Learning From One's Mistakes: A Dual Role for the Rostromedial Tegmental Nucleus in the Encoding and Expression of Punished Reward Seeking

Supplemental Information

Supplemental Methods

Progressive shock task: During initial lever-press training, each trial began with both levers extended and both cue lights illuminated. After every five presses (FR5) on the left (active) lever, both the left and right levers were retracted, the cue lights were extinguished, and a single food pellet was delivered. Responses on the inactive (right) lever were recorded, but had no programmed consequences, and the house light remained illuminated throughout the duration of each session. A 15sec intertrial interval (ITI) was imposed to allow for food consumption. Rats progressed to testing once they reliably completed each session (70 trials) with > 95% of trials completed within a latency of <15sec (Figure S1).

Rats then were tested for the suppressive effect of footshock on operant responding for food in a punishment task developed by our lab. Specifically, rats received twice-daily sessions that were identical to lever-press training except that after the first three trials of each session, completion of the fixed ratio yielded a single food pellet that was immediately followed by brief (33ms) footshock of gradually increasing intensity. Food consumption almost always occurred after footshock ceased and rats were reliably done eating by the start of the next trial. Further, food pellets were always found to be consumed at the end of each session, indicating that punished responding of food consumption did not occur. The intensity of footshock was mild in early trials of each session (0.25mA), but subsequently increased by approximately 30% after completion of every 3 trials thereafter. Failure to respond on the active lever for 30sec resulted in a time-out period in which the active and inactive levers remained extended to record behavioral responses, but the cue lights turned off and responses on the active lever yielded no consequence. After a 30sec timeout period, the cue lights were once again illuminated and a new trial initiated. After three consecutive timeouts, the session was terminated and responses were tallied. The last shock intensity in which subjects completed all three trials at that given intensity was considered the “shock breakpoint,” and data were derived from taking an average of each subject’s shock breakpoint across 6 consecutive sessions. While within-subject variability was rather low for this experiment, the averaging of data across multiple test sessions likely reflects a more precise measure of individual’s shock breakpoints through minimizing an effect of day-to-day variability. In a modified version of this task, a “fixed” intensity of shock was delivered in an identical manner to that described above, with the exception that rats experienced just one shock intensity per session and rather than enforcing a time-out period, sessions were terminated after 10min. In this extended shock task, the intensity of shock was increased across sessions using identical values as used in the “progressive” shock task, until subjects were found to reduce the number of trials completed per session by 85% of their no-shock baseline. The 85% threshold was used to approximate rate of suppression in the earlier progressive shock task. Subjects again were tested in twice-daily sessions, but each rat was exposed to each shock intensity in the escalating presentation only once.

In addition to the “progressive” and “extended” shock tasks, a third variation of this escalating shock paradigm was incorporated in which rats were trained to discriminate between two levers that yielded food rewards of different magnitude. Specifically, responses on the “high-cost” lever (FR5) yielded three food pellets (and footshock), whereas five presses on the “low-cost” lever yielded a single food pellet (and no punishment). The location of the large reward lever (left or right) was counterbalanced between subjects, and a cue light was illuminated above the lever that yielded the large reward. After completion of the response ratio both levers were retracted, the cue light was extinguished, and the reward was delivered. A 20sec timeout was imposed between trials to allow time for food consumption before the next trial began. Initial training of this two-lever shock paradigm consisted of four sessions of 60 trials each in which levers were randomly presented one at a time to establish the relationship between the large vs small reward levers. The location of the lever yielding the large reward (left or right side) was counterbalanced between subjects, but for any one rat the large reward lever remained constant. After the 4 sessions of “forced” trials, rats were administered 6 “no-cost” sessions comprising 30 forced trials (as described above) and 30 free choice trials in which both the large and small reward levers were presented and subjects’ preference for the large reward was assessed. In test sessions evaluating the propensity for footshock to bias rat’s decision-making toward the smaller “low-cost” alternative, choices on the large reward lever were accompanied by footshock administered immediately after delivery of the reward. In early punishment sessions, the shock intensity was relatively low (0.25mA/500ms duration), and the shock intensity was increased by approximately 40% each subsequent session until rats were found to shift their preference to the small (low-cost) reward option (>85% small reward preference). Due to the relatively high number of reinforcers that could potentially be earned in this experiment, subjects were run only once per day to avoid the potential confound posed by satiation.

Assessment of locomotor activity: Locomotor activity was recorded using small cameras mounted to the ceiling of the sound-attenuating cabinets where the operant chambers were housed. Cameras were positioned so as to capture subject's movements within the entire operant chamber, and data were collected using Virtual Dub (version 1.10.4) video tracking software. Locomotor tests were conducted in a different box from that which they were accustomed to in an effort to generate moderate levels of baseline activity in all subjects. Recordings lasted for 20min during which time the house light was illuminated. Activity was scored using custom-made video analysis software to determine the frame x frame difference in the X-Y coordinate of subjects in relation to an experimenter-defined region of interest comprising the grid flooring of each testing chamber.

Extinction training: Food training was conducted as described above in lever-press training for the progressive shock task. Rats progressed to testing once they reliably completed each session (70 trials) with > 95% of trials completed within a latency of <15sec. Immediately following training, RMTg- and sham-lesioned rats were tested once per day for their ability to suppress operant responding when lever pressing no longer resulted in delivery of the reinforcer. Specifically, in three 1-hr sessions, a house light was illuminated and both the active and inactive levers were extended and cue lights were present; however, responses on either the formerly active or inactive lever yielded no consequence.

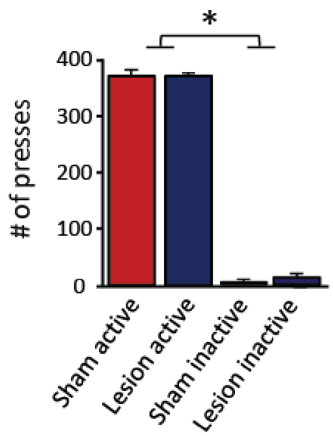

Figure S1. Mean lever presses during the final day of training prior to testing in the progressive shock task. Both lesioned rats and sham controls effectively discriminated between the active and inactive lever (n=9, 11; $F_{1,17}$=2173.102, $p<0.001$). We did not, however, detect any differences between groups in the number of lever responses (Lesion: $F_{1,17}$= 0.57, p= 0.461; Lesion x Lever: $F_{1,17}$= 0.739, p= 0.402), indicating that both lesioned and control rats were trained to a similar degree.

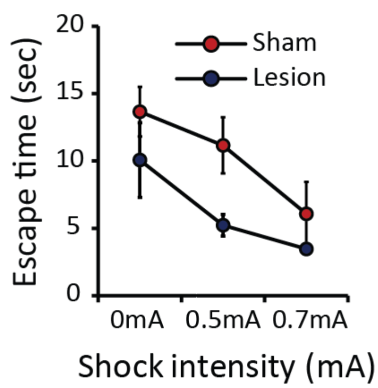

Figure S2. Mean escape latency in RMTg- and sham-lesioned rats. Rats (n=6) were placed into one side of a two-chamber shuttle box and current was passed across the grid floor at one of three shock intensities (0mA, 0.5mA, or 0.7mA). Latency to escape the shock-paired chamber to the opposite (unshocked) chamber was recorded. A repeated measures two-way ANOVA on median escape latency showed that RMTg-lesioned rats escaped the shocked chamber faster than sham-lesioned controls ($F_{1,10}$= 8.557, *p*= 0.015), and greater shock intensities caused rats to escape the shock-paired chamber faster ($F_{2,20}$= 6.454, *p*=0.007). We did not, however, detect a Lesion x Shock interaction ($F_{2,20}$= 0.612, *p*= 0.552) suggesting that the group differences in escape latency arose from different baseline activity.

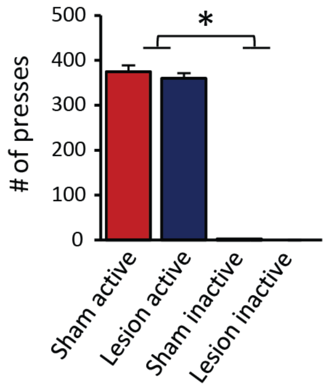

Figure S3. No differences were observed between lesioned rats and sham controls in lever pressing prior to extinction. A two-way ANOVA on total lever presses during the last food-training session prior to extinction demonstrated that both RMTg- and sham-lesioned rats pressed the active lever more ($F_{1,16}$=1379.057, p<0.001), but there was no difference in active or inactive presses between groups ($F_{1,16}$=0.689, p=0.419), nor was there a Lever x Condition interaction ($F_{1,16}$=0.552, p=0.468).