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Cocaine falls into oblivion during volitional initiation of choice trials

Running title: volitional choice of saccharin

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Abstract

When facing a choice, most animals quit drugs in favor of a variety of nondrug alternatives. We recently found, rather unexpectedly, that choice of the nondrug alternative is in fact inflexible and habitual. One possible contributing factor to habitual choice is the intermittency and uncontrollability of choice trials in previous studies. Here we asked whether and to what extent volitional control over the occurrence of choice trials could change animals' preference by preventing habitual choice. To do so, rats were trained to nosepoke in a hole to trigger the presentation of two operant levers: one associated with cocaine, the other with saccharin. Rats were then free to choose among the two levers to obtain the corresponding reward, after which both levers retracted until rats self-initiated the next choice trial. Overall, we found that volitional control over choice trials did not change preference. Most rats preferred saccharin over cocaine and selected this option almost exclusively. Intriguingly, after repeated choice and consumption of saccharin, rats transiently lost interest in this option (i.e., due to sensory-specific satiety), but they did not switch to cocaine, preferring instead to pause during long periods of time before reinitiating a choice trial for saccharin. This finding suggests that during volitional initiation of a choice trial, rats fail to consider cocaine as an option. We discuss a possible associative mechanism to explain this perplexing behavior.

1 Introduction:

2
3 There is growing awareness of the need to provide nondrug alternatives to the
4 drug in animal models of drug addiction ^{1–4}. The majority of studies examining choice
5 between drug and nondrug rewards have demonstrated that most animals prefer the
6 latter over the former. This preference for nondrug reward has been observed over
7 different drugs of abuse, including cocaine ^{5–13}, heroin ^{10,14,15}, alcohol ^{16,17}, nicotine ¹⁸
8 and also methamphetamine ^{19–22}. Such preference occurs even after escalation of
9 drug intake ^{5,19} and despite availability of large drug doses ⁵. It is observed with
10 different types of nondrug options, including saccharin ^{5,6,8,10}, sucrose ^{5,6}, water ²³,
11 food pellets ^{7,19,20,24} and playful social interactions ²². Punishment, drastic increase in
12 cost or delay, or decrease in nondrug reward concentration are needed to affect the
13 preference ^{5,6,13,22}. Despite this remarkable reproducibility, the finding that alternative
14 nondrug rewards are generally preferred over drugs remains nevertheless difficult to
15 explain and integrate with the unique ability of drugs to directly activate brain reward
16 circuits ²⁵.

17
18 All the experiments described above have assessed drug versus nondrug
19 choice in animals using a discrete-trial choice procedure where choice trials are
20 presented intermittently, typically spaced with relatively long intervals (i.e., 8-10 min),
21 and entirely independently from the animal's behavior. Furthermore, onset of choice
22 trials and thus, the opportunity to choose, is signaled by non-contingent
23 exteroceptive cues (e.g., the motor noise associated with lever insertion into the
24 operant cage). Spaced, choice trials triggered non-contingently and signaled by an
25 external cue may favor the development of habitual and inflexible nondrug
26 preference ²⁶, particularly after chronic cocaine exposure, which is known to promote
27 habitual responding for nondrug reward ^{27–30}. For instance, it was shown that reward-
28 predictive cues favor the development of habits in a discrete-trial fixed-ratio 5
29 procedure ²⁶. We recently tested this hypothesis using a discrete-trial choice
30 procedure and found that preference for the nondrug alternative is indeed inflexible
31 and habitual ²³. Specifically, after devaluation by satiation, rats persisted to choose
32 the nondrug option (i.e., water), even if they consumed little of it upon delivery ²³.
33 This finding suggests that rats are stuck in a habitual decision-making mode, unable

1 to return to a goal-directed mode upon experiencing a change in value of the
2 nondrug option.

3 Here we asked whether and to what extent volitional control over choice trials
4 could change preference by preventing habitual choice. Specifically, animals were
5 required to perform a heterogeneous instrumental chain that consisted of nose
6 poking in a central hole to obtain an opportunity to choose among two levers for
7 cocaine or saccharin rewards. Importantly, the hole in which rats had to poke their
8 nose to trigger a choice trial was continuously present and was not associated with
9 any discrete external cue. Choice trials must thus somehow be self-initiated
10 volitionally, that is, via an internally-generated goal-directed decision ³¹. We asked
11 whether the inclusion of this volitional trial initiation feature (i.e. the nose poke chain)
12 could prevent habitual preference for the nondrug alternative and/or change its
13 direction in favor of the drug.

15 Material and methods

16 Subjects

17 A total of 34 male Wistar rats (Charles River, L'Arbresle, France, 270-323 g at the
18 beginning of the experiment) were used in the present study (experiment 1, N=22;
19 experiment 2, N=12). Rats were housed in groups of three and were maintained in a
20 light- (12-h reverse light-dark cycle) and temperature-controlled vivarium (23° C). All
21 behavioral testing occurred during the dark phase of the light-dark cycle. Food and
22 water were freely available in the home cages throughout the duration of the
23 experiment (i.e., rats were neither food- nor water-restricted) and consisted of
24 standard rat chow A04 (SAFE, Scientific Animal Food and Engineering, Augy,
25 France) that contained 60% of carbohydrates (largely corn starch), 16% of proteins,
26 12% of water, 5% of minerals, 3% of fat and 4% of cellulose. No synthetic or refined
27 sugar was added. All experiments were carried out in accordance with institutional
28 and international standards of care and use of laboratory animals [UK Animals
29 (Scientific Procedures) Act, 1986; and associated guidelines; the European
30 Communities Council Directive (2010/63/UE, 22 September 2010) and the French
31 Directives concerning the use of laboratory animals (décret 2013-118, 1 February

2013)]. All experiments have been approved by the Committee of the Veterinary Services Gironde, agreement number B33-063-5.

Surgery

Anesthetized rats [mixture of xylazine (15 mg/kg, i.p., Merial, Lyon, France) and ketamine (110 mg/kg, i.p., Bayer Pharma, Lyon, France)] were surgically prepared with silastic catheters (Dow Corning Corporation, Michigan, USA) in the right jugular vein that exited the skin in the middle of the back about 2 cm below the scapulae. After surgery, catheters were flushed daily with 0.2 ml of a sterile antibiotic solution containing heparinized saline (280 IU / ml) and ampicilline (Panpharma, Fougères, France). When a catheter leakage was suspected, the patency of the catheter was checked by an intravenous administration of etomidate (1 mg/kg, Braun Medical, Boulogne-Billancourt, France), a short-acting non-barbiturate anesthetic. Behavioral testing began 7-10 days after surgery.

Apparatus

Twelve identical operant chambers (30 x 40 x 36 cm) were used for all behavioral training and testing (Imetronic, Pessac, France). All chambers were located away from the colony room in a dimly lit room. They were individually enclosed in wooden cubicles equipped with a white noise speaker (45 ± 6 dB) for sound-attenuation and an exhaust fan for ventilation. These chambers have been described in detail elsewhere ^{6,8}. Briefly, each chamber was equipped with 2 automatically retractable levers (Imetronic) located in the middle of each side wall of the chamber, a commercially-available lickometer circuit (Imetronic), two syringe pumps, a single-channel liquid swivel (Lomir biomedical Inc., Quebec, Canada), two pairs of infrared beams and, finally, a nosepoke hole equipped with an infrared detector (Imetronic) and a sliding door. The hole (diameter: 2 cm) was located 5 cm above the grid floor in the back wall at equidistance between the two retractable levers.

Initial training under a heterogeneous chain schedule of reinforcement

Rats were first trained over 2-5 weeks to lever press to self-administer either water sweetened with 0.2% saccharin (0.32 ml delivered over 20 s) or intravenous cocaine (0.25 mg delivered over 5 s) under a simple fixed-ratio (FR) 1 or 2 (time-out 20 s) schedule, as described in detail elsewhere ³². One lever was associated with cocaine reward (lever C), the other with saccharin reward (lever S). The dose of cocaine

1 tested in the present study (corresponding roughly to 0.75 mg/kg) has been
2 extensively used in many previous studies on cocaine self-administration in rats,
3 including our own initial choice experiments ^{5,6,8}. Note that higher doses of cocaine
4 produced the same outcomes as this dose, even after extended access to cocaine
5 for self-administration. Saccharin (a potent non-caloric sweetener) was selected as
6 the nondrug option because it is an inessential reward with no direct advantageous
7 effect on growth, survival and/or reproduction. The concentration of saccharin (i.e.,
8 0.2%) used in the present study is within the range of maximally rewarding
9 concentrations (0.1-1%) ^{6,33,34}.

10 Training sessions of cocaine or saccharin self-administration were alternated
11 between days and conducted 6 days a week. Sessions began with extension of one
12 single lever (C or S). If rats responded on the available lever, they were rewarded by
13 the corresponding reward (cocaine or saccharin). Reward delivery was signaled by a
14 20-s illumination of the cue-light above the lever during which time responses were
15 not rewarded (i.e., time-out period). Sessions ended after rats had earned a
16 maximum of 30 rewards or 3h had elapsed. The maximum number of saccharin or
17 cocaine rewards was limited to 30 per session to ensure approximately equal
18 exposure to both rewards before choice testing. The central nose poke hole was
19 closed (i.e., sliding door closed) throughout this initial training period.

20 After acquisition of lever pressing for cocaine and saccharin, rats were trained over
21 four additional weeks to self-administer cocaine or saccharin under heterogeneous
22 chain-schedule. Sessions of cocaine or saccharin self-administration were alternated
23 between days and conducted 6 days a week. Sessions began with the nose poke
24 hole open and with both levers retracted. Rats had to poke their nose in the hole to
25 extend the lever available on that day (lever C or S) and then, to press the extended
26 lever to obtain the corresponding reward (Fig 1A). Reward delivery was signaled by
27 retraction of the lever and illumination of the cue-light above it as described above.
28 Rats had to repeat this behavioral chain (i.e., nose poking followed by lever
29 pressing) to obtain each next reward. Rats were initially trained to perform one nose
30 poke followed by two lever presses (i.e., FR1-FR2). Nose poking requirement was
31 then gradually increased to reach the final FR10-FR2 schedule (i.e., 10 nose pokes
32 followed by 2 lever presses) (Fig 1C). The nose poke requirement was set to 10 – a
33 relatively effortful requirement – to promote volitional self-initiation of a trial. The FR

value for lever presses was set to 2 to avoid accidental responding during choice sessions. Sessions ended when rats obtained a total of 30 rewards or 3h had elapsed. Rats were trained under this final schedule for at least 10 consecutive sessions until stabilization of responding (i.e., no increasing or decreasing trend over 3 consecutive sessions for each type of reward and between-session variation < 10%).

Heterogeneous chain schedule of choice between cocaine and saccharin

After training, rats were allowed to choose between cocaine and saccharin under the same chain schedule of reinforcement. Specifically, during choice sessions, nose poke responses were followed by simultaneous presentation of both levers S and C, allowing rats to choose between the two by making two consecutive lever presses (Fig 1B). A response on the alternate lever before satisfaction of this response requirement reset it. Response resetting occurred very rarely, however. Reward delivery was signaled by the simultaneous retraction of both levers and illumination of the cue-light above the selected lever. To initiate each next choice trial, rats had to repeat the same behavioral chain (i.e., 10 nosepoke responses followed by 2 consecutive responses on the selected lever). If rats failed to respond on either lever within 5 min, both levers retracted and no cue-light or reward was delivered. This almost never happened, however. The number of trials per session was initially set to 30 in the experiment 1 but was rapidly increased to 70 to allow for saccharin satiation. Sessions ended when rats completed all 70 trials or 3h had elapsed.

Discrete-trial schedule of choice between cocaine and saccharin

Rats in the first experiment were then tested for 8 sessions in the discrete-trial choice schedule. The timeline of training and testing schedules is depicted in figure 1C. Each session comprised 12 trials spaced by 10-minutes inter trial intervals and consisted in 2 successive phases; sampling and choice. During the sampling phase composed of 4 trials, one lever was presented at a time in the following order: Lever C, Lever S, Lever C, Lever S. Two lever presses on the available lever resulted in delivery of the corresponding reward, illumination of the cue-light above the lever, lever retraction and initiation of a new 10-minutes inter-trial interval. During the choice phase composed of 8 trials, rats were allowed to choose between the levers S and C, simultaneously presented at the trial onset. Two consecutive lever presses on a lever resulted in the corresponding reward delivery, signaled by the illumination

of the cue-light above the selected lever and the retraction of the levers. In both phases, failure to respond on the lever(s) in 5 minutes was considered as an omission and resulted in lever(s) retraction with no cue-light nor reward delivery.

Pre-session access to saccharin

In the second experiment, rats were trained and tested under heterogeneous chain schedule as described above. We assessed the effect of pre-session free saccharin access on choice between cocaine and saccharin. Rats were first trained to choose between cocaine and saccharin under chain-schedule during 3 days to establish baseline preference (BL) and were then tested with free saccharin pre-session access during 3 additional choice sessions. During the 30-min saccharin access, the central nose poke hole was closed and levers were retracted. The drinking cup was initially filled with 0.08 ml of saccharin and rats could obtain additional volumes of saccharin by continuously licking the cup (i.e., 0.04 ml per 20 consecutive licks) which they did readily. We also assessed the effect of pre-session saccharin access on cocaine self-administration under FR10-FR2 chain schedule during cocaine training sessions.

Drugs

Cocaine hydrochloride (Coopération Pharmaceutique Française, Melun, France) was dissolved in a solution of NaCl (0.9%) which was filtered through a syringe filter (0.22µm) and kept at room temperature in a 500-ml sterile bag ($21 \pm 2^{\circ}\text{C}$). Drug doses were expressed as the weight of the salt. Sodium saccharin (Sigma-Aldrich, St Quentin-Fallavier, France) was dissolved in tap water at room temperature ($21 \pm 2^{\circ}\text{C}$). Sweet solutions were renewed each day.

Data Analysis

Relative cocaine choices were expressed as percent of total choices. Preference was compared against indifference using t-tests. Nose poke and lever press response rates were computed by dividing the response requirement (10 and 2, respectively) by the average duration of nose poke and lever press chains, respectively. Trial-by-trial variability in response rates and lever press latency was assessed by measuring the coefficient of variation for each variable (i.e. ratio of the standard deviation to the mean). Pauses in trial self-initiation were defined as inter-saccharin access intervals equal to or greater than 5 min. Post-saccharin pauses

followed by cocaine choice were very rare and were thus ignored. Statistical analyses were run using Statistica, version 7.1 (Statsoft Inc., Maisons-Alfort, France) and consisted in t-test or repeated measure analysis of variance, using sessions, reward or devaluation condition as within-subject factors. When the normality assumption was violated, we used the non-parametric tests Mann-Whitney and Wilcoxon. All analysis were conducted on the average of the last three sessions of the relevant experimental phase (training sessions, choice sessions, baseline, etc.).

Results:

Rats rapidly learned to self-administer saccharin and cocaine under chain-schedule and produced several hundreds of nose poke and lever press responses during the last stage of training (Fig 2A-B). Rats responded slightly less for cocaine than saccharin and earned less reward access during cocaine sessions (Fig 2A) (Number of access over the last 3 sessions: Wilcoxon $Z_{22}=3.18$, $p<0.01$ (inset)). Although the latency to initiate the first nose poke sequence did not differ between cocaine and saccharin ($Z_{22}=0.21$, $p>0.1$), the latency of the first reward access (from lever insertion) strongly differed as a function of reward, with rats responding on average 50 times faster for saccharin than cocaine (Fig 2C) (saccharin: 3.5 ± 0.5 ; cocaine: 164.7 ± 54.7 ; $Z_{22}=4.11$, $p<0.0001$). This difference in 1st access latency may have resulted from the emission of perseverative nose pokes (i.e. after insertion of the lever) during the first trial of cocaine sessions but not saccharin sessions (Fig 2D) ($Z_{22}=3.92$, $p<0.0001$). This perseverative behavior was particularly marked before the first cocaine access, when the reward had not yet been experienced, but also moderately persisted throughout the session (Fig 2D) (cocaine versus saccharin sessions: $Z_{22}=2.97$, $p<0.01$). While more perseverative nose pokes were emitted in cocaine sessions compared to saccharin sessions, nose poking response rate was lower (Fig 2E) ($Z_{22}=4.11$, $p<0.0001$). Furthermore, rats were systematically slower to press on the cocaine lever upon insertion (Fig 2F) ($Z_{22}=4.11$, $p<0.0001$), and completed the response requirement at a lower rate (Fig 2G) ($Z_{22}=4.11$, $p<0.0001$). These results demonstrate that rats were less eager to get cocaine reward than saccharin reward after lever presentation. Chains execution was more automatic and stereotyped in saccharin sessions compared to cocaine sessions, as shown by lower trial-by-trial variability in nose poke response rate (Fig 2H) ($F_{1,21}=43.2$, $p<0.0001$), lever press latency (Fig 2I) ($F_{1,21}=38.9$, $p<0.0001$) and lever press response rate (Fig

2J) ($F_{1,21}=9.2, p<0.01$). Together, these results suggest that chains of actions are executed with greater efficiency and regularity in saccharin sessions relative to cocaine sessions.

Rats expressed a robust preference for saccharin over cocaine from the first choice session (Fig 3A) (against indifference: t -values >6.5 , p -values <0.0001). Among saccharin-preferring rats, which represented 86% of the cohort ($N=19$), 16 never selected the cocaine option (Fig 3B). Rats expressing an exclusive preference for saccharin chose this reward in bouts of varying lengths, separated by relatively long pauses with no self-initiated trials (Fig 3C-E, S1). These pauses likely reflect periods of sensory-specific satiety during which saccharin is transiently devalued⁹. Importantly, when rats reinitiated a bout of saccharin choices after each pause, they consumed $97.0\pm0.6\%$ of the volume of saccharin available after each choice, suggesting that during a bout, saccharin had recovered most of its value. While there was substantial inter-individual variability, the average cumulated duration of satiety pauses exceeding 5 minutes reached 1.4 ± 0.1 h (Fig 3E). This total duration represents a significant opportunity cost since rats could have earned during this time 1 to 29 injections depending on individual injection rates during training sessions (injection rate in injection/h: range [2.2–15.6], median:10.4; opportunity cost: range [1-29], median: 8.7 injections) (Fig 3C, right). Additional control experiment (described below) shows that when there is no choice, satiety induced by pre-session ad libitum access to saccharin has no inhibitory effect on heterogeneous chain responding for cocaine (Fig S4).

During the last session, 3 rats expressed a non-exclusive preference for saccharin and 3 rats preferred cocaine. Like the majority of rats, these 6 rats initiated the session with saccharin choices, but after a variable number of saccharin choices, they eventually switched to cocaine choices (Fig S1). In general, once they made a cocaine choice, they persisted in choosing cocaine nearly exclusively throughout the session. As shown previously, this behavior is likely due to the anorexigenic effects of cocaine that is known to suppress motivation for saccharin^{9,35}. But why are these 6 rats selecting cocaine in the first place, when the majority (72%) failed to do so? To address this question, we examined performance of rats during training sessions, with respect to their choice pattern (i.e. switch to cocaine or not). During cocaine sessions, we found that rats that eventually switched their choice to cocaine (SC)

completed the nose poke sequence at a greater rate (Fig 4A) ($Z=2.14$, $p<0.05$), and were faster to press on the lever upon insertion than rats preferring saccharin exclusively without switching to cocaine (ES) (Fig 4B) ($Z=1.99$, $p<0.05$). Lever press response rate did not differ across groups (Fig 4C) ($Z=1.69$, $p=0.09$). Although SC rats tended to make less perseverative nose poke responses during the first cocaine trial, the effect was not significant ($Z=1.73$, $p=0.08$) (Fig 4D). Finally, SC rats self-administered more injections than ES rats during training sessions (Fig 4E) ($Z=2.43$, $p<0.05$). SC and ES rats did not differ in behavior during saccharin sessions (Fig S2). Together these results suggest that SC rats selected cocaine during choice sessions because they made a stronger association between nose pokes and presentation of the cocaine lever. In contrast, ES rats might not have selected cocaine during choice sessions because this option was not part of the associative structure of the task, due to the weak association between the first (nosepoke) and second (lever press) link of the instrumental chain.

When tested in the classic discrete-trial choice schedule, preference for cocaine stabilized at 17.5% of cocaine choice during the last 3 sessions (against indifference: $t_{22}=7.96$, $p<0.0001$) (Fig 5A-B). Although average preference was similar between choice schedules ($Z_{22}=1.21$, $p>0.1$), the proportion of rats expressing exclusive preference for saccharin was lower under the classical discrete-trial choice schedule (6 out of 22) compared to the heterogeneous chain schedule (16 out of 22) (Fig 5B and 3B).

To demonstrate that choice trials were initiated under volitional goal-directed control in the chain schedule task, we trained another group of rats in this task and measured the effect of 30-min pre-session saccharin access on saccharin self-administration and preference. Saccharin satiety was induced by allowing rats to freely consume saccharin in the operant chamber by licking in the receptacle (1 volume 0.04mL per 20 licks) during 30 minutes immediately before each session, for 3 sessions.

During training, the results described above were replicated (Fig S3). Specifically, performance was more efficient and automated on saccharin sessions relative to cocaine sessions, with higher nose poke and lever press response rates, less perseverative nose pokes and shorter lever press latencies. On devaluation

1 sessions, rats consumed 17.2 ± 0.6 mL of saccharin during pre-session access to it in
2 the operant box. As expected, the number of saccharin access decreased during
3 devaluation sessions compared to baseline but the number of cocaine injections
4 remained unchanged (Fig 6A) (saccharin access: $Z_{12}=3.06$, $p<0.01$; cocaine access:
5 $Z_{12}=0.34$, $p>0.1$). As a result, preference was not significantly affected by satiety (Fig
6 6B-C) ($Z_{12}=1.69$, $p>0.05$). The decrease in the number of saccharin access was
7 associated with an increase in the cumulated pause duration, further indicating
8 reliable saccharin devaluation by satiety (Fig 6D-E) ($Z_{12}=2.35$, $p<0.05$). The nose
9 poke response rate was also decreased (Fig 6F) ($F_{(1,11)}=8.35$, $p<0.05$) and the
10 latency to press on the saccharin lever upon insertion was slightly but significantly
11 increased (Fig 6G) ($Z_{12}=2.35$, $p<0.05$). However, the response rate on the saccharin
12 lever was not significantly impacted by pre-session saccharin (Fig 6H) ($F_{(1,11)}=1.21$,
13 $p>0.1$). In addition, rats consumed $92.6 \pm 3.1\%$ of the volume of saccharin available
14 after each choice, suggesting that when selected, saccharin had apparently
15 recovered most of its value. Thus, although pre-session access to saccharin
16 considerably reduced self-initiation of choice trials, it did not affect what animals
17 eventually chose in a given trial.

18 Importantly, when cocaine was the only option available, free saccharin access had
19 no effect on cocaine self-administration under chain-schedule (Fig S4) (number of
20 injection, $F_{(1,11)}=1.21$, $p>0.1$). More specifically, though saccharin satiety delayed the
21 first cocaine injection during the first trial of the sessions, it had no subsequent
22 impact on chain responding for cocaine (Fig S4). This result clearly shows that
23 saccharin satiety *per se* has no inhibitory effect on instrumental chain responding for
24 cocaine.

25 Discussion

26
27 Overall, volitional initiation of choice trials had no impact on rats' preference,
28 compared to previous research where choice trials were presented intermittently and
29 non-contingently. During volitionally-initiated choice trials, rats preferred saccharin
30 over cocaine, with a large majority expressing an exclusive preference for the
31 nondrug alternative (i.e., they never initiated a trial that ended up with a cocaine
32 choice). Devaluation of saccharin by satiety considerably decreased initiation of
33 choice trials and increased inter-trial pauses, indicating goal-directed control ^{36,37}

1 and, thus, confirming the volitional nature of choice trial initiation in our task ³¹.
2 However, on those few self-initiated trials following devaluation, saccharin remained
3 the preferred option and was fully consumed. This observation suggests that
4 saccharin devaluation was not steady but fluctuated over time and that saccharin
5 transiently recovered its value during choice, making interpretation in terms of habit
6 uncertain in comparison to our previous study²³. In theory, after devaluation of the
7 preferred nondrug option, rats could self-initiate choice trials and then switch their
8 choice to cocaine, but most did not, preferring pausing during long periods of time
9 before reinitiating a choice trial for saccharin. Only few rats were able eventually to
10 switch their choice to cocaine. To explain this intriguing behavior, we propose that
11 rats may have preferentially associated initiation of choice trial with saccharin
12 outcome, leading to oblivion of the cocaine option.

13
14 During choice sessions, most rats expressed an exclusive preference for
15 saccharin and self-administered this reward in bouts of saccharin accesses.
16 Surprisingly, when saccharin temporally lost its value by specific satiety due to
17 repeated saccharin choices, rats preferred pausing during long periods of time
18 before reinitiating a choice trial for saccharin. This resulted in an opportunity cost
19 since many cocaine injections could have been earned during these pauses. One
20 may argue that cocaine would be so low on the value ladder of rats that it would still
21 remain lower than the saccharin option, even in saccharin-sated rats. However, this
22 is unlikely for several reasons. First, most of the rats expressing an exclusive
23 preference for saccharin in the volitional choice procedure chose the cocaine option
24 at least once per session when tested in the classic discrete-trial procedure (12 out
25 of 16 rats). Secondly, when cocaine and saccharin levers are continuously presented
26 under concurrent schedule of reinforcement, 90% of the rats eventually switch to
27 cocaine and choose this option exclusively until the end of the session ⁹. These
28 comparisons suggest that some specific features of the volitional choice procedure
29 favors the expression of exclusive preference for the nondrug option.

30
31 Performing the nose poke chain is required to initiate both cocaine and
32 saccharin trials during training under chain-schedule. Thus, the same response is
33 associated with two distinct outcomes of different value. Analysis of performance
34 during training sessions suggests that nose poke responses were preferentially

1 associated with saccharin accesses. Indeed, chains of action were rapidly executed
2 with high efficiency and consistency (low trial by trial variability) in saccharin
3 sessions relative to cocaine sessions. Furthermore, rats rapidly stopped nose poking
4 when the lever extended during saccharin sessions but persisted during cocaine
5 sessions. This suggests that insertion of the saccharin lever reliably predicted
6 saccharin availability and instantly triggered approach of the lever and lever presses,
7 with a short latency. On the other hand, weaker association between nose pokes
8 and insertion of the cocaine lever resulted in perseverative nose pokes and longer
9 lever press latencies, as if rats were expecting and waiting for the insertion of the
10 saccharin lever. It is therefore possible that during choice sessions, in which cocaine
11 and saccharin levers are concurrently presented upon completion of the nose poke
12 chain, the cocaine lever is not considered in the associative structure of the task. In
13 other words, rats would only initiate choice trial for saccharin accesses, as if they
14 failed to consider the cocaine option.

15
16 As would be predicted from this hypothesis, only rats making stronger
17 association between nose pokes and insertion of the cocaine lever during cocaine
18 training sessions did occasionally selected the cocaine option during choice
19 sessions. Concurrent training in alternate cocaine and saccharin sessions may have
20 promoted preferential association between nose poke responses and saccharin
21 access. Alternatively, cocaine reinforcement may not be strong enough to promote
22 consistent association between nose pokes, presses on the cocaine lever and the
23 cocaine injection. However, previous studies showing reliable cocaine self-
24 administration under heterogeneous chain-schedule make this hypothesis unlikely
25 ^{38–40}. To investigate further these intriguing results, it would be interesting to
26 determine whether extended cocaine training under heterogeneous chain-schedule,
27 followed by limited saccharin training would mitigate the bias observed in this study
28 and favor cocaine preference during choice sessions.

29
30 Though pre-session access to saccharin considerably reduced self-initiation
31 of choice trials, saccharin remained nevertheless the preferred option and was fully
32 consumed after being chosen. This suggests that though saccharin devaluation was
33 effective, it was not steady but fluctuated over time and that saccharin recovered

1 most of its value during choice. However, it is important to point out that when choice
2 trials were initiated, there was evidence for differential sensitivity to devaluation
3 across links in the chain of actions. Devaluation had the greatest effect on nose
4 poking, small but significant effect on lever press latency, and no effect on lever
5 press response rate. This differential sensitivity of sequence subcomponents to
6 motivational control has been previously reported ⁴¹ and is partially consistent with
7 the hierarchical decision-making model proposed by Dezfouli and colleagues ^{42–44}. In
8 this model, the authors suggest that individual actions are concatenated to form
9 unitary sequence which can be initiated under goal-directed control but executed
10 under habitual control. In our study, the performance during saccharin training and
11 choice sessions was characterized by high response rates, short lever press
12 latencies and low trial-by-trial variability suggesting great regularity and automaticity
13 of instrumental performance and concatenation of individual actions into behavioral
14 sequences (action chunking)^{45–47}.

15 It is worth noting that cocaine exerts anorexigenic properties which can
16 impede responding for saccharin under certain choice settings, as shown previously
17 ⁹. It is thus possible that when sated on saccharin, most rats prefer to pause instead
18 of initiating a cocaine choice to avoid that its anorexigenic effects interfere with
19 subsequent saccharin choices. However, such avoidance behavior is unlikely as it
20 would require rats to anticipate the saccharin-suppressing effects of cocaine. We
21 have previously shown that rats are unable to associate cocaine self-administration
22 with suppression of saccharin, even in a procedure optimally designed to promote
23 this learning ⁹.

24 To conclude, this study highlights the importance of the task structure in
25 understanding choice between drug and nondrug rewards. There are multiple
26 interactions between cues, actions and outcomes and the picture becomes even
27 more complex when subjects are required to choose between outcomes of different
28 values. We have shown that intermittent and uncontrollable presentation of levers
29 favors habitual lever pressing behavior and promote preference inflexibility ²³. Here,
30 we found that allowing volitional control over the occurrence of choice trials failed to
31 favor cocaine preference. On the contrary, introduction of the nose poke chain
32 feature abolished entirely cocaine choice in most rats, probably because of a
33 preferential association of this feature with the alternative nondrug option at the

1 expense of the cocaine option. The resulting choice pattern departed in important
2 ways from that seen in discrete-trial or concurrent choice schedules. Introducing
3 volitional control over the occurrence of choice trials may better reflect the human
4 situation which often involves intentional choices and planning to procure and
5 consume drugs. In addition, our results using the chain-schedule task suggest more
6 complex hierarchical decision-making mechanisms. Interestingly, it seems that when
7 animals are free to self-initiate choice trials, this seems to protect them further from
8 drug choices compared to more constrained choice settings such as discrete-trial
9 choice schedules. Clearly, future research is needed to better understand the factors
10 underlying this phenomenon and to evaluate its clinical relevance and significance
11 for addiction.

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13
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Figure legends

Figure 1: Experimental design and timeline. (A-B) Schematic representation of hierarchical chain-schedule during training (A) and choice (B) sessions. The black circle represents the nose poke hole and black rectangles represent levers. (C) Timeline of experiments 1 and 2.

Figure 2: Differential performance during cocaine and saccharin training sessions under chain-schedule. (A) Mean number of saccharin (black circles) and cocaine (white circles) accesses (\pm SEM) across training sessions. Inset: average across the last 3 training sessions. * $p < 0.05$, Difference between cocaine and saccharin. (B) Mean number of nose poke (NP) responses (\pm SEM) during saccharin (sac) and cocaine (coc) sessions. (C) Mean latency (\pm SEM) of first nose poke and first reward access in saccharin (black bars) versus cocaine (white bars) sessions. ** $p < 0.0001$ compared to cocaine. (D) Mean number of perseverative nose poke responses per trial (\pm SEM) during the first and next trials of saccharin and cocaine sessions. ** $p < 0.0001$ and * $p < 0.01$ compared to cocaine. (E-G) Mean (\pm SEM) nose poke response rate (E), lever press latency (F) and lever press response rate (G) in saccharin versus cocaine sessions. * $p < 0.0001$ compared to cocaine. (H-J) Mean coefficient of variation (\pm SEM) of nose poke response rate (H), lever press latency (I) and lever press response rate (J) in saccharin versus cocaine sessions. ** $p < 0.0001$ and * $p < 0.01$ compared to cocaine. All bar graphs represent the average performance over the last three training sessions under FR10-FR2 chain schedule. Response rates are expressed in response per second.

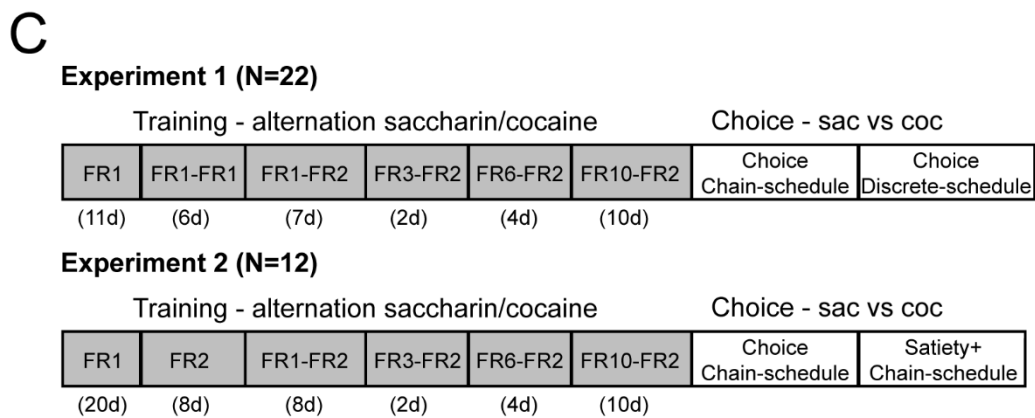
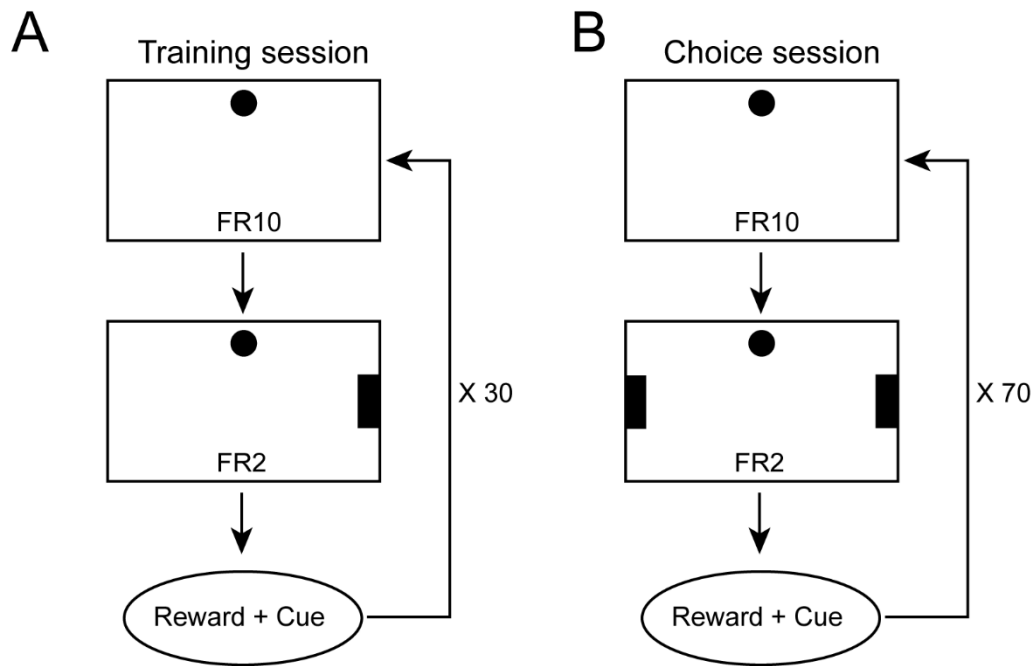
Figure 3: Rats preferred saccharin under heterogeneous chain-schedule. (A) Mean percentage of cocaine choice (\pm SEM) across choice sessions under chain-schedule. The red dotted line represents indifference. (B) Individual preference score (in % of cocaine choice) averaged over the last three choice sessions. Red dots represent preference scores of rats preferring cocaine. (C) Examples of individual choice patterns during choice (left) and cocaine training (right) sessions. Vertical bars above or below the horizontal line indicate saccharin and cocaine choices, respectively. Pink boxes illustrate the opportunity cost associated with pauses in initiation of choice trials during choice sessions. Based on the pattern of cocaine self-administration during training sessions (right), several cocaine injections could have been earned during these pauses. (D) Cumulative saccharin accesses during the last choice session under chain-schedule. Grey lines represent individual rats expressing an exclusive preference for saccharin (N=16). The red line represents the average of the group. (E) Cumulated duration of inter-saccharin access pauses longer than

5 minutes, for each individual rat, sorted in ascending order. Red bars represent cocaine-preferring rats.

Figure 4: Greater cocaine self-administration performance during training in rats that switched their choice from saccharin to cocaine. (A-C) Mean (\pm SEM) nose poke response rate (A), lever press latency (B) and lever press response rate (C) during cocaine training sessions, in rats that preferred saccharin exclusively without switching to cocaine (ES, black) and in rats that switched their choice from saccharin to cocaine (SC, white). Response rates are expressed in response per second. * $p < 0.05$. (D) Mean number of perseverative nose poke responses (\pm SEM) in ES and SC rats. (E) Mean number of cocaine injection (\pm SEM) in ES and SC rats. * $p < 0.05$.

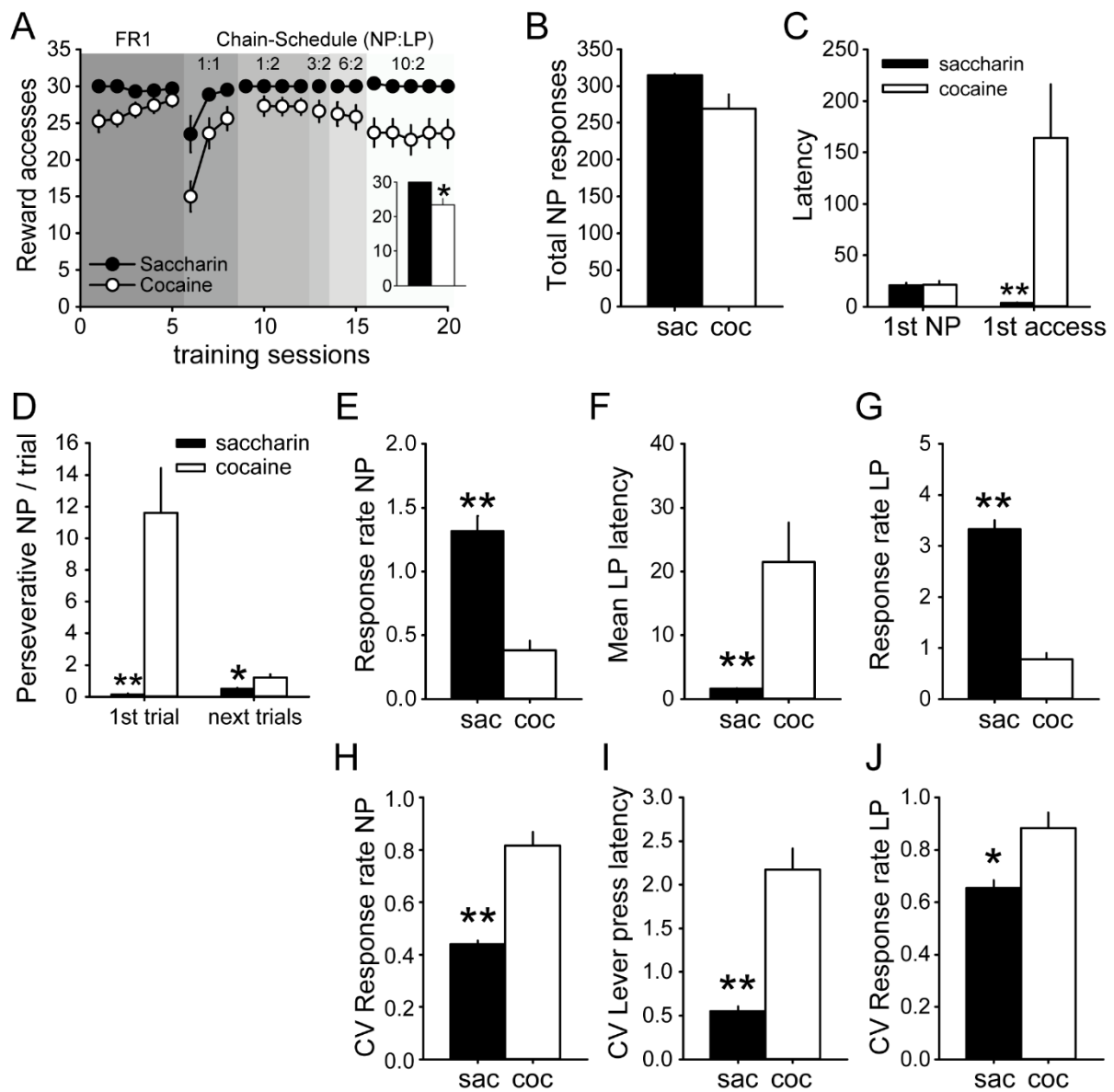
Figure 5: Non-exclusive preference for saccharin under discrete-trial choice schedule. (A) Mean percentage of cocaine choice (\pm SEM) across choice sessions under discrete-trial choice schedule. The red dotted line represents indifference. (B) Individual preference score (in % of cocaine choice) averaged over the last three choice sessions.

Figure 6: Preference insensitivity to saccharin devaluation by specific satiety. (A) Mean number of saccharin (black) and cocaine (white) accesses (\pm SEM) during baseline choice sessions (BL) and following devaluation by specific satiety (SAT). * $p < 0.01$ cocaine versus saccharin; # $p < 0.01$ satiety versus baseline. (B) Mean percentage of cocaine choice (\pm SEM) under baseline and satiety conditions. * $p < 0.05$ against indifference. (C) Individual preference scores (in % of cocaine choice) averaged over baseline (left) and satiety (right) sessions under chain-schedule. The red dotted line represents indifference. Rats choosing saccharin exclusively during baseline sessions are indicated with white circles (ES) whereas rats switching to cocaine during baseline sessions are indicated with red circles (SC). (D) Examples of individual choice patterns during baseline (left) and satiety (right) choice sessions under chain-schedule. Vertical bars above or below the horizontal line indicate saccharin and cocaine choices, respectively. (E) Mean cumulated duration (\pm SEM) of inter-saccharin access pauses longer than 5 min during baseline and satiety sessions. * $p < 0.05$. (F-H) Mean (\pm SEM) nose poke response rate (F), lever press latency (G) and lever press response rate (H) during baseline and satiety choice sessions. Response rates are expressed in response per second. * $p < 0.05$.



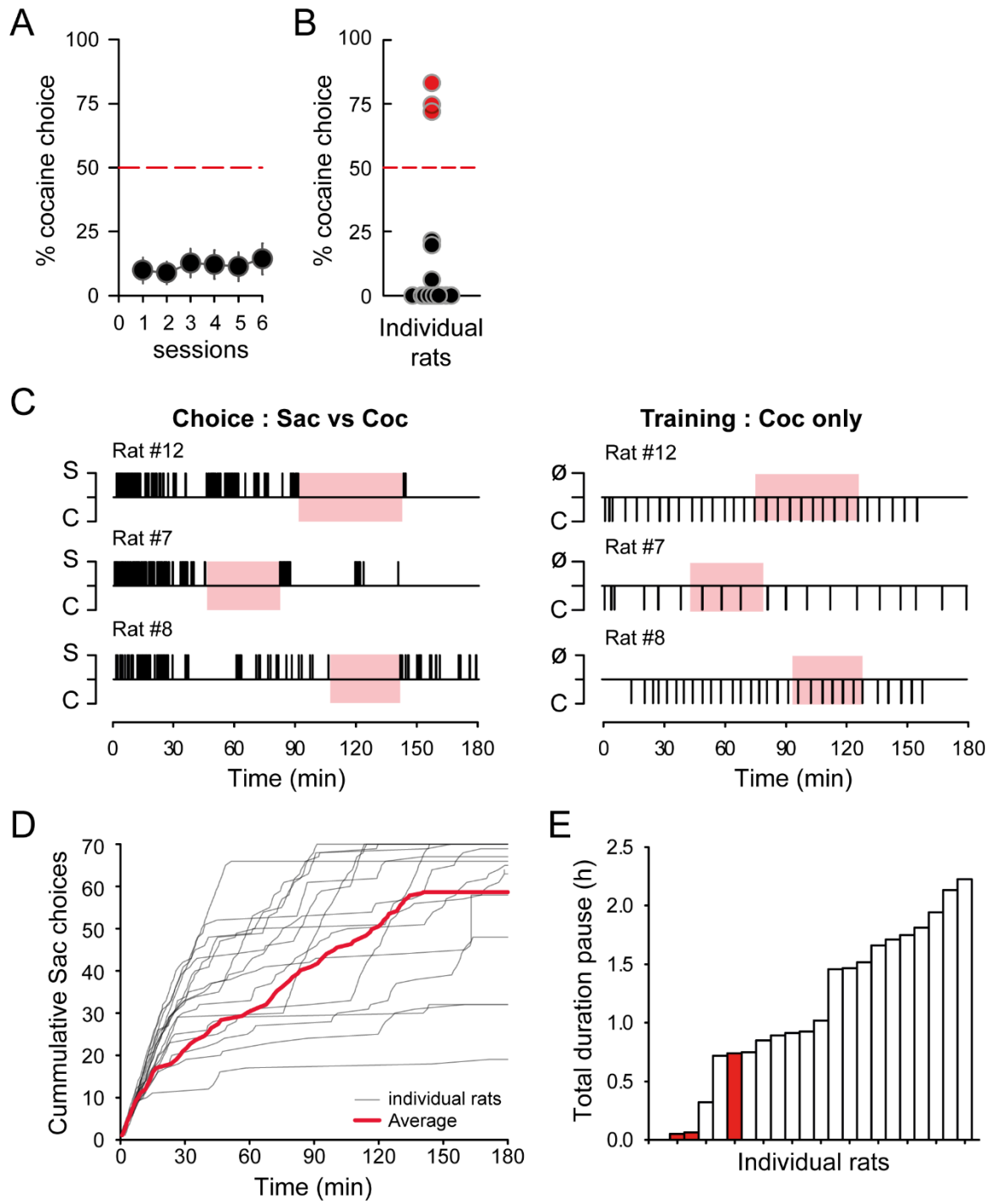
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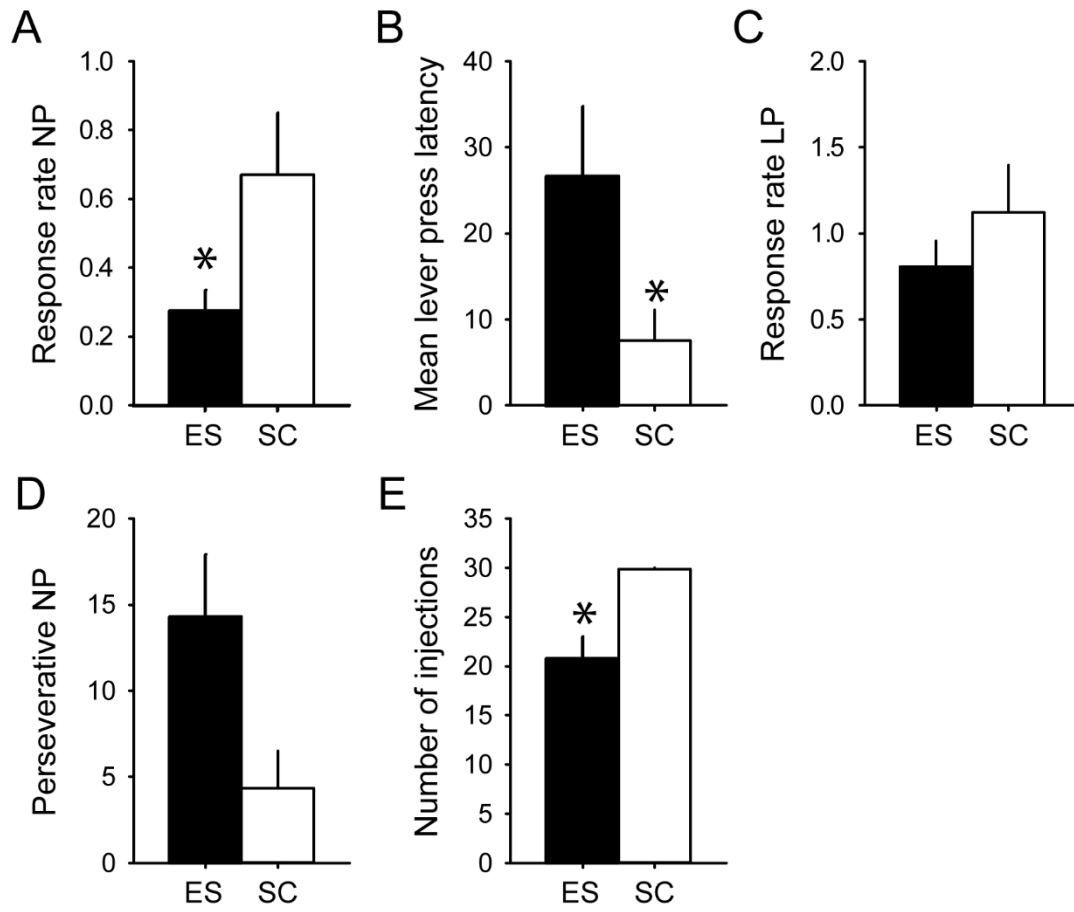
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