Figures and figure supplements

Discriminative stimuli are sufficient for incubation of cocaine craving

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Figure 1. Incubation of discriminative stimulus-controlled cocaine seeking. (A) Experimental timeline. (B) Schematic showing the timing of individual events during a single 3 min DS trial, and the differences between the two trial types during discrimination training for cocaine reward. Rats received...
cocaine reward (0.75 mg/kg/infusion) during DS+ trials but did not receive cocaine reward during DS- trials (n = 16). (C) Training data. Self-administration: Rats learned to self-administer cocaine over six sessions. Mean (±SEM) number of cocaine infusions and lever presses during each 3 hr session. Trial training: Mean (±SEM) number of DS+ or DS- trials with at least one lever press (denoted as trials), and number of lever presses during the 3 hr sessions (denoted as lever presses) with 30 trials of a single-trial type (DS+ trials in the AM session, DS- trials in the PM session). Discrimination training: Over 10 sessions, rats learned to discriminate DS+ from DS- trials. Mean (±SEM) number of trials and lever presses during the 3 hr discrimination training session with 30 trials each of DS+ and DS- trials presented in a pseudorandomized manner. *indicates significant difference (p<0.05) between responding during DS+ and DS- trial types (n = 14). (D) Relapse test. Incubation of lever responding during DS+, but not DS-, trials peaked at 60 days of abstinence and returned to basal levels over 400 days. Mean (±SEM) number of trials and lever presses during the 3 hr relapse test sessions (30 trials each of DS+ and DS- presented in a pseudorandomized manner) under extinction conditions. *denotes significant (p<0.05) difference from responding during day 1. Columns indicate mean (±SEM) for the group, while dots indicate values for individual rats. #denotes significant (p<0.05) difference between DS+ and DS- responding during the test sessions (n = 11–14). (E) Reinstatement test. Rats reinstated DS-controlled cocaine-seeking in response to IP injections of cocaine (10 and 20 mg/kg), but not saline. Mean (±SEM) number of trials and lever presses during the 3 hr saline- or cocaine-primed reinstatement test sessions (30 trials each of DS+ and DS- presented in a pseudorandomized manner) under extinction conditions. Columns indicate mean (±SEM) for the group, while dots indicate values for individual rats. *denotes significant (p<0.05) difference from responding on the first saline-prime test session (cocaine dose = 0 mg/kg). #denotes significant difference (p<0.05) between DS+ and DS- responding during the test session (n = 11). See Figure 1—figure supplement 1 for behavioral data from the experiment, disaggregated by sex. See Figure 1—figure supplement 2 for subject body weights, disaggregated by sex. See Figure 1—source data 1 for a table of statistical output relating to the experiment.

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Figure 1—figure supplement 1. Behavioral data disaggregated by sex. (A, B) Self-administration: Rats learned to self-administer cocaine over six sessions. No main effects or interactions involving Sex were observed. Mean (±SEM) number of cocaine infusions and lever presses during each 3 hr interval.

C. Discrimination training
D. Discrimination training

E. Relapse test
F. Relapse test

G. Reinstatement test
H. Reinstatement test

Figure 1—figure supplement 1 continued on next page
Discrimination training: Over 10 sessions, rats learned to discriminate DS+ from DS- trials. We observed a three-way interaction between DS, Session, and Sex, in both trials and lever presses measures as male rats pressed more during DS- trials in initial sessions. Mean (±SEM) number of trials and lever presses during the 3 hr discrimination training session. (E, F) Relapse test: Rats showed incubation of lever responding during DS+, but not DS-, trials during abstinence. We observed an interaction between Sex and DS in the trials measure but no interaction involving Sex and Day. Further, we observed no main effects or interactions involving Sex in the lever presses measure. This indicates that while female rats pressed on more DS+ trials than male rats across all test sessions, male and female rats did not differ in the incubation of DS-controlled cocaine-seeking. Mean (±SEM) number of trials and lever presses during the 3 hr relapse test sessions under extinction conditions. Columns indicate mean (±SEM) for the group, while dots indicate values for individual rats. (G, H) Reinstatement test: Rats reinstated DS-controlled cocaine-seeking in response to IP injections of cocaine (10 and 20 mg/kg), but not saline. No main effects or interactions involving Sex were observed. Mean (±SEM) number of trials and lever presses during the 3 hr saline- or cocaine-primed reinstatement test sessions. Columns indicate mean (±SEM) for the group, while dots indicate values for individual rats.

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A. Subject body weights disaggregated by sex

![Graph showing body weight of subjects over the course of Experiment 1, disaggregated by sex.](image)

**Figure 1—figure supplement 2.** Subject body weights disaggregated by sex. (A) Mean (±SEM) body weight of subjects over the course of Experiment 1, disaggregated by sex.

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Figure 2. Abatement of discriminative stimulus-controlled palatable food seeking. (A) Experimental timeline. (B) Schematic showing the timing of individual events during a single 2-min DS trial, and the differences between the two trial types during discrimination training for palatable food reward (45 mg high carbohydrate pellets). Rats received food reward during DS+ trials but did not receive reward during DS- trials (n = 16). (C) Training data. Self-administration: Rats learned to self-administer palatable food over three sessions. Mean (±SEM) number of palatable food pellets received and lever presses.
Figure 2 continued

lever presses during each 1 hr session. **Trial training:** Mean (±SEM) number of DS+ or DS- trials with at least one lever press (denoted as trials), and number of lever presses during the 2 hr sessions (denoted as lever presses) with 30 trials of a single trial type (DS+ trials in the AM session, DS- trials in the PM session). **Discrimination training:** Over 11 sessions, rats learned to discriminate DS+ from DS- trials. Mean (±SEM) number of trials and lever presses during the 2 hr discrimination training session with 30 trials each of DS+ and DS- trials presented in a pseudorandomized manner. *indicates significant difference (p<0.05) between responding during DS+ and DS- trials (n = 15). **(D) Relapse test:** Lever responding during DS+, but not DS-, trials peaked at 1 day of abstinence and abated over 200 days. Mean (±SEM) number of trials and lever presses during the 2 hr relapse test sessions (30 trials each of DS+ and DS- presented in a pseudorandomized manner) under extinction conditions. *denotes significant (p<0.05) difference from responding during day 1. Columns indicate mean (±SEM) for the group, while dots indicate values for individual rats. #denotes significant (p<0.05) difference between DS+ and DS- responding during the test (n = 15). See **Figure 2—figure supplement 1** for behavioral data from the experiment, disaggregated by sex. See **Figure 2—figure supplement 2** for food rewards earned during discrimination training, disaggregated by sex. See **Figure 2—figure supplement 3** for subject body weights, disaggregated by sex. See **Figure 2—source data 1** for a table of statistical output relating to the experiment.

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Figure 2—figure supplement 1. Behavioral data disaggregated by sex. (A, B) Self-administration: Rats learned to self-administer palatable food pellets over three sessions. No main effects or interactions involving Sex were observed. Mean (±SEM) number of cocaine infusions and lever presses during each 1 hr session. (C, D) Discrimination training: Over 11 sessions, rats learned to discriminate DS+ from DS- trials. We observed an interaction between Sex and DS in the lever presses but not trials measure, as female rats pressed more during DS+ trials across all sessions. Mean (±SEM) number of trials and lever presses during the 2 hr discrimination training session. (E, F) Relapse test: Rats’ lever responding during DS+, but not DS-, trials peaked at 1 day of abstinence and abated over 200 days. We observed an interaction between Sex and Day but no interaction involving Sex and DS in the trials measure as male rats pressed more during both DS trial types on some days. Further we observed no main effects or interactions involving Sex in the lever presses measure. This indicates that while male rats pressed during more trials on some days, male and female rats did not differ in the abatement of DS-controlled palatable food-seeking. Mean (±SEM) number of trials and lever presses during the 2 hr relapse test sessions under extinction conditions. Columns indicate mean (±SEM) for the group, while dots indicate values for individual rats.

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Figure 2—figure supplement 2. Food rewards earned disaggregated by sex. (A) Mean (±SEM) number of palatable food pellets earned during discrimination training, disaggregated by sex. (B) Mean (±SEM) amount of palatable food (g/kg) earned during discrimination training, disaggregated by sex.
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Figure 2—figure supplement 3. Subject body weights disaggregated by sex. (A) Mean (±SEM) body weight of subjects over the course of Experiment 1, disaggregated by sex.

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