Supporting Information

Hollon et al. 10.1073/pnas.1419770111

SI Materials and Methods

Subjects and Surgery. All procedures were approved by the University of Washington Institutional Animal Care and Use Committee. A total of 41 male Sprague–Dawley rats (Charles River Laboratories), 250–300 g upon arrival, were used for this study. Fourteen rats in the first cohort and 14 in the second cohort completed recording sessions included in this study; four additional rats were excluded because of electrode misplacement, three because of failure of electrodes to satisfy criteria for dopamine detection, and six because of postsurgical complications (e.g., headcap loss). Rats were maintained on a 12-h light/dark cycle (lights on at 0700), with all behavioral testing occurring during the light phase. Rats were pair-housed until surgery, after which they were housed individually. Rats were anesthetized with isoflurane for bilateral implantation of carbon-fiber microelectrodes (1) targeting the nucleus accumbens core (1.3 mm anterior, 1.3 mm lateral, 6.8–7.0 mm ventral to bregma) and a Ag/AgCl reference electrode. After at least 1 wk of recovery postsurgery, rats were food-restricted to 90% their ad libitum body weight; for all subsequent behavioral procedures, each rat received a total of ∼15 g of food/d consisting of pellets earned as reward during behavioral sessions plus standard laboratory chow after these sessions. Water was available ad libitum in the animals' home cages.

Initial Behavioral Training. In their home cages before the first session of training, the food-restricted rats were exposed to the 45-mg food pellets (Bio-Serv dustless precision pellets) that served as rewards for all subsequent sessions. All training sessions took place between 0800 and 1800 in one of four standard operant chambers (Med Associates). Each chamber was equipped with a central food magazine and magazine light, a retractable lever on either side of the magazine (6 cm above the grid floor), a cue light above each lever, a house light at the top back left corner of the chamber, and a ventilation fan on the back wall of the sound-attenuating cabinet around the operant chamber.

Rats underwent one session of magazine training in which a total of 60 food pellets were delivered noncontingently, one at a time with a variable time interval (60 ± 20 s). Training to press levers for food pellets began the next day. One of the two cue lights was illuminated (side counterbalanced between rats), the corresponding lever was extended continuously for the duration of the session, and each press was reinforced on a fixed ratio (FR)-1 continuous reinforcement schedule until rats received 100 pellets in a 2-h session. If rats did not press the lever within 15–20 min, a pellet was placed behind the lever to encourage the rat to interact with the lever. In the next session, the other cue light was illuminated, and the lever was extended for another 100-pellet session reinforced on a continuous FR-1 schedule.

All subsequent sessions consisted of training on discrete trials: After the rat completed the response requirement, the cue light turned off, the lever retracted, a pellet was delivered into the food magazine, and the magazine light was illuminated for 6 s, followed by a variable ITI. At this stage of training, only one lever was available on each trial (all trials were forced), and each session consisted of 80 total trials (40 for each lever). For the first cohort of rats, each trial began at the end of the ITI with the simultaneous onset of a cue light and extension of the corresponding lever. For the second cohort, the cue light came on 5 s before lever extension. Rats completed one session of FR-1 training with a 20 ± 5 s ITI and unlimited time to initiate responding on each trial. For all subsequent behavioral sessions, rats were connected to a head-stage containing a voltammetric amplifier to habituate them to the equipment used for eventual recording sessions. While tethered, subsequent training consisted of one session each of FR-1 with a 20 ± 5 s ITI, FR-4 with a 30 ± 10 s ITI, and FR-8 and FR-16 with a 45 ± 15 s ITI for all sessions thereafter. Starting in the FR-16 session, failure to initiate responding within 10 s of lever presentation resulted in an unrewarded "Miss." Training on FR-16 sessions continued until rats completed more than 90% of the trials in a session. Rats then performed behavioral decision-making sessions that included blocks of four single-option forced and four dual-option choice trials, during which either the reward magnitude or effort requirement differed between the two options, as in our previous study that included independent manipulations of reward and effort (2). For the reward-manipulation sessions, each option required four lever presses, with one lever yielding four pellets and the other yielding one pellet; for the effort-manipulation sessions, each option yielded one food pellet, with one requiring four lever presses and the other requiring 32 presses. The contingencies assigned to each lever side were reversed between each session, and each rat performed daily sessions of either the reward or effort manipulation (order counterbalanced) until it reached criterion (75% choice in a sliding window of 12 choice trials) in fewer than 80 trials for both lever side assignments. After completing both the reward- and effort-manipulation stages, rats then advanced to the mixed-contingency decision-making task described below.

Mixed-Contingency Decision-Making Task. All sessions consisted of blocks of four single-option forced trials in which only one of the two options was available followed by four choice trials in which both options were available concurrently. In choice trials, the unchosen lever retracted and the cue light turned off once the rat made an initial press on the chosen lever. A 45 ± 15 s variable ITI separated each discrete trial, with a maximum of 120 trials per session. As in prior training, for the first cohort of rats each trial began immediately after the ITI with the onset of one or both cue lights and the simultaneous extension of the corresponding lever(s), and for the second cohort the lever(s) extended 5 s after the onset of the cue light(s).

The mixed-contingency decision-making task consisted of two types of sessions: moderate-cost and high-cost conditions (Fig. 1*A*). In both conditions, one lever served as a low value/low effort (LL) reference option, yielding one food pellet for four lever presses. The alternative option yielded a high-value reward (four pellets) for a medium-effort requirement (eight presses) in the moderate-cost condition (high value/medium effort: HM) or for a high-effort requirement in the high-cost condition (high value/high effort: HH). Before voltammetric recordings were conducted, this high-effort requirement was determined individually for each rat so that the rat preferred the LL option. For the first cohort, the lever side assigned to the low- vs. high-value options was reversed every two behavioral sessions; if a rat did not reliably prefer the LL option in both side configurations, the effort requirement for the HH option was increased by eight presses for the subsequent high-cost session, but the effort requirement always remained constant within a given session. The same procedure was used to determine the high-effort requirement for the second cohort, except the lever side assignments were reversed pseudorandomly every one to three sessions. The final high-effort requirements used in recording sessions ranged from 32–48 lever presses for rats in the first cohort and from 32–128 presses for rats in the second cohort. For both cohorts, recordings were conducted after rats had performed at least eight behavioral

sessions of a given condition (2) and always were conducted on the second session with a given lever side assignment. The behavioral criterion was defined as 75% choice for the HM option in the moderate-cost condition and for the LL option in the high-cost condition within a sliding 12-choice window. After reaching this criterion, rats performed four additional blocks (32 trials) that provided the primary data analyzed from each recording session. We also obtained recordings from high-cost sessions in which rats did not reach the intended criterion for the LL option and instead reached the opposite criterion, preferring the HH option. The high-effort requirements from these HH-preferred high-cost sessions ranged from 32–64 presses for the first cohort and 32–128 presses for the second cohort.

Fast-Scan Cyclic Voltammetry Recording Sessions. The chronically implanted carbon-fiber microelectrodes were connected to a head-mounted voltammetric amplifier for dopamine detection by fast-scan cyclic voltammetry as previously described (1). A potential of −0.4 V (versus the Ag/AgCl reference electrode) was applied to the carbon-fiber microelectrode and ramped to +1.3 V and back at a rate of 400 V/s. This voltammetric scan was applied at a frequency of 60 Hz for ∼40 min before the behavioral sessions were recorded and then at 10 Hz for ∼20 min before and throughout the recording session. To confirm that electrodes were capable of detecting chemically verified dopamine, a series of unexpected food pellets was delivered before and after each recording session. The voltammetry data from a recording session were included in the analysis only if the pre- and postsession pellet deliveries elicited dopamine release whose cyclic voltammogram (electrochemical signature) achieved a high correlation ($r^2 \geq 0.75$ by linear regression) with that of a dopamine standard.

Statistical Analyses. Postcriterion choice proportions were normalized with the arcsine transformation and compared with indifference using two-tailed, one-sample *t* tests in SPSS (IBM). Voltammetry data analysis was carried out using software written in LabView and Matlab. Following 2000-Hz low-pass filtering, dopamine was isolated from the background (1 s before cue onset)-subtracted voltammetric signal using chemometric analysis (3) using a standard training set based on stimulated dopamine release detected by chronically implanted electrodes (1). Dopamine concentration was estimated based on the average postimplantation electrode sensitivity. Noise spikes >1.5 nA versus the immediately preceding and following time points were removed (2), and the data were smoothed using a 0.5-s moving average.

The discriminability of cue-evoked dopamine responses in the different forced trial types was analyzed at each time point using the auROC, an approach from signal-detection theory (4). The high-value option (HM or HH) always was coded as the positive case in comparisons with the LL option, and auROC values were not rectified around 0.5 (i.e., if the LL option had evoked a greater dopamine response, the auROC values would have been less than 0.5). Significant discriminability at each time point was determined using a random permutation test, shuffling the trial types and recomputing the auROC and repeating this process for 2,000 permutations to generate a null distribution. After correcting for multiple comparisons across time using a supra-threshold cluster-correction technique (5, 6), all time points outside the 95% confidence interval were considered statistically significant. For graphical display purposes, all auROC values were transformed to a dopamine discriminability index ranging from −1 to 1: discriminability index = 2 × (auROC − 0.5).

To test the relationship between dopamine-associated cached values and subjective preference, we computed a dopamine discriminability index and a choice index to summarize each recorded session. The mean change in dopamine concentration over 5 s following cue onset for each postcriterion trial was used to calculate the auROC for a given session (HM or HH trials as positive cases, LL trials as negative cases), and each session's auROC was transformed to a dopamine discriminability index as above: discriminability index = 2 × (auROC − 0.5). Thus, a dopamine discriminability index approaching 1 indicates a greater dopamine response to the high-value option (HM or HH), whereas an index of −1 indicates a greater response to the LL option, and an index of 0 indicates equivalent dopamine release to either option. Likewise, the choice index was calculated by transforming the postcriterion choice behavior in each session: choice index = 2 × [p(H) − 0.5], where p(H) is the proportion of choices for the high-value option (HM or HH), so that a choice index of 1 corresponds to 100% choice for the high-value option, −1 indicates 100% choice for the LL option, and 0 indicates indifference between the two options. Categorical models of expected utility and expected benefits were evaluated with binomial tests of the number of sessions violating or satisfying the models' predictions, and regression models were evaluated by comparing the goodness-of-fit using the second-order AICc (7, 8) based on the residual sum of squares.

Finally, to test for the possibility of direction-selective encoding by mesolimbic dopamine, we examined the counterbalanced pairs of recorded sessions from the first cohort of rats to compare the cue-evoked dopamine response during forced trials for each option when it was assigned to the lever side ipsilateral versus contralateral to the hemisphere of the recording electrode. Within each trial type, the dopamine responses were indistinguishable between the two lever side assignments (Fig. S2). Moreover, we observed the same pattern of greater dopamine transmission for the high-value option regardless of the lever assignment configuration. Because these results do not reflect direction encoding by mesolimbic dopamine, for the second cohort we included all recorded sessions meeting the electrochemical and behavioral criteria regardless of whether the counterbalanced pair was obtained.

Histological Verification of Recording Site. Animals were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg), and the recording site was marked by passing a current (∼70 μA) through the carbon-fiber microelectrode for 20 s to make a small electrolytic lesion. Animals were perfused transcardially with physiological saline and then with 4% (wt/vol) paraformaldehyde in PBS, in which brains also were postfixed after removal from the skull. Brains were sunk in 15% (wt/vol) sucrose solution in PBS for 24 h, in 30% (wt/vol) sucrose for at least 72 h, flash frozen in dry ice, sectioned coronally (30–60 μm) on a cryostat, mounted on slides, and stained with a 0.5% cresyl violet solution.

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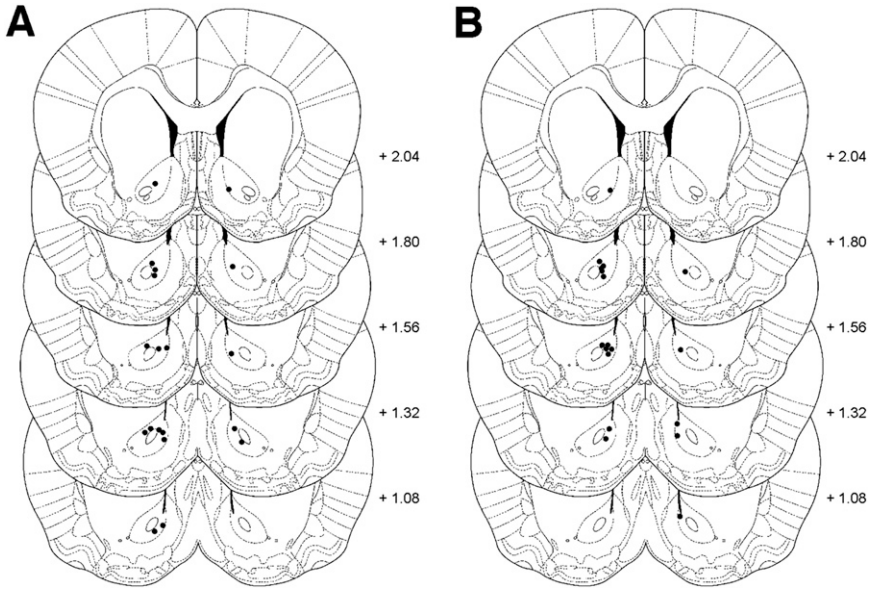

Fig. S1. Recording locations in the nucleus accumbens core in the first (*A*) and the second (*B*) cohorts of rats. The numbers next to each section indicate distance in millimeters anterior to bregma. Adapted from Paxinos G, Watson C (2005) *Rat Brain in Stereotaxic Coordinates* (Elsevier Academic, Burlingame, MA) 5th Ed.

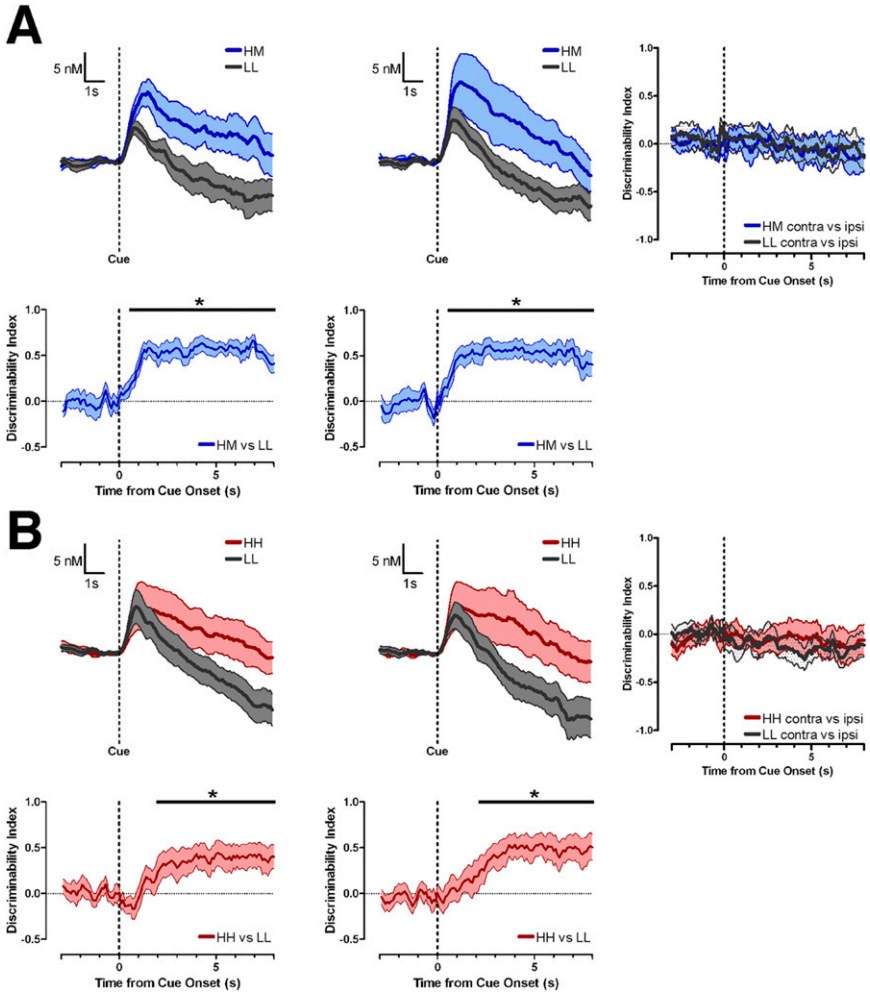

Fig. S2. Lack of direction-selective encoding by mesolimbic dopamine. (*A*, *Upper*) Mean (± SEM) cue-evoked dopamine release during forced trials from the side-counterbalanced pairs of moderate-cost sessions recorded in rats in the first cohort, separated by lever side assignment. (*Upper Left*) Sessions in which the HM option (blue) was assigned to the lever ipsilateral to the hemisphere of the carbon-fiber microelectrode and the LL option (gray) was contralateral. (*Upper Center*) Sessions in which the HM option (blue) was assigned to the lever contralateral to the hemisphere of the electrode and the LL option (gray) was ipsilateral. (*Upper Right*) Mean (± SEM) discriminability index time series comparing forced trials from the side-counterbalanced pairs. Neither the HM contralateral vs. HM ipsilateral comparison (blue) nor the LL contralateral vs. LL ipsilateral comparison (gray) ever reached significance. (*Lower*) Mean (± SEM) discriminability index time series comparing HM vs. LL forced trials within each session, with lever assignments defined as above. Horizontal bars indicate time points of significant discriminability (*$P < 0.05$, permutation tests). (*B*, *Upper*) Mean (± SEM) cue-evoked dopamine release during forced trials from the side-counterbalanced pairs of high-cost sessions recorded in rats in the first cohort reaching behavioral criterion for preferring the LL option, separated by lever side assignment. (*Upper Left*) Sessions in which the HH option (red) was assigned to the lever ipsilateral to the hemisphere of the carbon-fiber microelectrode and the LL option (gray) was contralateral. (*Upper Center*) Sessions in which the HH option (red) was assigned to the lever contralateral to the hemisphere of the electrode and the LL option (gray) was ipsilateral. (*Upper Right*) Mean (± SEM) discriminability index time series comparing forced trials from the side-counterbalanced pairs. Neither the HH contralateral vs. HH ipsilateral comparison (red) nor the LL contralateral vs. LL ipsilateral comparison (gray) ever reached significance. (*Lower*) Mean (± SEM) discriminability index time series comparing HH vs. LL forced trials within each session, with lever assignments defined as above. Horizontal bars indicate time points of significant discriminability (*$P < 0.05$, permutation tests).

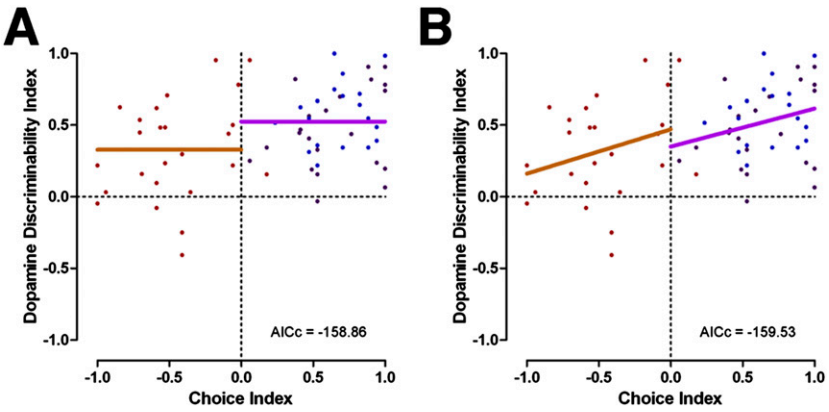

Fig. S3. Additional models testing the relationship between dopamine-associated cached values and subjective preferences. (*A*) Modeling the discriminability index with two constants, depending on whether the low- or high-value option was preferred (AICc = −158.86). (*B*) Separate linear regressions for cases in which the low- vs. high-value option was preferred (AICc = −159.53). Each provided a better fit than either the origin-constrained utility model (AICc = −97.30, Fig. 3*C*) or the single-constant without slope (AICc = −154.27, Fig. 3*D*) but was not better than the standard linear regression model (AICc = −163.45, Fig. 3*F*). As in Fig. 3, blue points represent moderate-cost sessions, red points are LL-preferred high-cost sessions, and purple points are HH-preferred high-cost sessions.

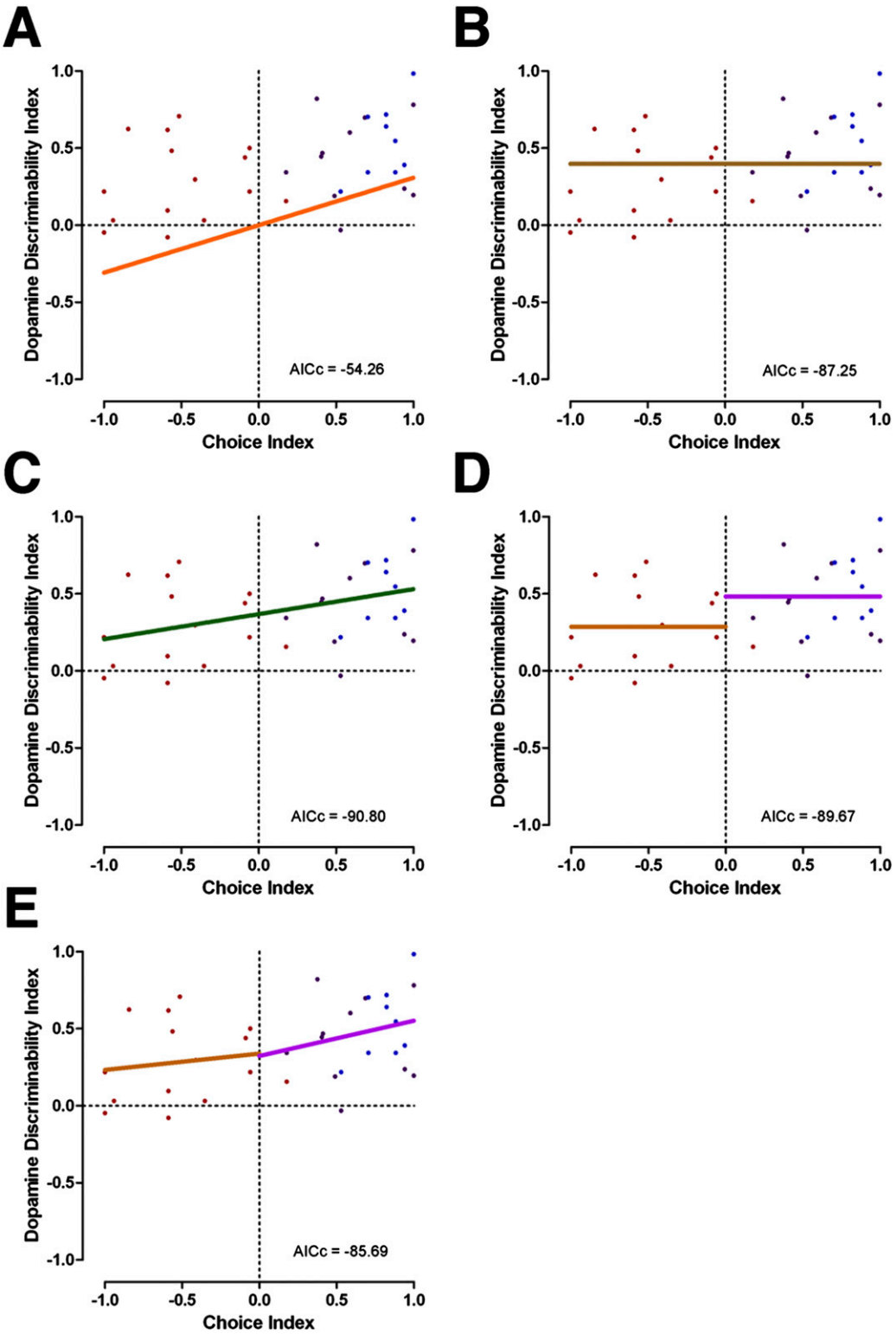

Fig. S4. Models from Fig. 3 and Fig. S3 including only the data from the second cohort (5-s cue-to-lever delay). (*A*) The expected utility regression model: constrained through origin (AICc = −54.26). (*B*) The constant line without slope (AICc = −87.25). (*C*) The standard linear regression (AICc = −90.80; $r^2 = 0.1564$). Both the slope and intercept differ significantly from 0 ($\beta_1 = 0.162 \pm 0.066$, $t = 2.473$, $P = 0.019$; $\beta_0 = 0.368 \pm 0.045$, $t = 8.210$, $P = 1.76 \times 10^{-9}$). (*D*) Two constants, depending on whether the low- or high-value option was preferred (AICc = −89.67). (*E*) Separate linear regressions for cases in which the low- vs. high-value option was preferred (AICc = −85.69). In all panels, blue points are moderate-cost sessions, red points are LL-preferred high-cost sessions, and purple points are HH-preferred high-cost sessions.

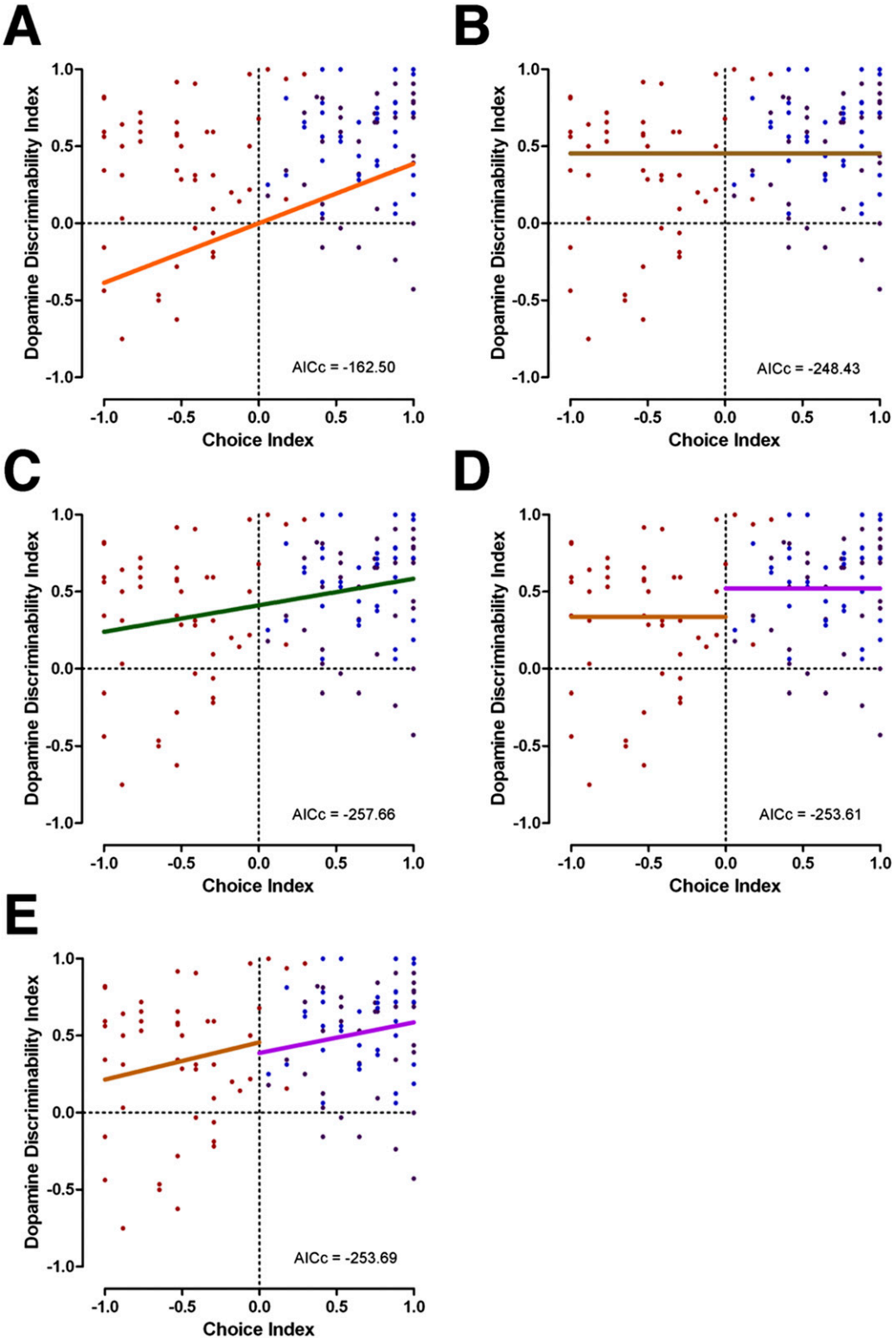

Fig. S5. Models from Fig. 3 and Fig. S3 splitting the pairs of counterbalanced sessions and treating each as an independent data point. (*A*) Expected utility regression model: constrained through origin (AICc = −162.50). (*B*) Constant line without slope (AICc = −248.43). (*C*) Standard linear regression (AICc = −257.66; $r^2 = 0.0822$). Both the slope and intercept differ significantly from zero ($\beta_1 = 0.172 \pm 0.050$, $t = 3.413$, $P = 8.56 \times 10 - 4$; $\beta_0 = 0.411 \pm 0.035$, $t = 11.900$, $P = 1.52 \times 10 - 22$). (*D*) Two constants, depending on whether the low- or high-value option was preferred (AICc = −253.61). (*E*) Separate linear regressions for cases in which the low- vs. high-value option was preferred (AICc = −253.69). In *A*–*E*, blue points are moderate-cost sessions, red points are LL-preferred high-cost sessions, and purple points are HH-preferred high-cost sessions.

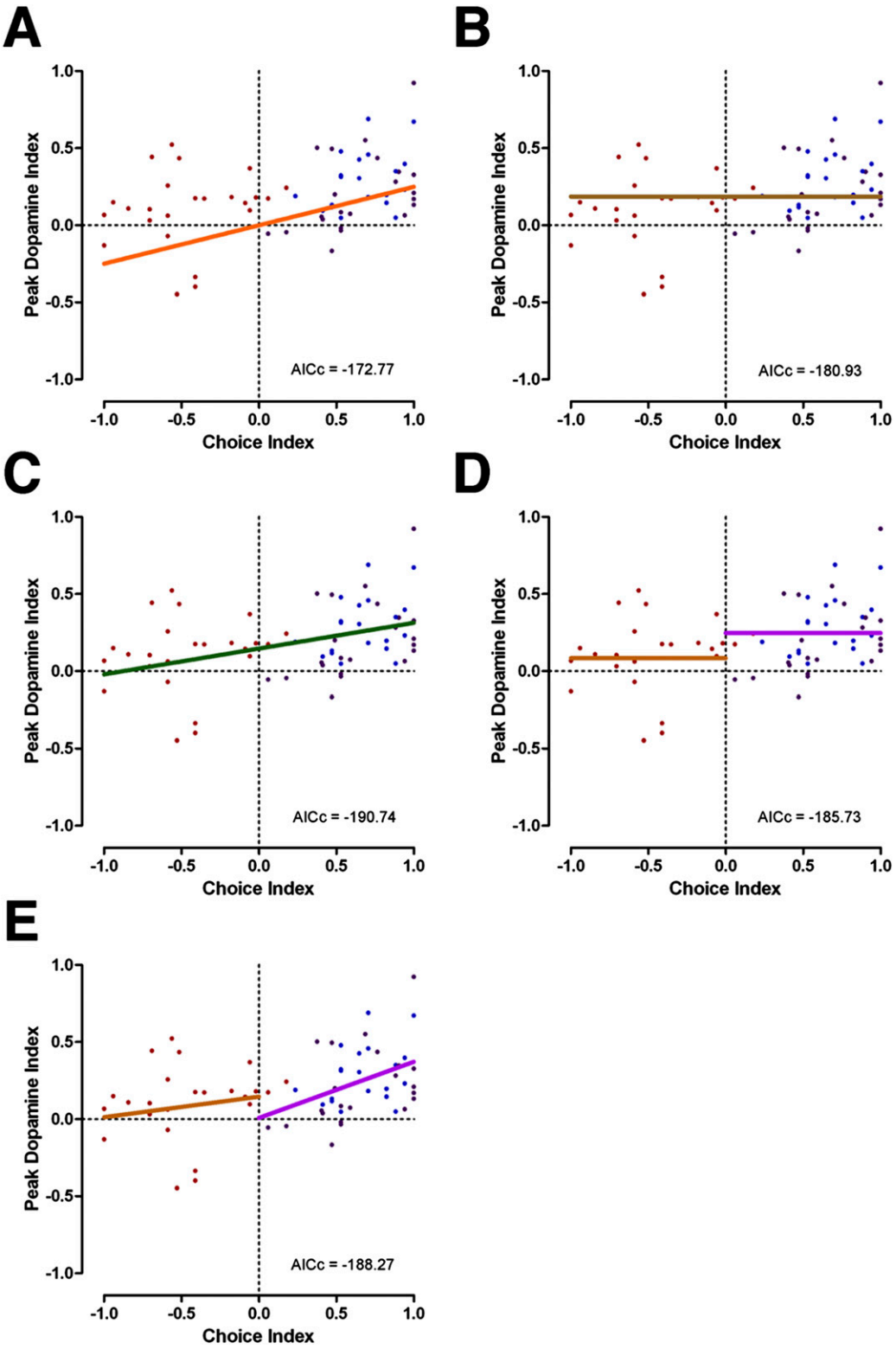

Fig. S6. Models from Fig. 3 and Fig. S3 using a peak dopamine index [(H − L)/(H + L)] instead of the auROC-based discriminability index. (*A*) Expected utility regression model: constrained through origin (AICc = −172.77). (*B*) Constant line without slope (AICc = −180.93). (*C*) Standard linear regression (AICc = −190.74; $r^2 = 0.1639$). Both the slope and intercept differ significantly from zero ($\beta_1 = 0.167 \pm 0.047$, $t = 3.570$, $P = 6.77 \times 10 - 4$; $\beta_0 = 0.146 \pm 0.031$, $t = 4.776$, $P = 1.06 \times 10 - 5$). (*D*) Two constants, depending on whether the low- or high-value option was preferred (AICc = −185.73). (*E*) Separate linear regressions for cases in which the low- vs. high-value option was preferred (AICc = −188.27). In *A*–*E*, blue points are moderate-cost sessions, red points are LL-preferred high-cost sessions, and purple points are HH-preferred high-cost sessions.

Table S1. AICc and weights of evidence for each model from Fig. 3 and Fig. S3

Model	Free parameters	AICc	Weight for model
Origin-constrained slope	2	−97.30	3.45×10^{-15}
Constant with no slope	2	−154.27	0.0081
Standard linear regression	3	−163.45	0.7991
Two constants	3	−158.86	0.0802
Two linear regressions	5	−159.53	0.1125

Table S2. AICc and weights of evidence for each model from Fig. S4

Model	Free parameters	AICc	Weight for model
Origin-constrained slope	2	−54.26	6.39×10^{-9}
Constant with no slope	2	−87.25	0.0932
Standard linear regression	3	−90.80	0.5511
Two constants	3	−89.67	0.3130
Two linear regressions	5	−85.69	0.0427

Table S3. AICc and weights of evidence for each model from Fig. S5

Model	Free parameters	AICc	Weight for model
Origin-constrained slope	2	−162.50	1.69×10^{-21}
Constant with no slope	2	−248.43	0.0077
Standard linear regression	3	−57.66	0.7821
Two constants	3	−253.61	0.1029
Two linear regressions	5	−253.69	0.1073

Table S4. AICc and weights of evidence for each model from Fig. S6

Model	Free parameters	AICc	Weight for model
Origin-constrained slope	2	−172.77	9.11×10^{-5}
Constant with no slope	2	−180.93	0.0054
Standard linear regression	3	−190.74	0.7244
Two constants	3	−185.73	0.0593
Two linear regressions	5	−188.27	0.2108