Supporting Information

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SI Materials and Methods

Subjects and Surgery. Individually housed male Sprague–Dawley rats (n = 12) maintained on a reverse light–dark cycle and within 85% of presurgical weight were run in experiments during their dark (active) cycle, with procedures approved by the Massachusetts Institute of Technology Committee on Animal Care. For optogenetics, AAV5-CaMKIIα-eNpHR3.0-EYFP (halorhodopsin) or AAV5-CaMKIIα-EYFP (control) was injected bilaterally into the IL cortex [0.2–0.7 μL/20 min per injection; anterior–posterior (AP), 3.1 mm; medial–lateral (ML), ±0.6 mm; dorsal–ventral (DV), 5.3 mm]. A dual-ferrule optical fiber (diameter, 200 μm; Doric Lenses) was implanted bilaterally in the dorsal IL cortex, anchored, and shielded. For recording light-induced firing changes, rats were implanted with recording head stages carrying 12–24 tetrodes and two optical fibers aimed at the IL cortex. Recording head stages were implanted as described (1–4).

T-Maze Apparatus and Training. A T-maze was used for the experiment and was identical to one described previously (1–3). Reward was manually delivered via tubing to troughs at the end-arm goal sites. Rats were habituated to task conditions and rewards (30% sucrose solution and chocolate-flavored whole milk) as described (1–4). Training proceeded in daily 40-trial sessions consisting of the following: the rat waited on a platform, a warning click sounded, the start gate was lowered, the rats traversed the maze, and instruction cues (1 or 8 kHz) sounded as the rat approached the decision point and remained on until a goal was reached, where the rat was rewarded (about 0.3 mL) for correct performance (*ca.* 1-min intertrial interval). Each reward was assigned to only one arm per rat; turn, tone, and reward assignments were pseudorandom across rats. Training continued through acquisition (72.5% accuracy criterion, χ^2; $P <$ 0.01 compared with chance) and overtraining (10+ additional sessions at or above criterion). Photobeams placed every *ca.* 17.5 cm on the maze-tracked behavior. Task control was by a MED-PC program (Med Associates). Nearly all trials terminated with goal-reaching. Very rare trials in which rats stopped at the tone were accepted but did not contribute to tallies of correct or wrong-way runs. Some rats eventually chose not to run during the unrewarded probe session (i.e., not all rats reached 40 probe trials); the session was ended at these instances.

Reward Devaluation. Rats were given 45-min access to one maze reward (e.g., chocolate milk) in their home cage and then received an injection of lithium chloride (0.6 M 5 mL/kg or 0.3 M 10 mL/kg, i.p.). Three devaluation procedures at 48-h intervals were given in multiple laboratory rooms, although never in the maze room, and efficacy was confirmed by reduced intake. Devalued reward identity was pseudorandomly assigned across rats. Rats were then given a probe session without rewards given, followed by normal rewarded sessions. The purpose of these rewarded sessions was to confirm that the taste aversion developed in the home-cage environment generalized to the task environment, as well as to assess behavioral plasticity occurring after encounter with the devalued reward in the maze task.

Session Staging. Training sessions were staged as follows: stages 1–2 (first two sessions); stages 3–4 (pairs of sessions ≥60% correct); stage 5 (first pair of sessions ≥72.5%); and stages 6+ (subsequent pairs of sessions ≥72.5%). Stages for comparing post-devaluation days of IL light delivery were as follows: probe (IL light delivery, unrewarded); stage PP1 (first one to two rewarded sessions with IL light delivery); stages PP2–PP5 (subsequent sessions without IL light delivery); stage PP6 (session with light delivery); stages PP7–PP8 (sessions without IL light delivery); stage PP9 (final IL light delivery sessions); and stage PP10 (final sessions without light delivery).

Optogenetic Light Delivery. Light was delivered to the IL cortex from a laser (593.5-nm diode-pumped solid-state laser; OEM Laser Systems) from warning cue to goal arrival (*ca.* 3-s duration; 2.5–5.0 mW). Fibers and equipment were connected during light-off training and test days. Light effects on reward consumption were measured in two, pseudorandomly ordered 40-min tests with rats placed in their home cage on a table in the maze room. The devalued reward was delivered via tubing into a maze trough placed in the cage while illumination was given (5 mW per fiber; 3-s-on/10-s-off pulses) or not given. The same illumination parameters were used for tests of firing rate changes evoked by light delivery, during which rats were allowed to freely explore the maze for 40+ illumination trials while IL activity was recorded.

Electrophysiological Data Acquisition. Tetrodes lowered to recording targets over 7 postsurgical days were left in place or moved in <0.04-mm steps. Electrical signals were amplified at 100–10,000, sampled at 32 kHz, filtered at 600–6,000 Hz, and recorded by a Cheetah data acquisition system (Neuralynx) as described (1–4). Single units were identified as isolated waveform clusters using Offline Sorter (Plexon).

Analysis. Performance (percentage of correct, incorrect, and incomplete trials) and reward consumption were analyzed by ANOVA (P < 0.05) to compare across learning stages, trial subtypes (e.g., devalued and nondevalued trials), and conditions of IL light delivery. Within sessions, we also assessed the time scale of runs to the devalued goal and drinks: first trial in which it occurred, number of two consecutive trials (repeat doublets), average number of repeated runs and drinks, and volume of drinks (*ca.* 0.3 mL per reward). Bonferroni-corrected post hoc comparisons were made when significance was obtained for the main effect of variables and/or interaction between variables. For neuronal recordings, per-unit firing 3 s before, during, and 3 s after light delivery was compared using ANOVA on time epoch and firing rate and separate ANOVAs for comparing first and last trials blocks within a session. Responsive units were those with a significant change in firing rate during the light-on period compared with 3 s prior.

Histology. Tetrode and fiber cannula tracks were identified histologically (1–4). For double immunostaining, sections were immunostained for YFP (GFP antibody) and activated microglia (CD11b/c antibody).

1. Barnes TD, Kubota Y, Hu D, Jin DZ, Graybiel AM (2005) Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. *Nature* 437(7062): 1158–1161.
2. Barnes TD, et al. (2011) Advance cueing produces enhanced action-boundary patterns of spike activity in the sensorimotor striatum. *J Neurophysiol* 105(4):1861–1878.
3. Thorn CA, Atallah H, Howe M, Graybiel AM (2010) Differential dynamics of activity changes in dorsolateral and dorsomedial striatal loops during learning. *Neuron* 66(5): 781–795.
4. Kubota Y, et al. (2009) Stable encoding of task structure coexists with flexible coding of task events in sensorimotor striatum. *J Neurophysiol* 102(4):2142–2160.