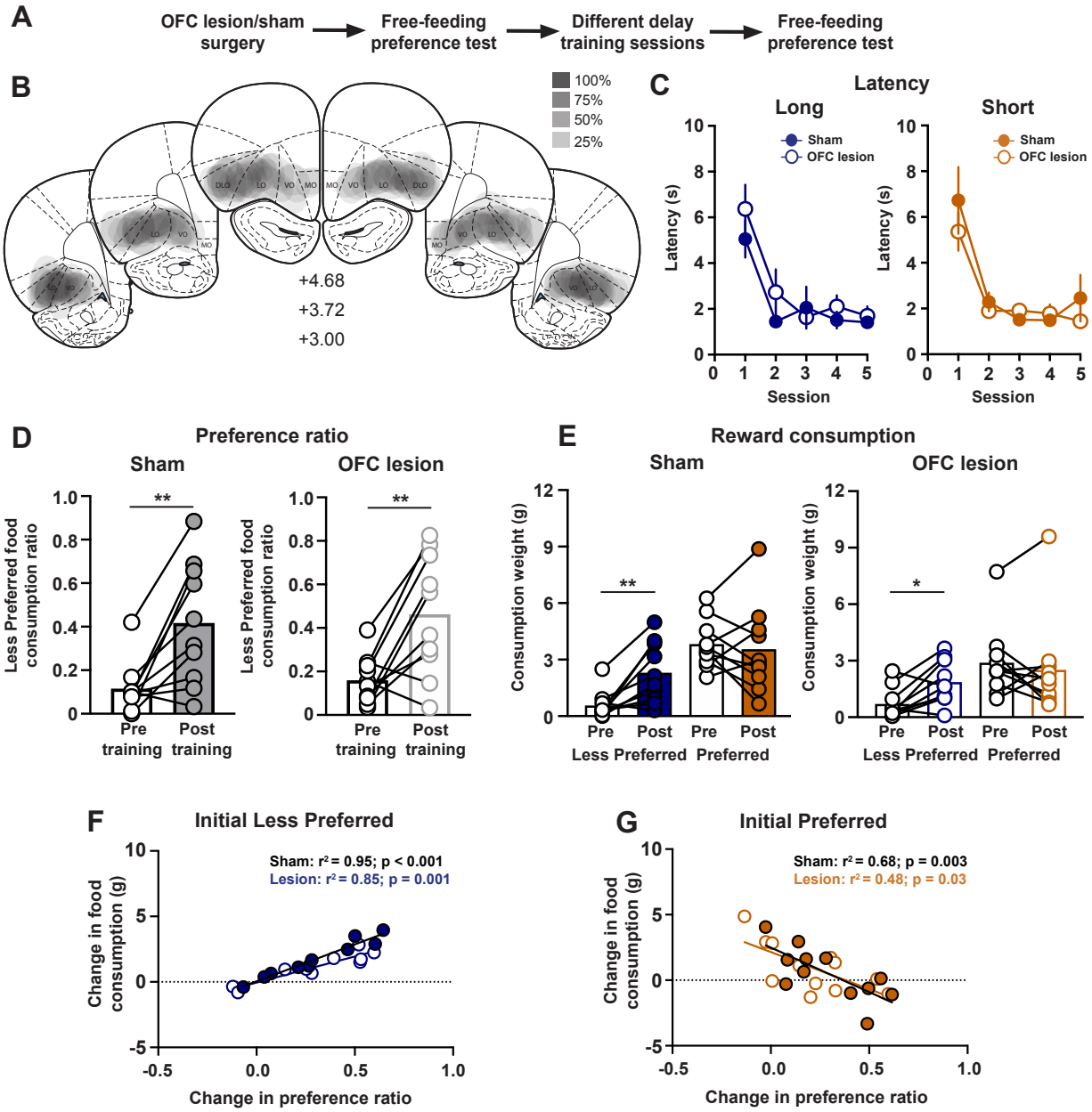

Figure 4. The orbitofrontal cortex (OFC) is not required for the change in reward preference. (A) Training schematic. (B) The extent of OFC lesions across three coronal planes with the anterior distance from bregma (millimeters) indicated. (C) Latency to make a head entry into the food port after a reward is delivered during training sessions in sham or OFC-lesioned rats. (D) Preference ratio in sham (left) or OFC-lesioned (right) rats. (E) Reward consumption for each flavor during the preference tests in sham (left) or OFC-lesioned (right) rats. (F,G) Linear regression relating the change in food consumption as a function of the change in the preference ratio. * $p < 0.05$, ** $p < 0.01$.

Increasing evidence highlights the BLA is a critical nucleus that contributes to learning, reward valuation, and reward seeking[26-30]. As such, the BLA could potentially mediate the change in reward preference following the different delay training regimen. Rats underwent a surgery to lesion the BLA or a sham procedure prior to the initial preference test (**Fig. 5A-B**).

There was no difference in the latency to retrieve a food pellet between sham surgery and BLA-lesioned rats across training sessions (three-way mixed-effect analysis; session effect: $F_{(4,56)} = 71.96$, $p < 0.0001$; treatment effect: $F_{(1,14)} = 0.19$, $p = 0.6702$; delay effect: $F_{(1,14)} = 0.26$, $p = 0.6190$; interaction effect: $F_{(4,56)} = 0.05$, $p = 0.9948$; n = 8 sham rats, n = 8 lesion rats; **Fig. 5C**). Lesioning the BLA prevented the change in preference following training (two-way mixed-effects analysis; training effect: $F_{(1,14)} = 30.69$, $p < 0.0001$; treatment effect: $F_{(1,14)} = 3.55$, $p = 0.0805$; interaction effect: $F_{(1,14)} = 15.17$, $p = 0.0016$; **Fig. 5D**). Sham surgery rats exhibited an enhanced preference for the Initial Less Preferred reward (post-hoc Sidak's test; sham: $t_{(14)} = 6.67$, $p < 0.0001$; **Fig. 5D**) which was due to a selective increase in the consumption of the Initial Less Preferred reward (post-hoc Sidak's test; Initial Less Preferred flavor: $t_{(7)} = 5.99$, $p = 0.0011$; Initial Preferred flavor: $t_{(7)} = 2.2$, $p = 0.1233$; **Fig. 5E**). In contrast, BLA lesions prevented the change in reward preference (post-hoc Sidak's test; lesion: $t_{(14)} = 1.16$, $p = 0.4588$; **Fig. 5D**), as rats selectively increased the consumption of the Initial Preferred reward (post-hoc Sidak's test; Initial Less Preferred flavor: $t_{(7)} = 2.4$, $p = 0.0931$; Initial Preferred flavor: $t_{(7)} = 4.13$, $p = 0.0088$; **Fig. 5E**). The change in reward preference in sham surgery rats was inversely related to the change in consumption of the Initial Preferred reward (Initial Less Preferred: $r^2 = 0.02$, $p = 0.14$; Initial Preferred: $r^2 = 0.60$, $p = 0.02$; **Fig. 5F-G**). A similar trend was observed in BLA-lesioned rats (Initial Less Preferred: $r^2 = 0.38$, $p = 0.1$; Initial Preferred: $r^2 = 0.52$, $p = 0.04$; **Fig. 5F-G**). These data collectively illustrate that the BLA is required for the change in reward preference elicited by sunk temporal costs.

The RSC is another brain region that could participate in sustained changes in reward preference given its role in learning, and that RSC neurons respond to rewards to encode value-related signals[32-35]. To address this possibility, rats underwent an RSC lesion or a sham surgery prior to the initial preference test (**Fig. 6A-B**). There was no difference in the post-reward latency to retrieve a food pellet between sham surgery and RSC-lesioned rats across training sessions (three-way mixed-effect analysis; session effect: $F_{(4,56)} = 43.99$, $p < 0.0001$; treatment effect: $F_{(1,53)} = 0.18$, $p = 0.6756$; delay effect: $F_{(1,14)} = 0.59$, $p = 0.4543$; interaction effect: $F_{(4,53)} = 1.26$, $p = 0.2952$; n = 8 sham rats, n = 8 lesion rats; **Fig. 6C**). Lesioning the RSC prevented the change in preference following training (two-way mixed-effects analysis; training effect: $F_{(1,14)} = 10.64$,

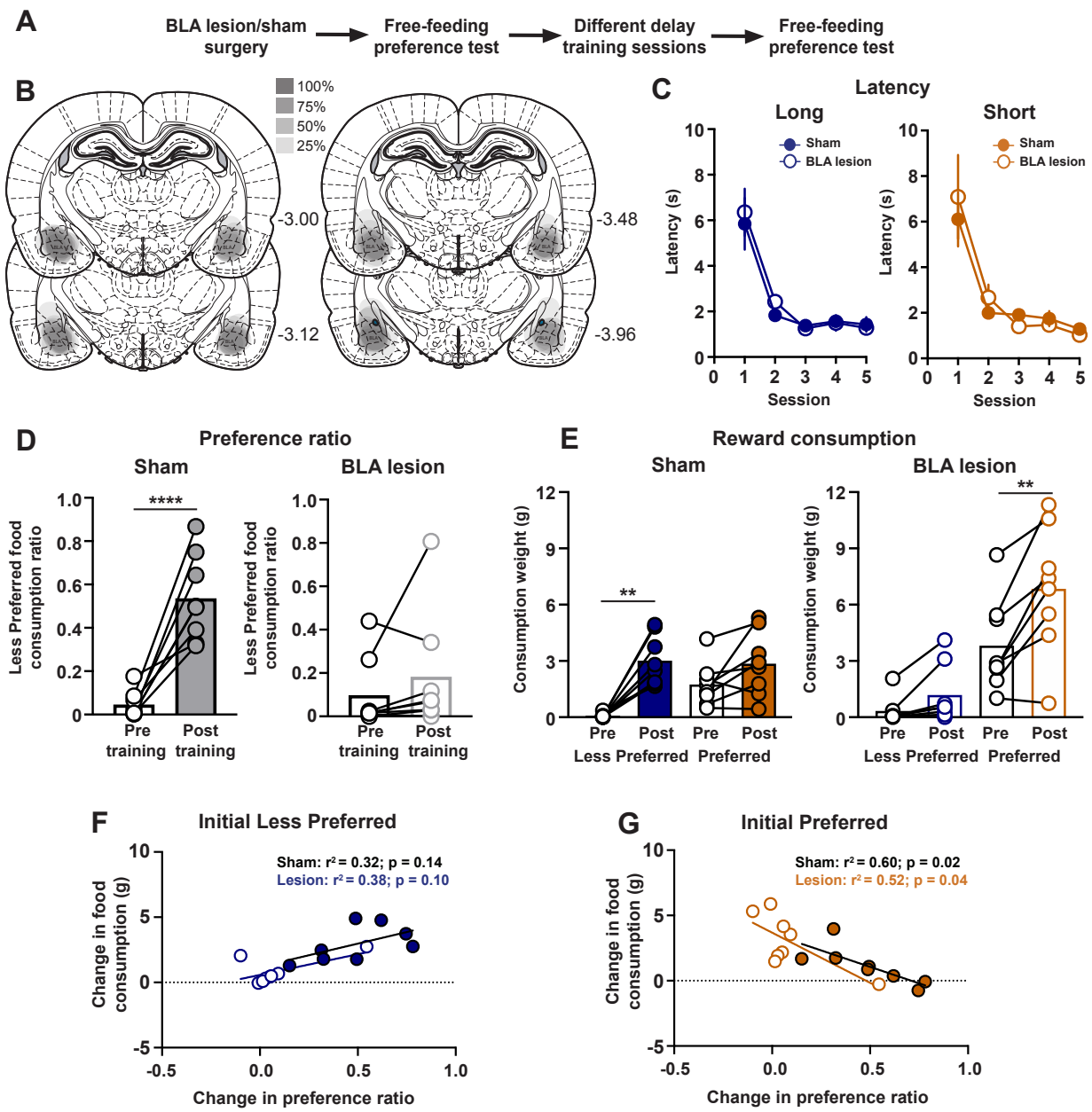

Figure 5. The basolateral amygdala (BLA) is required for the change in reward preference. (A) Training schematic. (B) The extent of BLA lesions across four coronal planes with the anterior distance from bregma (millimeters) indicated. (C) Latency to make a head entry into the food port after a reward is delivered during training sessions in sham or BLA-lesioned rats. (D) Preference ratio in sham (left) or BLA-lesioned (right) rats. (E) Reward consumption for each flavor during the preference tests in sham (left) or BLA-lesioned (right) rats. (F,G) Linear regression relating the change in food consumption as a function of the change in the preference ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

p = 0.0057; treatment effect: $F_{(1,14)}$ = 12.69, p = 0.0031; interaction effect: $F_{(1,14)}$ = 17.36, p = 0.001; **Fig. 6D**). Sham rats exhibited an enhanced preference for the Initial Less Preferred reward (post-hoc Sidak's test; sham: $t_{(14)}$ = 5.25, p = 0.0002; **Fig. 6D**), which was accompanied by an increased consumption of both rewards following training (post-hoc Sidak's test; Initial Less

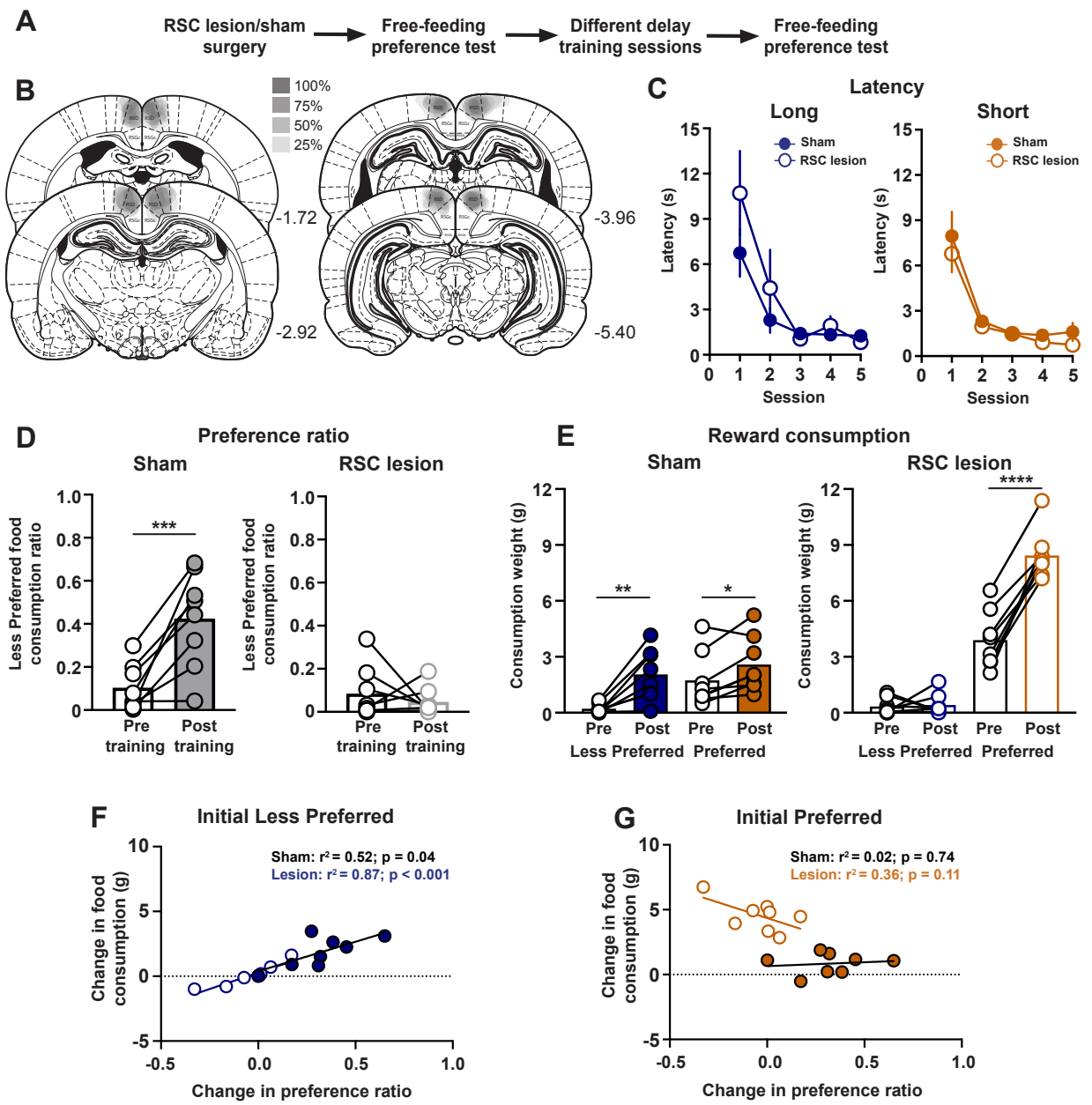

Figure 6. The retrosplenial cortex (RSC) is required for the change in reward preference. (A) Training schematic. (B) The extent of RSC lesions across four coronal planes with the anterior distance from bregma (millimeters) indicated. (C) Latency to make a head entry into the food port after a reward is delivered during training sessions in sham or RSC-lesioned rats. (D) Preference ratio in sham (left) or RSC-lesioned (right) rats. (E) Reward consumption for each flavor during the preference tests in sham (left) or RSC-lesioned (right) rats. (F,G) Linear regression relating the change in food consumption as a function of the change in the preference ratio. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Preferred flavor: $t_{(7)} = 4.31$, $p = 0.0071$; Initial Preferred flavor: $t_{(7)} = 2.95$, $p = 0.0424$; **Fig. 6E**). In contrast, there was no change in preference in the RSC lesion group (post-hoc Sidak's test; lesion: $t_{(14)} = 0.64$, $p = 0.7814$; **Fig. 6D**), as rats selectively increased the consumption of the Initial Preferred reward (post-hoc Sidak's test; Initial Less Preferred flavor: $t_{(7)} = 0.28$, $p =$

0.9548; Initial Preferred flavor: $t_{(7)} = 10.63$, $p < 0.0001$; **Fig. 6E**). The relative change in reward preference and consumption of the Initial Less Preferred reward was positively correlated across RSC lesion and sham groups (Initial Less Preferred sham: $r^2 = 0.52$, $p = 0.04$, lesion: $r^2 = 0.87$, $p < 0.001$; Initial Preferred sham: $r^2 = 0.02$, $p = 0.74$; lesion: $r^2 = 0.36$, $p = 0.11$; **Fig. 6F-G**). Our results highlight that both the BLA and RSC are necessary for increasing the preference toward an initial less desirable reward option.

When examining all subjects trained on the different delay regimen, the change in reward preference was positively related to the consumption of the Initial Less Preferred reward ($r^2 = 0.76$, $p < 0.001$) and inversely related to the consumption of the Initial Preferred reward ($r^2 = 0.47$, $p < 0.001$; n = 88 rats). This suggests that rats exhibiting a robust bias toward the Initial Less Preferred reward could potentially decrease the consumption of the Initial Preferred reward following training. We therefore analyzed the relative food consumption across subjects based upon if there was a mild bias toward the Initial Preferred reward (**Fig. 7A**, orange), a mild bias toward the Initial Less Preferred reward (**Fig. 7A**, light blue), or a robust bias toward the Initial Less Preferred reward (**Fig. 7A**, dark blue). The relative change in food consumption for each reward differed according to the change in reward preference (two-way mixed-effects analysis; flavor effect: $F_{(1,85)} = 3.89$, $p = 0.0517$; change in preference effect: $F_{(2,85)} = 5.78$, $p = 0.0044$; interaction effect: $F_{(2,85)} = 116.2$, $p < 0.0001$; **Fig. 7B-D**). Rats exhibiting a mild bias toward the original reward preference selectively increased the consumption of the Initial Preferred reward (one-sample t-test relative to 0: Initial Less Preferred: $t_{(15)} = 0.69$, $p = 0.5007$; Initial Preferred: $t_{(15)} = 8.39$, $p < 0.0001$; **Fig. 7B**). Rats with a mild bias toward the Initial Less Preferred reward increased the consumption of both rewards (one-sample t-test relative to 0: Initial Less Preferred: $t_{(44)} = 88.88$, $p < 0.0001$; Initial Preferred: $t_{(44)} = 5.47$, $p < 0.0001$; **Fig. 7C**). Interestingly, rats with a robust bias toward the Initial Less Preferred reward increased the consumption of the Initial Less Preferred and *decreased* the consumption of the Initial Preferred reward (one-sample t-test relative to 0: Initial Less Preferred: $t_{(26)} = 16.99$, $p < 0.0001$; Initial Preferred: $t_{(26)} = 2.23$, $p = 0.0348$; **Fig. 7D**). Together, these results highlight that sunk temporal costs toward one reward can positively impact the value of that reward as well as negatively impact the value of the alternative option.

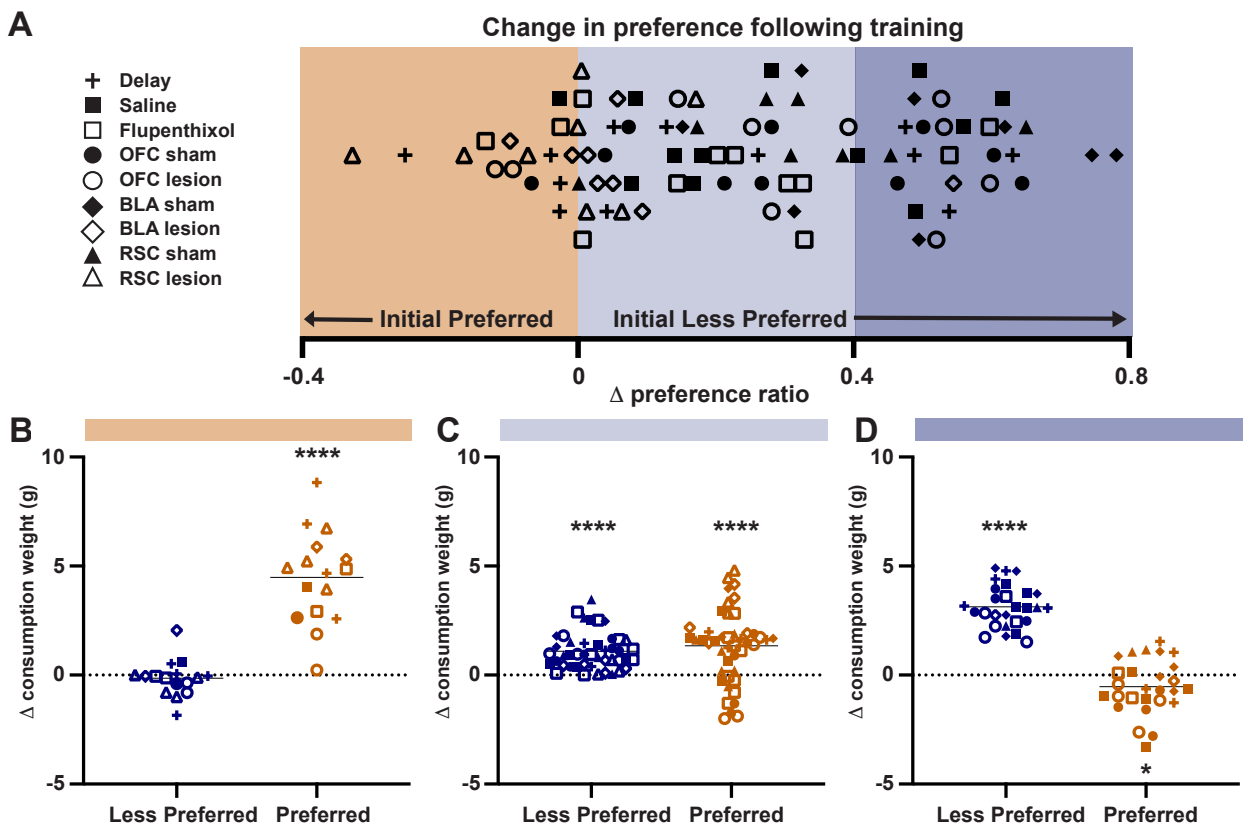

Figure 7. Relating the change in preference to the change in the food consumption. (A) Change in the preference ratio across all rats that underwent the different delay training sessions. Color overlays denote a mild bias toward the Initial Preferred reward (orange; change in the preference ratio < 0), a mild bias toward the Initial Less Preferred reward (light blue; change in the preference ratio between 0 and 0.4), and a robust bias toward the Initial Less Preferred reward (dark blue; change in the preference ratio > 0.4). (B) Change in the food consumption in rats that displayed a mild bias toward the Initial Preferred reward. (C) Change in the food consumption in rats that displayed a mild bias toward the Initial Less Preferred reward. (D) Change in the food consumption in rats that displayed a robust bias toward the Initial Less Preferred reward. * $p < 0.05$, **** $p < 0.0001$.

Discussion:

Sunk costs are irrecoverable past costs that can influence future decisions. Human studies illustrate behavioral consequences from a variety of sunk costs, including embarrassment, political, personal, and financial, though these costs are challenging to model in animals[10,12,38-40]. Research in pigeons and humans demonstrate that subjects will display increased preference for an outcome that follows greater effort[5-7,9]. However, increasing effort (e.g. more responses) often requires more time, thereby confounding effort costs with temporal costs[41]. As such, exclusively manipulating temporal costs provides an attractive strategy to examine the impact of sunk costs

across species[11,42]. Recent studies have examined the behavioral impact of sunk temporal costs using a choice-based foraging task[11,42,43]. In this foraging task, choosing one option comes at the expense of a potential alternative outcome, so one cannot determine if sunk costs increase the value of the chosen outcome and/or decrease the value of the alternative outcome. To determine how sunk temporal costs impact reward value, we quantified the change in reward consumption and reward preference using a free-feeding assay. We found that sunk temporal costs resulted in increased consumption of the Initial Less Preferred reward after training. Interestingly, we observed that a robust change in preference toward the Initial Less Preferred reward were a product of changes in consumption of *both* rewards: increased consumption of the Initial Less Preferred reward as well as decreased consumption of the Initial Preferred reward. Together, these findings indicate that sunk temporal costs toward one reward can positively impact the value of that reward as well as negatively impact the value of the alternative option.

Prior research has identified a number of factors that can alter reward preference. For example, allowing free access to one reward option prior to a preference test will devalue that reward and elicit a transient change in preference[44-46]. Long term changes in preference can be induced by pairing a reward with an aversive outcome[47]. Here, we utilized a long temporal delay before the delivery of an initial less desirable option to subsequently increase preference for that reward. Critically, this post-training preference test is performed in a distinct context and on the day following the last training session. Taken together, these results demonstrate that the temporal costs imposed during training sessions can induce a sustained change in preference without the need for altering satiety or pairing the outcome with an aversive stimulus.

Antagonizing dopamine receptors during training sessions increased the latency to retrieve the reward, consistent with dopamine's role in regulating locomotor activity[48,49]. While the dopamine system is not required for preference to update in our task, it has been previously found that dopamine signals reflect changes in preference induced by state-specific satiety, and that dopamine receptor antagonism impairs preference changes arising from conditioned taste aversion[45,46,50]. We also found that OFC lesions failed to prevent changes in reward preference. These results are consistent with previous reports demonstrating that lesions to the OFC did not alter outcome preference or behavioral flexibility[51,52]. However, prior research has found that

OFC lesions impair the ability to update choices following selective satiation[23]. In addition, OFC lesions do not prevent changes in preference induced by taste aversion, but can disrupt devaluation of the associated cue[53]. While the dopamine system and the OFC are active participants in other forms of preference changes, our findings suggest these two systems do not regulate changes in preference elicited by sunk temporal costs.

Our results demonstrate that lesioning the BLA prior to training sessions prevented sunk temporal costs from influencing changes in preference. Previous studies have implicated BLA neurons in reward learning and memory formation[26,27]. However, the BLA also has a role in reward preference as studies demonstrate the BLA maintains representations of appetitive and economic value[30,54,55]. BLA neurons additionally encode the value of a chosen reward and inactivating the BLA decreased choice for a more preferred option[56,57]. Moreover, BLA inactivation impairs choice following reinforcer devaluation[57,58]. Our findings coupled with prior work collectively highlight a critical role for the BLA in updating reward value.

The RSC has been well-studied for its contributions to memory formation and spatial navigation[32,59,60]. However, increasing evidence illustrates that the RSC also contains reward-responsive neurons that encode reward value[33-35]. Furthermore, inactivation of the RSC impaired the ability to adapt behavior based on the reward history[34]. Consistent with the RSC's role in reward-based behavior, we find that RSC lesions prior to training sessions prevented the change in preference induced by sunk temporal costs.

Lesions of the BLA or the RSC led to similar behavioral outcomes: preventing the change in reward preference without producing overt motor impairments. These results indicate the presence of a circuit involving the BLA and RSC to update reward value. In support, the BLA sends direct projections to the RSC[61]. We note that the lesion experiments demonstrate the necessity of the BLA and RSC in changing reward preference. However, future studies are needed to determine if the BLA and RSC are responsible for acquiring and/or expressing changes in reward preference. Taken together, our data highlights a previously unappreciated role for the BLA and RSC in mediating changes in preference following sunk temporal costs.

Acknowledgements:

This work was supported by National Institutes of Health grants DA033386 (MJW) and DA042362 (MJW) and the Mind Science Foundation Research Award (MJL).

Competing Interest Statement: The authors declare no competing interests.

Author Contributions:

MJL, APM, JMG, MRL performed the experiments and analyzed the data. MJL and MJW designed the experiments and wrote the manuscript.

References:

1 Phillips, P. E., Walton, M. E. & Jhou, T. C. Calculating utility: preclinical evidence for cost-benefit analysis by mesolimbic dopamine. *Psychopharmacology (Berl)* **191**, 483-495, doi:10.1007/s00213-006-0626-6 (2007).

2 Rangel, A., Camerer, C. & Montague, P. R. A framework for studying the neurobiology of value-based decision making. *Nat Rev Neurosci* **9**, 545-556, doi:10.1038/nrn2357 (2008).

3 Kalenscher, T. & Pennartz, C. M. Is a bird in the hand worth two in the future? The neuroeconomics of intertemporal decision-making. *Prog Neurobiol* **84**, 284-315, doi:10.1016/j.pneurobio.2007.11.004 (2008).

4 Loewenstein, G., Rick, S. & Cohen, J. D. Neuroeconomics. *Annu Rev Psychol* **59**, 647-672, doi:10.1146/annurev.psych.59.103006.093710 (2008).

5 Alessandri, J., Darcheville, J. C., Delevoye-Turrell, Y. & Zentall, T. R. Preference for rewards that follow greater effort and greater delay. *Learn Behav* **36**, 352-358, doi:10.3758/LB.36.4.352 (2008).

6 Clement, T. S., Feltus, J. R., Kaiser, D. H. & Zentall, T. R. "Work ethic" in pigeons: reward value is directly related to the effort or time required to obtain the reward. *Psychon Bull Rev* **7**, 100-106, doi:10.3758/bf03210727 (2000).

7 Friedrich, A. M. & Zentall, T. R. Pigeons shift their preference toward locations of food that take more effort to obtain. *Behav Processes* **67**, 405-415, doi:10.1016/j.beproc.2004.07.001 (2004).

8 Marsh, B. & Kacelnik, A. Framing effects and risky decisions in starlings. *Proc Natl Acad Sci U S A* **99**, 3352-3355, doi:10.1073/pnas.042491999 (2002).

9 Navarro, A. D. & Fantino, E. The sunk cost effect in pigeons and humans. *J Exp Anal Behav* **83**, 1-13, doi:10.1901/jeab.2005.21-04 (2005).

10 Fujino, J. *et al.* Neural mechanisms and personality correlates of the sunk cost effect. *Sci Rep* **6**, 33171, doi:10.1038/srep33171 (2016).

11 Sweis, B. M. *et al.* Sensitivity to "sunk costs" in mice, rats, and humans. *Science* **361**, 178-181, doi:10.1126/science.aar8644 (2018).
12 Haller, A. & Schwabe, L. Sunk costs in the human brain. *Neuroimage* **97**, 127-133, doi:10.1016/j.neuroimage.2014.04.036 (2014).
13 Lak, A., Stauffer, W. R. & Schultz, W. Dopamine prediction error responses integrate subjective value from different reward dimensions. *Proc Natl Acad Sci U S A* **111**, 2343-2348, doi:10.1073/pnas.1321596111 (2014).
14 Gan, J. O., Walton, M. E. & Phillips, P. E. Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. *Nat Neurosci* **13**, 25-27, doi:10.1038/nn.2460 (2010).
15 Tobler, P. N., Fiorillo, C. D. & Schultz, W. Adaptive coding of reward value by dopamine neurons. *Science* **307**, 1642-1645, doi:10.1126/science.1105370 (2005).
16 Fiorillo, C. D., Tobler, P. N. & Schultz, W. Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* **299**, 1898-1902, doi:10.1126/science.1077349 (2003).
17 Wise, R. A. Dopamine, learning and motivation. *Nat Rev Neurosci* **5**, 483-494, doi:10.1038/nrn1406 (2004).
18 Steinberg, E. E. *et al.* A causal link between prediction errors, dopamine neurons and learning. *Nat Neurosci* **16**, 966-973, doi:10.1038/nn.3413 (2013).
19 Izquierdo, A. Functional Heterogeneity within Rat Orbitofrontal Cortex in Reward Learning and Decision Making. *J Neurosci* **37**, 10529-10540, doi:10.1523/JNEUROSCI.1678-17.2017 (2017).
20 Stalnaker, T. A., Cooch, N. K. & Schoenbaum, G. What the orbitofrontal cortex does not do. *Nat Neurosci* **18**, 620-627, doi:10.1038/nn.3982 (2015).
21 Padoa-Schioppa, C. & Conen, K. E. Orbitofrontal Cortex: A Neural Circuit for Economic Decisions. *Neuron* **96**, 736-754, doi:10.1016/j.neuron.2017.09.031 (2017).
22 Padoa-Schioppa, C. Neuronal origins of choice variability in economic decisions. *Neuron* **80**, 1322-1336, doi:10.1016/j.neuron.2013.09.013 (2013).
23 Rhodes, S. E. & Murray, E. A. Differential effects of amygdala, orbital prefrontal cortex, and prelimbic cortex lesions on goal-directed behavior in rhesus macaques. *J Neurosci* **33**, 3380-3389, doi:10.1523/JNEUROSCI.4374-12.2013 (2013).
24 Schoenbaum, G., Roesch, M. R., Stalnaker, T. A. & Takahashi, Y. K. A new perspective on the role of the orbitofrontal cortex in adaptive behaviour. *Nat Rev Neurosci* **10**, 885-892, doi:10.1038/nrn2753 (2009).
25 McDannald, M. A., Lucantonio, F., Burke, K. A., Niv, Y. & Schoenbaum, G. Ventral striatum and orbitofrontal cortex are both required for model-based, but not model-free, reinforcement learning. *J Neurosci* **31**, 2700-2705, doi:10.1523/JNEUROSCI.5499-10.2011 (2011).
26 Morse, A. K. *et al.* Basolateral Amygdala Drives a GPCR-Mediated Striatal Memory Necessary for Predictive Learning to Influence Choice. *Neuron* **106**, 855-869 e858, doi:10.1016/j.neuron.2020.03.007 (2020).
27 Namburi, P. *et al.* A circuit mechanism for differentiating positive and negative associations. *Nature* **520**, 675-678, doi:10.1038/nature14366 (2015).

28 Malvaez, M., Shieh, C., Murphy, M. D., Greenfield, V. Y. & Wassum, K. M. Distinct cortical-amygdala projections drive reward value encoding and retrieval. *Nat Neurosci* **22**, 762-769, doi:10.1038/s41593-019-0374-7 (2019).
29 Ambroggi, F., Ishikawa, A., Fields, H. L. & Nicola, S. M. Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron* **59**, 648-661, doi:10.1016/j.neuron.2008.07.004 (2008).
30 Winstanley, C. A., Theobald, D. E., Cardinal, R. N. & Robbins, T. W. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J Neurosci* **24**, 4718-4722, doi:10.1523/JNEUROSCI.5606-03.2004 (2004).
31 Maren, S. Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J Neurosci* **19**, 8696-8703 (1999).
32 Todd, T. P., Fournier, D. I. & Bucci, D. J. Retrosplenial cortex and its role in cue-specific learning and memory. *Neurosci Biobehav Rev* **107**, 713-728, doi:10.1016/j.neubiorev.2019.04.016 (2019).
33 Fischer, L. F., Mojica Soto-Albors, R., Buck, F. & Harnett, M. T. Representation of visual landmarks in retrosplenial cortex. *Elife* **9**, doi:10.7554/eLife.51458 (2020).
34 Hattori, R., Danskin, B., Babic, Z., Mlynaryk, N. & Komiyama, T. Area-Specificity and Plasticity of History-Dependent Value Coding During Learning. *Cell* **177**, 1858-1872 e1815, doi:10.1016/j.cell.2019.04.027 (2019).
35 Vedder, L. C., Miller, A. M. P., Harrison, M. B. & Smith, D. M. Retrosplenial Cortical Neurons Encode Navigational Cues, Trajectories and Reward Locations During Goal Directed Navigation. *Cereb Cortex* **27**, 3713-3723, doi:10.1093/cercor/bhw192 (2017).
36 Powell, A. L. *et al.* The rat retrosplenial cortex as a link for frontal functions: A lesion analysis. *Behav Brain Res* **335**, 88-102, doi:10.1016/j.bbr.2017.08.010 (2017).
37 Fonzi, K. M., Lefner, M. J., Phillips, P. E. M. & Wanat, M. J. Dopamine Encodes Retrospective Temporal Information in a Context-Independent Manner. *Cell Rep* **20**, 1765-1774, doi:10.1016/j.celrep.2017.07.076 (2017).
38 Aronson, E. & Mills, J. The effect of severity of initiation on liking for a group. *J Abnormal and Social Psych* **59**, 177-181, doi:doi:10.1037/h0047195 (1959).
39 Staw, B. M. Knee-deep in the big muddy: a study of escalating commitment to a chosen course of actiona. *Organizational Behavior and Human Performance* **16**, 27-44, doi:doi.org/10.1016/0030-5073(76)90005-2 (1976).
40 Strube, M. J. The decision to leave an abusive relationship: empirical evidence and theoretical issues. *Psychol Bull* **104**, 236-250, doi:10.1037/0033-2909.104.2.236 (1988).
41 Wanat, M. J., Kuhnen, C. M. & Phillips, P. E. Delays conferred by escalating costs modulate dopamine release to rewards but not their predictors. *J Neurosci* **30**, 12020-12027, doi:10.1523/JNEUROSCI.2691-10.2010 (2010).
42 Wikenheiser, A. M., Stephens, D. W. & Redish, A. D. Subjective costs drive overly patient foraging strategies in rats on an intertemporal foraging task. *Proc Natl Acad Sci U S A* **110**, 8308-8313, doi:10.1073/pnas.1220738110 (2013).
43 Abram, S. V., Breton, Y. A., Schmidt, B., Redish, A. D. & MacDonald, A. W., 3rd. The Web-Surf Task: A translational model of human decision-making. *Cogn Affect Behav Neurosci* **16**, 37-50, doi:10.3758/s13415-015-0379-y (2016).

44 Roesch, M. R., Calu, D. J. & Schoenbaum, G. Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. *Nat Neurosci* **10**, 1615-1624, doi:10.1038/nn2013 (2007).
45 Cone, J. J. *et al.* Physiological state gates acquisition and expression of mesolimbic reward prediction signals. *Proc Natl Acad Sci U S A* **113**, 1943-1948, doi:10.1073/pnas.1519643113 (2016).
46 Papageorgiou, G. K., Baudonnat, M., Cucca, F. & Walton, M. E. Mesolimbic Dopamine Encodes Prediction Errors in a State-Dependent Manner. *Cell Rep* **15**, 221-228, doi:10.1016/j.celrep.2016.03.031 (2016).
47 Colby, J. J. & Smith, N. F. The effect of three procedures for eliminating a conditioned taste aversion in the rat. *Learning and Motivation* **8**, 404-413, doi:10.1016/0023-9690(77)90060-1 (1977).
48 Trost, A. & Hauber, W. Dopamine D1/D2 receptors do not mediate the expression of conditioned place preference induced by the aftereffect of wheel running. *BMC Neurosci* **15**, 124, doi:10.1186/s12868-014-0124-4 (2014).
49 Pitts, S. M. & Horvitz, J. C. Similar effects of D(1)/D(2) receptor blockade on feeding and locomotor behavior. *Pharmacol Biochem Behav* **65**, 433-438, doi:10.1016/s0091-3057(99)00249-x (2000).
50 Fenu, S., Bassareo, V. & Di Chiara, G. A role for dopamine D1 receptors of the nucleus accumbens shell in conditioned taste aversion learning. *J Neurosci* **21**, 6897-6904 (2001).
51 Keiflin, R., Reese, R. M., Woods, C. A. & Janak, P. H. The orbitofrontal cortex as part of a hierarchical neural system mediating choice between two good options. *J Neurosci* **33**, 15989-15998, doi:10.1523/JNEUROSCI.0026-13.2013 (2013).
52 Rudebeck, P. H., Saunders, R. C., Prescott, A. T., Chau, L. S. & Murray, E. A. Prefrontal mechanisms of behavioral flexibility, emotion regulation and value updating. *Nat Neurosci* **16**, 1140-1145, doi:10.1038/nn.3440 (2013).
53 Gallagher, M., McMahan, R. W. & Schoenbaum, G. Orbitofrontal cortex and representation of incentive value in associative learning. *J Neurosci* **19**, 6610-6614 (1999).
54 Cador, M., Robbins, T. W. & Everitt, B. J. Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. *Neuroscience* **30**, 77-86, doi:10.1016/0306-4522(89)90354-0 (1989).
55 Leathers, M. L. & Olson, C. R. In monkeys making value-based decisions, amygdala neurons are sensitive to cue value as distinct from cue salience. *J Neurophysiol* **117**, 1499-1511, doi:10.1152/jn.00564.2016 (2017).
56 Jezzini, A. & Padoa-Schioppa, C. Neuronal Activity in the Primate Amygdala during Economic Choice. *J Neurosci* **40**, 1286-1301, doi:10.1523/JNEUROSCI.0961-19.2019 (2020).
57 Hart, E. E. & Izquierdo, A. Basolateral amygdala supports the maintenance of value and effortful choice of a preferred option. *Eur J Neurosci* **45**, 388-397, doi:10.1111/ejn.13497 (2017).

58 West, E. A. *et al.* Transient inactivation of basolateral amygdala during selective satiation disrupts reinforcer devaluation in rats. *Behav Neurosci* **126**, 563-574, doi:10.1037/a0029080 (2012).
59 Vann, S. D., Aggleton, J. P. & Maguire, E. A. What does the retrosplenial cortex do? *Nat Rev Neurosci* **10**, 792-802, doi:10.1038/nrn2733 (2009).
60 Miller, A. M. P., Mau, W. & Smith, D. M. Retrosplenial Cortical Representations of Space and Future Goal Locations Develop with Learning. *Curr Biol* **29**, 2083-2090 e2084, doi:10.1016/j.cub.2019.05.034 (2019).
61 Buckwalter, J. A., Schumann, C. M. & Van Hoesen, G. W. Evidence for direct projections from the basal nucleus of the amygdala to retrosplenial cortex in the Macaque monkey. *Exp Brain Res* **186**, 47-57, doi:10.1007/s00221-007-1203-x (2008).