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Correlates of recovery from incentive downshift: A preliminary selective breeding study

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Rats exposed to a downshift in the concentration of a sucrose solution from 32% to 4% exhibit a transient suppression of consummatory behavior relative to an unshifted control group always exposed to 4% sucrose. One explanation of this effect, known as consummatory successive negative contrast (cSNC), explains consummatory suppression as arising from an emotional state of frustration that redirects behavior away from the source of the devalued solution. A preliminary selective breeding protocol consisting of three experiments was performed. Experiment 1 reports results from 5 generations of selected breeding for either high (H) or low (L) recovery rates from cSNC. A control line of randomly (R) mated rats was included. cSNC was reduced in H rats, but L and R rats did not differ across generations. H rats also provided no evidence of behavioral activation in acquisition or increased persistence in extinction after partial reinforcement, rather than continuous reinforcement. L and R rats, by contrast, showed both of these effects. H rats were also significantly smaller in body size than R rats, but did not differ in terms of water intake, sucrose sensitivity, open-field activity, or responding to sucrose solutions before the downshift. In Experiment 2, H infants from the sixth selected generation showed increased bandwidth in vocalizations induced by mother-infant separation relative to L and R rats. Experiment 3 showed that H rats failed to show increased response to incentive downshift after treatment with the nonselective opioid antagonist naloxone, as done by L and R rats. The results, if replicated, may provide support for the interpretation of a significant role of frustration in cSNC.

An unexpected reduction in incentive magnitude or quality can lead to a variety of effects that have collectively been referred to as frustrating (Amsel, 1992; Papini & Dudley, 1997). Incentive downshifts have been extensively studied using a procedure known as consummatory successive negative contrast (cSNC; Flaherty, 1996). In a typical cSNC experiment, a group of rats receives free access to 32% sucrose during 10 daily trials and then is downshifted to 4% sucrose during Trials 11-15. During these final trials, the consummatory behavior of downshifted rats is lower than that of a control group exposed to 4% sucrose in all trials. This consummatory suppression reflects the detection of a mismatch between the magnitude of the current incentive and the reactivated memory of the incentive previously received under similar conditions (Papini & Pellegrini, 2006). A large enough mismatch (e.g., an 8-to-1 ratio of the preshift to postshift magnitudes) triggers an emotional response, alternative behaviors, and conflict (Flaherty, 1996). Papini (2003; Papini, 2006; Papini, Wood, Daniel, & Norris, 2006; Wood, Daniel, & Papini, 2005) applied Amsel's (1992) frustration theory to the special case of cSNC. Frustration theory uniquely predicts that consummatory suppression has two dissociable sources: (1) *Primary frustration*, the unconditioned reaction to a negative mismatch, which provides one source of consummatory suppression; and (2) *Secondary frustration*, a conditioned, anticipatory reaction elicited by stimuli (including taste stimuli) paired with primary frustration, which provides a second source of suppression. The intensity of primary frustration is a main determinant of the initial size of the cSNC (i.e., on the first downshift trial, usually Trial 11), whereas the intensity of secondary frustration determines the rate of recovery from cSNC (i.e., on the second and subsequent downshift trials, usually Trials 12-15). The intensity of both states of frustration is assumed to be positively related to the

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size of the ratio of postshift to preshift incentive magnitudes, at least for the sucrose concentrations typically used in these experiments (Papini & Pellegrini, 2006).

This theoretical framework was suggested by evidence pointing to the operation of different mechanisms underlying cSNC during the first vs. second downshift trials (typically Trials 11 and 12, respectively). For example, consummatory behavior during Trial 11 is modulated by the delta opioid receptor agonist DPDPE (Wood et al., 2005) and antagonist naltrindole (Pellegrini, Wood, Daniel, & Papini, 2005), but not by the kappa opioid receptor agonist U50,488H (Wood, Norris, Daniel, & Papini, 2008). Conversely, DPDPE and naltrindole do not affect behavior on Trial 12, but U50,488H has a dose-dependent effect on consummatory behavior in this trial. Similarly, benzodiazepine anxiolytics reduce cSNC on Trial 12, but tend to have no effects when administered before Trial 11 (Becker, 1986; Flaherty et al., 1990; Flaherty & Rowan, 1989; Ortega, Glueck, Daniel, Prado-Rivera, White, & Papini, 2014). Moreover, rats exhibit hypoalgesia when tested in the hot plate immediately after Trial 12, but no change in pain sensitivity when tested after Trial 11 (Mustaca & Papini, 2005). Lesion data are also consistent with this view. For example, damage to the anterior cingulate cortex does not affect consummatory performance on Trial 11, but it retards recovery from cSNC on subsequent trials (Ortega, Uhelski, Fuchs, & Papini, 2011).

There is also evidence consistent with the general postulate that genetic and/or epigenetic factors play a role in the emergence of the cSNC effect. For example, individual differences in recovery from incentive downshift are related to sensitivity to opioid blockage in an unrelated task. Pellegrini et al. (2005) matched rats in terms of their performance on Trial 11 and then segregated them according to the extent of their recovery on Trial 12, thus generating fast- and a slow-recovery groups. When tested in an activity test in a dark, walled enclosure designed to minimize unconditioned anxiety of the type observed when exposing rats to bright, open spaces (e.g., Braun, Skelton, Vorhees, & Williams, 2011), slow-recovery rats (but not fast-recovery rats) showed significant behavioral suppression induced by the nonselective opioid receptor antagonist naloxone. Pellegrini et al. (2005) also reported that the probability of littermates to be assigned to either the fast- or the slow-recovery groups was greater than that expected by chance. In the absence of cross-fostering and genetic data it is not possible to determine whether these individual differences reflect genetic or epigenetic influences on the adjustment to incentive downshift.

Several studies have also shown strain differences in cSNC. For example, Roman high-avoidance rats, selected for good performance in a two-way active avoidance task and generally shown to be low in emotionality, recover faster from a 22%-to-4% sucrose downshift event than Roman low-avoidance rats, generally shown to be high in emotionality (Gómez, Escarabajal, de la Torre, Tobeña, Fernández-Teruel, & Torres, 2009). Interestingly, both Roman strains exhibit similar performance on Trial 11. A similar pattern was reported for Syracuse high and low avoidance strains (Flaherty & Rowan, 1989). In both Roman and Syracuse lines, strain differences were not observed in anticipatory negative contrast (Flaherty & Rowan, 1989; Gómez et al., 2009), a situation similar to cSNC, but known to involve little or no emotional reactivity (see Flaherty, 1996). However, selection for emotional reactivity does not always yields predictable results. For example, the Maudsley reactive strain, selected for high defecation in an open field (arguably an index of emotional reactivity; Broadhurst, 1975), exhibited a reduced cSNC effect relative to the Maudsley nonreactive strain, selected for low defecation rate, the opposite of what would be expected (Rowan & Flaherty, 1991). There is also little evidence that well-established rat strains (Wistars, Sprague-Dawley, and Long-Evans) differ in terms of the cSNC effect, although they may differ in terms of their recovery (Flaherty, Troncoso, & Deschu, 1979).

The most direct evidence that genetic and/or epigenetic factors affect the cSNC effect comes from a study in which selective breeding was implemented on the basis of the ratio of lick frequency on Trial 11 relative to Trial 10 (Flaherty, Krauss, Rowan, & Grigson, 1994). Five males and five females from the lower and higher ends of the ratio distribution were selected and tested over 7 generations. This study had two limitations: (1) unshifted controls were not included in the initial six selected generations, $S_{1\text{-}6}$, thus no data on

the evolution of behavioral performance are available, and (2) a random breeding control line was not included, thus preventing conclusions as to the specificity of the change relative to the selective criterion. These limitations notwithstanding, when animals from S_7 were tested, whereas high and low ratio rats did not differ in preshift performance (32% vs. 4% sucrose), high-ratio rats exhibited less suppression on Trial 11 and faster recovery from cSNC on Trial 12 than low-ratio rats. As in previous experiments with selected strains, testing in anticipatory negative contrast yielded no evidence of strain differences.

A preliminary selective breeding approach was used in the present study in order to evaluate the plausibility of a future selective breeding study with more extensive samples. There is reason to anticipate that selective breeding for recovery from cSNC should shape the process in less than 6 generations, as many selective breeding studies report differences between the selected lines during this timeframe (e.g., Dichter, Brunelli, & Hofer, 1996; Flaherty et al., 1994; Freudenberg, Dieckmann, Winter, Koch, & Schwabe, 2007; Scott, Cierpial, Kilts, & Weiss, 1996) Three lines of breeding where developed depending on the performance on three key downshift trials: Trials 11, 12, and 15 (see below). One line was selected for high recovery rate, another for low recovery rates, and a third one in which animals were bred independently of their recovery rate (a random control line). The present study has two main goals. The first goal was to assess some variables that could correlate with the effects of selective breeding on recovery from cSNC without reference to changes in emotional reactivity, including water intake, sucrose sensitivity, and locomotor activity. To assure rapid selection effects, a relatively small parental population was used: three pairs per line were mated on each generation. Thus, pairing with close conspecifics was widespread during the experiment. This protocol has the potential problem of random genetic drift effects that may overcome the selection effects or deleterious inbreeding effects (Garland, 2003). However, these potential problems were monitored with the use of a line from the same original population that underwent random selection mating. Both inter- and intra-strain comparisons were used.

The second goal was to evaluate if the preliminary selective breeding procedure for recovery from cSNC correlates with changes in other tasks involving incentive downshifts as predicted by frustration theory (Amsel, 1992; Wood et al., 2005). The tasks selected were partial reinforcement training (Experiment 1), mother-infant separation (Experiment 2), and the effects of naloxone on consummatory behavior during incentive downshifts (Experiment 3). The rationales are presented in the introduction to each experiment.

Experiment 1

Starting with a parental population, S_0, animals were selected for six generations, $S_{1\text{-}6}$, according to the proportion of total recovery (defined as the difference between Trial 15 and Trial 11) from incentive downshift observed between the first two downshift trials (defined as the difference between Trials 11 and 12). Based on this recovery ratio (RR), three lines were selected: low (L), random (R), and high (H). Several variables were measured in each generation, including weight, water intake, sucrose sensitivity, and locomotor activity. In addition, animals from each generation were randomly assigned to a 32%-to-4% sucrose downshift group or to a 4%-to-4% sucrose unshifted control to assess the cSNC effect. Only rats randomly assigned to the downshifted condition contributed to the next generation as a function of their RRs. Independently of the number of animals produced in each generation, 3 male-female pairs were selected for breeding to produce the following generation. For L and H lines, selected pairs were those showing the lowest and highest RRs in each generation. For the R line, three pairs were selected randomly, that is, irrespective of their RRs.

To implement the second goal, a partial vs. continuous reinforcement task, in an autoshaping situation, was applied to all the animals of S_5. Autoshaping involves signaling the occurrence of the unconditioned stimulus (US; food pellets) by the presentation of a retractable lever. Despite some individual variability (e.g., Flagel, Watson, Robinson, & Akil, 2007), most rats develop lever pressing, even though there are no response requirements (i.e., a Pavlovian procedure). Autoshaping in rats was selected because it is a preparation that

seems to be especially sensitive to the consequences of unexpected incentive downshifts, including response invigoration after surprising nonreward, successive contrast effects, and extinction effects (Boughner & Papini 2006, 2008; Dudley & Papini 1995, 1997; Papini, Ludvigson, Huneycutt, & Boughner, 2001; Pellegrini, Lopez-Seal, & Papini, 2008; Thomas & Papini, 2001). In addition, autoshaping differs substantially from the consummatory preparation used to test for cSNC. For example, the response is anticipatory rather than consummatory, there is a clear signal, it involves multiple trials per session rather than just one, and the reinforcer is solid rather than liquid. Similar learning outcomes in these two situations would suggest shared mechanisms, rather than shared performance effects.

According to Amsel's (1992) theory, unexpected incentive downshifts have at least two behavioral consequences of interest for the present experiment. First, downshifts induce an internal emotional state of frustration (i.e., primary frustration) with immediate invigorating behavioral consequences (see Stout, Boughner, & Papini, 2003). In the cSNC situation, primary frustration is assumed to invigorate a switch from consummatory to searching (Wood et al., 2005). In the partial reinforcement situation, primary frustration is assumed to invigorate the instrumental response leading to a phenomenon known as the partial reinforcement acquisition effect (PRAE). The PRAE is defined as higher response output in a partially reinforced group than in a continuously reinforced group (Goodrich, 1959). For the present situation, it was predicted that the PRAE would be greater in L than R rats, and smaller in H than R rats. This prediction stems from the expectation that primary frustration would be strongest in L rats and weakest in H rats, provided, of course, that these lines respond to the selective breeding protocol.

Second, primary frustration is associated to prevailing stimuli to generate an anticipatory response termed secondary frustration, with response suppressive effects. The occasional pairings between secondary frustration and food leads to the counterconditioning of secondary frustration, which is assumed to underlie the increased persistence in extinction typically observed after partial, rather than continuous, reinforcement. The strength of counterconditioning in turn depends on the strength of secondary frustration. Counterconditioning accounts for the partial reinforcement extinction effect (PREE), or greater persistence in extinction after partial reinforcement training, a phenomenon observed in autoshaping with rats (Boughner & Papini, 2006, 2008). As a result of this hypothesized common mechanism, it was predicted that L rats would exhibit a stronger PREE than R rats, whereas H rats would exhibit a weaker PREE than R rats. This prediction derives from the presumption that L rats would develop the strongest level of counterconditioning and H rats the weakest; of course, this will be the case if these lines respond to the selective breeding protocol as expected.

These predictions are based on the assumption that selective breeding would affect behavior in the cSNC situation in the expected direction. Explicitly, that L rats would exhibit retardation of recovery from cSNC, whereas H rats would exhibit acceleration of recovery from cSNC, in both cases relative to R rats. If either strain failed to respond to selective breeding (i.e., behaving similarly to R rats in the cSNC situation), then it is predicted that the performance of such rats in the partial reinforcement situation would be similar to that of R rats. Additional dependent measures were assessed to determine the extent of the effects of the artificial selection protocol (e.g., body weight, water consumption, sensitivity to sucrose solutions, and activity).

Method

Subjects. Thirty-three Long-Evans rats, 16 males and 17 females, served as the parental population (S_0). These animals were purchased from Harlan Laboratories (Indianapolis, IN). A rearing and testing protocol was applied to all the generations bred for the present study (S_0-S_5), as described in Table 1. Dams and their pups were maintained in polycarbonate tubs. Pups were weaned around postnatal day (PND) 21, and placed in same-sex groups of 2-4 individuals in polycarbonate tubs for about 10 additional days. Between PNDs 30-40, juveniles were transferred to a new room and individually housed in wire-bottom cages. Each cage contained a rodent retreat as an enrichment device, 15 × 9 × 9 cm (L × H × W), made of dark red Plexiglas. Food and water were available ad libitum in the cage. cSNC and subsequent testing started once the youngest litter of the generation reached PND 90 and animals reached the target deprivation weight (81-84% of the ad libitum weight). Food deprivation was never started before PND 90. Animals were given

a smaller amount of food each day and weighed daily until their body weight reached the target percentage; food deprivation took approximately 5-7 days. Throughout the experiment, animals were under a 12:12 h light:dark cycle (lights on at 07:00 h), under constant room temperature (22-24 °C) and humidity (50-60%).

Selective breeding criterion. In a typical cSNC experiment, rats receive 10 preshift trials followed by 5 postshift trials; trials are administered at a rate of 1 trial/day. Pellegrini et al. (2005) reported that the majority of the recovery from cSNC occurred between the first and second postshift trials (here Trials 11 and 12). Taking this information into account and controlling for the total amount of recovery as indexed by consummatory performance on the last postshift trial (Trial 15), a recovery rate (RR) index was calculated individually according to the following formula:

$$RR = \frac{\text{Score on Trial 12 - Score on Trial 11}}{|\text{Score on Trial 15 - Score on Trial 11}|} \quad \text{Eq. 1}$$

Each term in this formula refers to the cumulative contact time (measured in 0.05-s units) for the appropriate trial. This formula assesses the proportion of recovery on Trials 12 - 11, relative to the absolute (i.e., positive) number for the total amount of recovery (module of Trials 15 - 11). An RR> 1 indicates that all the recovery occurred between Trials 11 and 12; an RR< 1 indicates that the animal shows partial recovery from the downshift during the initial two trials; and an RR< 0 indicates behavioral deterioration after the downshift (RR is negative when the numerator is negative; the denominator is always positive).

Table 1
Rearing and behavioral testing schedule

Test/Activity	PND (Approx.)	Duration/Frequency	Generation
Weaning, group housing	21		$S_0 - S_5$
Individually housing	30-40		$S_0 - S_5$
Body weight	40	Every 3 days	S_5
Daily water intake	60	3 days	S_5
Food deprivation	90	7 days	$S_0 - S_5$
cSNC	98	15 days	$S_0 - S_5$
Sucrose sensitivity	125	3 days	S_5
Open-field activity	135	1 day	S_5
Autoshaping	140	20 days	S_5

Twelve rats from the initial population, six males and six females, were selected as the starting population (S_0). Two pairs of rats were chosen randomly to develop the random recovery (R) line. After those rats were selected, two pairs of the rats with the highest RR scores were assigned to the high recovery (H) line, whereas the two pairs of rats with the lowest RR scores were assigned to the low recovery (L) line. Male-female selected pairs were formed randomly. The selection of breeders for each subsequent generation followed the same procedure, except that three pairs per line were selected in each generation.

Body weight (S_0-S_5). As part of the process of food deprivation, ad libitum weights were obtained for each animal, in each generation. This measure assessed potential line differences in body size. In addition, all animals in S_1-S_5 were weighed every third day starting in PND 40-46 to assess the potential for developmental effects of the selective breeding protocol. Only the developmental profile in body weight for S_5 will be presented here. All weights were taken in the housing room in an automated scale (Vicon 4100G x 1G, Daigger, Vernon Hills, IL) that saved the weights to an adjacent computer.

Water consumption (S_1-S_5). This test assessed possible changes in water consumption resulting from selective breeding in the cSNC situation, which is based on consummatory behavior. The water consumption test was performed on generations S_1-S_5. During 3 days, between PNDs 57-73, the total amount of water consumed by each rat during the entire day was measured using graduated bottles (0.1-ml units). The automatic delivery system was occluded for the duration of this test and a single bottle containing a known amount of tap water was inserted in the home cage. Bottles were placed each day at 9:00 h and their content was recorded the following day at the same time. Bottles were refilled and inserted again in the cage until data from 3 consecutive days were obtained.

cSNC (S_0-S_5). Four conditioning boxes (Coulbourn Instruments, Whitehall, PA) were used for cSNC training. Each conditioning box was made of aluminum and Plexiglas (29.3 × 21.3 × 26.8 cm, L × H × W). The floor consisted of steel rods running parallel to the feeder wall. A tray with corncob bedding was placed below the floor to collect feces and urine. The sipper tube (1 cm in diameter) was inserted through a hole (1-cm wide, 2-cm high, and 4 cm from the floor) in the feeder wall. When fully inserted, the sipper tube was flush against the feeder wall. Illumination was provided by a house light located in the center of the ceiling. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube. When a rat made contact with the sipper tube, a circuit involving the steel rods in the floor was closed, thus generating a signal that was recorded by the computer. This

provided a measure of cumulative contact with the sipper tube, called goal-tracking time (0.05-s units). Each conditioning box was placed inside a sound-attenuating chamber containing a speaker that delivered white noise and a fan that provided ventilation. Together, the speaker and fan produced noise with an intensity of 80.1 dB (SPL, scale C).

For all generations and lines, rats were randomly assigned to two groups, matched as far as possible by sex, weight, and water consumption. One group in each strain received 10 trials of access to 32% sucrose (w/w, prepared by mixing 32 g of commercial sugar for every 68 g of distilled water), followed by 5 trials of access to 4% sucrose (w/w, prepared by mixing 4 g of commercial sugar for every 96 g of distilled water). The other group in each strain received 15 trials of access to 4% sucrose. One trial per day was administered. Each trial lasted 5 min starting with the first recorded contact with the sipper tube. Rats were trained in squads of four; the squads remained constant across the experiments, but the order of squads varied across days.

Sensitivity for sucrose solutions (S_5). This test assessed possible changes in sucrose sensitivity resulting from the selective breeding protocol applied to these lines. Sucrose sensitivity was assessed on generation S_5. After cSNC testing and during 3 full days, two graduated bottles (0.1 ml units) were administered in the home cage following a procedure described by Dess (2000). One bottle contained a sucrose solution whereas the other bottle contained distilled water. Three sucrose concentrations were administered across three days in counterbalanced order across subjects: 0.125, 0.5, or 1.0 g/ml. Every morning at 09:00 h, an experimenter recorded the amount consumed and refilled the bottles with the appropriate solution. These tests were scheduled after cSNC training to avoid potential interactions between the concentrations of sucrose used here with those used in the main consummatory testing.

Activity (S_5). Selective breeding can lead to correlated changes in activity (Stohr, Wermeling, Weiner, & Feldon, 1998). To test for this possibility, activity in the open field test was assessed on generation S_5. Four open field chambers were used (MED Associates, St. Albans, VT). Testing took place between 9:00 and 15:00 h. The dimensions of each chamber were 43 × 30 × 43 cm (L × H × W). Rats were tested in squads of four. At the start of the trial, each rat was placed in the center of the open field. General locomotor activity was automatically recorded in 5-min bins during a single 20-min trial. The open field was cleaned immediately after each trial. The dependent measure was the distance traveled, measured in cm.

Autoshaping (S_5). Autoshaping training was used to test whether selective breeding for recovery from cSNC would also affect behavior acquired under partial reinforcement training. Autoshaping training under partial and continuous reinforcement (PR, CR) was carried out on generation S_5. Four standard conditioning boxes were used (MED Associates, St. Albans, VT). The dimensions of each chamber were 28 × 20.5 × 20.1 cm (L × H × W). The floor was made of steel rods running parallel to the feeder wall. A tray with corncob bedding was placed below the floor to collect feces and urine. A recessed magazine, 2 cm from the floor, was located in the center of the front wall, into which pellets (45-mg Noyes pellets, rat formula A/I) were delivered automatically. A retractable lever made of aluminum (4.8-cm wide, 1.9-cm deep, and 7 cm above the floor) was located 2 cm to the left of the magazine. Lever insertion or retraction took 0.2 s. A light bulb (GE 1820) attached to the ceiling of the chamber was positioned opposite to the magazine, and provided diffuse illumination. Each conditioning box was placed in a sound-attenuating chamber that contained a speaker to deliver white noise and a fan for ventilation (SPL 80.1 dB, scale C).

Training consisted of 20 daily sessions, ran between 09:00 and 17:00 h, 7 days per week. Rats in each strain were randomly assigned to either Group PR or CR. Each session started when the house light was turned on and ended when the house light was turned off. There were 10 trials per session separated by a variable intertrial interval averaging 90 s (range: 60-120 s). Regular intertrial intervals were selected before the first and after the last trial of each session. Each trial began with the insertion of a retractable lever. A computer recorded lever-pressing responses during the 10 s of lever presentation and then the lever was retracted. There were 10 acquisition sessions. For Group CR, each lever presentation ended with the response-independent delivery of 5 pellets (45-mg, rat formula; Bio-Serv, Frenchtown, NJ) on the magazine cup. For Group PR, a random 5 trials were selected by the computer program to end with the delivery of 5 pellets, whereas the other 5 trials ended without food delivery. Individual pellets were delivered at a rate of one per 0.2 s. Then, all animals received 10 extinction sessions, each under the same conditions as during acquisition, except that no pellets were delivered. The number of responses recorded per trial was transformed to responses per minute for statistical analysis.

Results

The results are presented in the same ontogenetic order in which they were recorded.

Body weight (S_0-S_5). Rats are sexually dimorphic and thus differences in body weight were expected. Figure 1 shows the ad libitum body weights of rats within each line and across all generations, as measured between PND 90-105. In all the figures similar to this one (i.e., expressing data across generations), a regression line was fit and the slope parameter and coefficient of determination were added in the figure as descriptors. Visual inspection of this figure shows a reduction in body weights across generations for both

selected lines, but especially marked for the H line, whereas R rats showed less change. These data were analyzed in a Generation × Line × Sex analysis of variance for independent samples. This analysis provided significant effects for the generation by sex, $F(5, 429) = 42.29$, $p < 0.001$, and generation by line interactions, $F(10, 429) = 3.24$, $p < 0.001$. All three main effects for generation, sex, and line were also significant, Fsn > 5.82, ps < 0.001. However, the sex by line and the triple interactions were not significant, Fs < 1.56, ps > 0.21. Post hoc LSD pairwise tests indicated that all lines differed from each other, ps < 0.01. The average weights (±SEM) including all generations, for males and females, were the following: L rats, 354.38 (±5.0) and 244.70 (±4.1) g; R rats, 370.13 (±6.3) and 256.06 (±4.1) g; and H rats: 322.23 (±7.3) and 228.24 (±6.6) g. Thus, the trend for ad libitum weights was R > L > H.

To determine whether these line differences were present from the beginning of the study, independent Line × Sex analyses were computed on the adult weights of the first selected generation, S_1. There was a significant difference between males and females, $F(1, 38) = 258.83$, $p < 0.001$, but nonsignificant effects across lines and for the line by sex interaction, Fs < 2.38, ps > 0.10. Thus, line differences emerged as a result of the selective breeding protocol.

The differences in weight across lines observed in ad libitum weights recorded when animals were approximately 90-105 days old open the question of the developmental origin of such differences. To clarify this issue, ad libitum weights recorded in PND 40 were plotted in Figure 2. The figure shows data from S_1 to S_5 (no such data were available for the parental population, S_0). At that age, sexual dimorphism is apparent, but not yet fully developed. As shown in this figure, generational trends are not strong, as reflected by relatively lower coefficients of determination. However, the slope for H females is the only one that was negative and the largest change was observed in S_2; thereafter weights showed no clear trend. A Generation × Line × Sex analysis provided the following results. Whereas the triple interaction effect was not significant, $F < 1$, all double interaction effects and main effects were significant. Thus, there were significant interactions between generation and line, $F(8, 338) = 2.39$, $p < 0.02$; generation and sex, $F(4, 338) = 2.79$, $p < 0.03$; and line and sex, $F(2, 338) = 3.10$, $p < 0.05$. There were also significant differences across generations, $F(4, 338) = 19.41$, $p < 0.001$; between lines, $F(2, 338) = 92.95$, $p< 0.001$; and between males and females, $F(1, 338) = 200.43$, $p < 0.001$. Mean weights (±SEM) including all generations, for males and females, were the following: L rats, 145.54 (±1.8) and 123.76 (±1.2) g; R rats, 157.54 (±1.7) and 132.42 (±1.0) g; and H rats: 126.79 (±3.4) and 109.16 (±2.5) g. Thus, differences in body weight apparent from a juvenile stage of development followed the same order as in the adults: R > L > H.

To determine whether these juvenile line differences were already observed in the first selected generation, S_1, a Line × Sex analysis was calculated. There was a significant sex difference, $F(1, 38) = 8.48$, $p < 0.01$, but the differences across lines and the line by sex interaction were not significant, Fs < 1.94, ps > 0.15. Thus, line differences in body weight were the result of selective breeding beyond S_1 and were apparent from PND 40.

Water consumption (S_1-S_5). Water consumption was evaluated using the averages of drinking behavior for the three days of the test and for generations S_1 to S_5 (no water consumption data were recorded for the parental generation). Figure 3 shows a general trend to increase water consumption across generations, but without apparent differences across lines. A Generation × Line × Sex analysis confirmed these conclusions. The increase across generations was significant, $F(4, 337) = 44.06$, $p < 0.001$, and males drank more water than females, $F(1, 337) = 132.62$, $p < 0.001$. None of the interactions and the main effect of line were significant, Fs < 2.40, ps > 0.08.

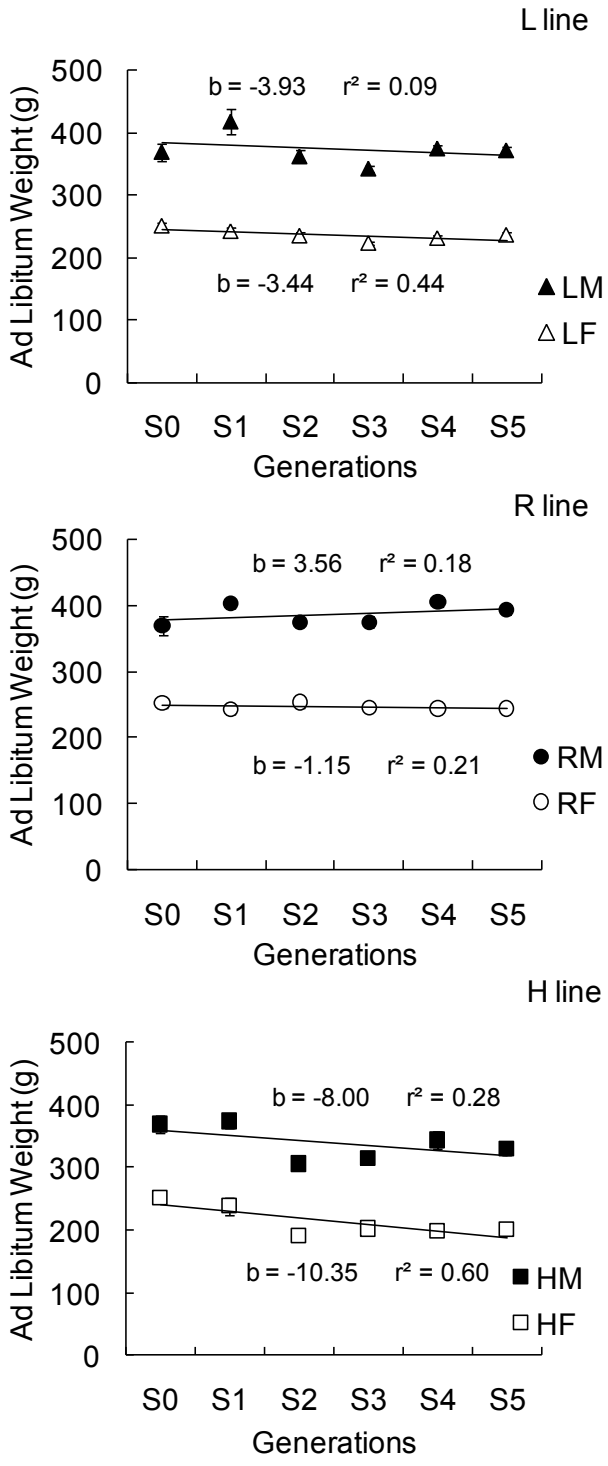

Figure 1. Ad libitum weights measured around PND 90, before food deprivation started, in rats from the low (L), random (R), and high (H) lines across generations. Results for males (M) and females (F) are presented separately for each line. The slope parameter (*b*) of the regression line and the coefficient of determination (r^2) are presented for descriptive purposes. Results from Experiment 1.

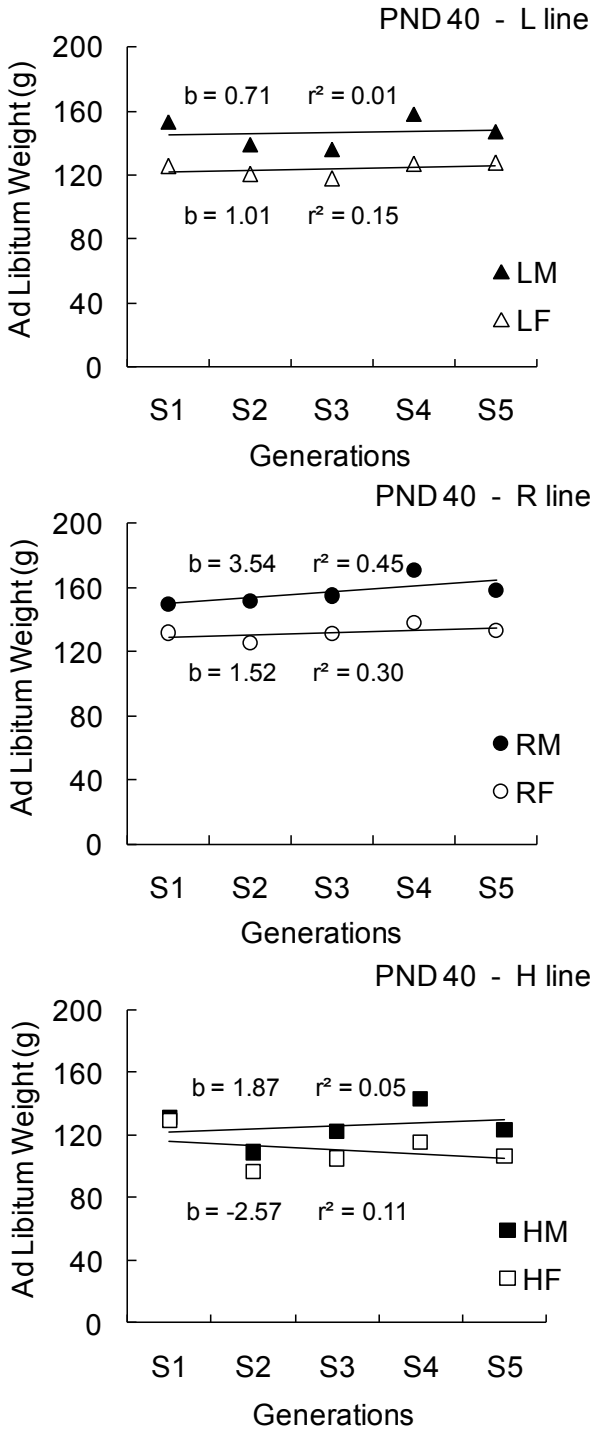

Figure 2. Ad libitum weights measured around PDN 40. See Figure 1 for further details.

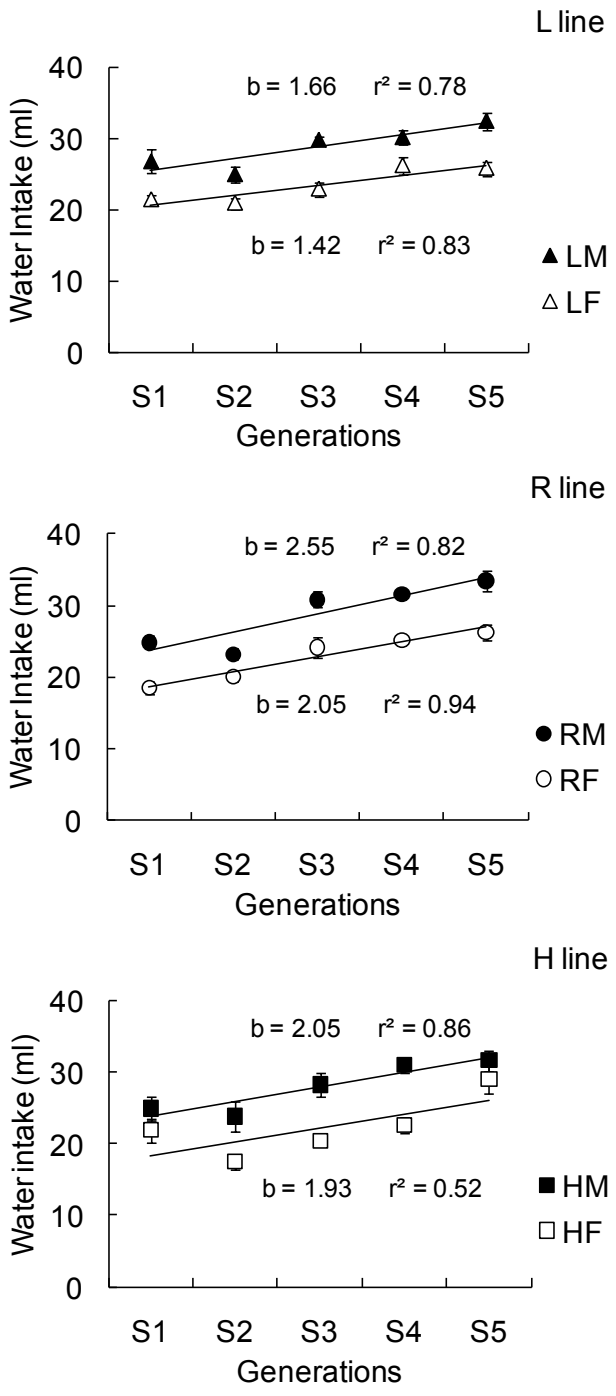

Figure 3. Mean water intake (ml) per day taken over three days for each line (L, R, and H), and separately for males (M) and females (F), across the five selected generations. See Figure 1 for further details.

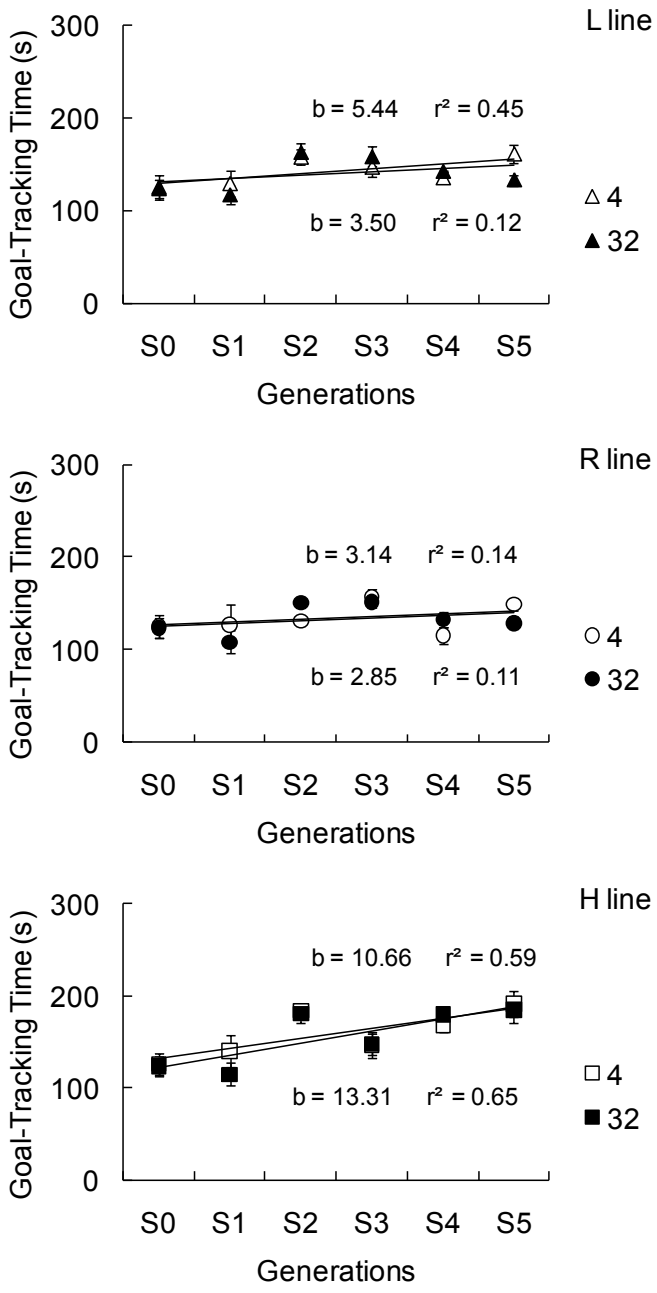

Figure 4. Goal-tracking times (s) for each line (L, R, and H) during preshift trials (Trials 1-10) of groups with access to either 32% or 4% sucrose solution in each selected generation. See Figure 1 for further details.

cSNC (S_0-S_5): Preshift performance (Trials 1-10). Figure 4 shows the mean goal-tracking times across Trials 1 to 10, for each generation and line, and for animals with access to 32% and 4% sucrose. These overall means are not different for the two sucrose concentrations, a fact that has been observed in previous studies (Flaherty, 1996). The slopes are all positive, but the *b* values for H rats are the highest. Coefficients of determination, r^2, are also highest for H rats. These results suggest two points: first, that goal-tracking times increased across generations independently of the line and behavioral treatment, and, second, that the effects of selective breeding on preshift performance were most noticeable for the H line.

These data were analyzed with a Generation × Line × Sex × Contrast analysis (Sex is not included in Figure 4, but it was included in the analysis). The 4-way interaction and the triple interactions were all nonsignificant, Fs< 1.88, ps> 0.15. Generation interacted significantly with line, $F(10, 381) = 2.74$, $p < 0.005$, with sex, $F(5, 381) = 3.39$, $p < 0.01$, and with contrast condition, $F(5, 381)= 2.96$, $p< 0.02$. All four main effects were also significant, Fs > 6.55, ps < 0.001. LSD pairwise comparisons for lines, including both contrast conditions, indicated that H rats were significantly different from R rats, $p < 0.005$; the other pairwise comparisons were not significant, ps > 0.09. Of these effects, it is the interaction between generation and line that captures the steeper change in goal-tracking times in H rats compared to L and R rats.

cSNC (S_0-S_5): Postshift performance (Trials 11-15). RRs were obtained only from animals randomly assigned to the downshift groups in each generation (i.e., 32-4 treatment). In those animals, no detectable changes were observed in the RRs across generations. A Generation × Line analysis yielded nonsignificant effects for all factors, Fs < 1.72, ps > 0.14. A closer inspection of the data suggests dissociation between RRs and consummatory behavior. This dissociation can be explained in terms of changes in the initial downshift trials (Trials 11 and 12) across generations. Weak or nonexistent response to reward downshift, like the scores shown before for animals in the H line, resulted in negative values for the RR numerator, which turned the RR into a negative number. Such negative values do not describe a slower rate of recovery, but a decrease in performance on Trial 12 relative to Trial 11. The specific RR formula was aimed at selecting for high vs. low recovery rates from cSNC, while simultaneously ensuring that there was a cSNC effect. However, the RR index was not a good descriptor of the observed changes in consummatory behavior in the present study, although it was a good index for the selection of differential rates of recovery from cSNC, as is shown below. Thus, Figure 5 shows goal-tracking times for both contrast groups (unshifted, 4% sucrose, and downshifted, 32%-to-4% sucrose) and for the three key trials included in the computation of RRs: Trials 11, 12, and 15.

Trial 11 is the first downshift trial and thus assesses possible changes in the initial detection of the incentive downshift event. Although animals were not specifically selected for their response on this trial (unlike in a previous study by Flaherty et al., 1994), there were line differences across generations in terms of their response during this trial (Figure 5, left panel). Slope and coefficients of determination provide a general picture of line differences on Trial 11. Thus, whereas goal-tracking times for R rats fluctuated across generations, downshifted (but not unshifted) L rats showed a tendency to *decrease* responding across generations, whereas downshifted (but not unshifted) H rats showed a tendency to *increase* responding across generations. Of the three downshifted groups, only H rats showed a positive slope and it was the steepest of all slopes for Trial 11. These trends were analyzed by a Generation × Line × Contrast × Sex analysis. The only two significant interactions were those between generation and line, $F(10, 381)= 2.04$, $p< 0.03$, and line and contrast, $F(3, 381) = 3.74$, $p < 0.03$ (for all other interactions: Fs < 2.03, ps > 0.07). All four main effects were also significant: Fs > 2.85, ps < 0.02. LSD pairwise comparisons combining both contrast conditions indicated that H rats were different from both L and R rats, ps < 0.02, which did not differ from each other, $p > 0.69$.

The behavior of rats from the three selected lines on Trial 12 was similar to what was observed on the previous trial, with the caveat that the divergent trend toward an increase cSNC effect across generations was more pronounced in R rats (Figure 5, middle panel). Of the three downshifted conditions, only H rats exhibited a positive slope and, as in the previous trial, the slope parameter b was the highest of all groups. These trends were analyzed using the same approach as above. In this case, the triple interaction between generation, line, and contrast achieved significance, $F(10, 381) = 1.87$, $p < 0.05$. In addition, the double interactions between generation and line, $F(10, 381) = 2.13$, $p < 0.03$, and generation and sex, $F(5, 381) = 2.86$, $p < 0.02$, were also significant. All other interactions failed to achieve significance, Fs < 2.04, ps > 0.07. As in the previous trial, on Trial 12 the four main effects were significant, Fs > 4.21, ps < 0.005. Pairwise LSD comparisons involving both downshifted and unshifted conditions indicated that H

rats were different from R rats, $p < 0.005$, but did not differ from L rats, $p < 0.07$; L and R rats did not differ either, $p > 0.30$.

By Trial 15, downshifted rats have typically recovered from the cSNC effect and exhibit goal-tracking times similar to those of unshifted controls, as seen in the parental groups, S_0, in Figure 5 (right panel). Across generations, there was a trend in L and R rats toward an increased cSNC effect on Trial 15, but this effect was much reduced in H rats. As in the other trials, among the three downshifted conditions, H rats were the only ones exhibiting a positive slope, albeit rather small in comparison with previous trials. A similar analysis to those previously calculated provided the following outcomes. There were significant interactions between generation and line, $F(10, 381) = 2.34$, $p < 0.02$, and generation and contrast, $F(5, 381) = 2.46$, $p < 0.04$. All other interactions were nonsignificant, Fs < 2.05, ps > 0.13, as was the main effect of sex, $F(1, 381) = 2.93$, $p > 0.08$. The main effects for generations, line, and contrast were all significant, Fs > 3.35, ps < 0.007.

From the results presented in Figure 5 and the corresponding statistical data, we arrive at two conclusions. First, L rats displayed a tendency toward an increased cSNC effect across generations, but so did R rats. Therefore, it is tentatively concluded that the L-line trend is a nonspecific effect of the selective breeding protocol implemented in this study, rather than an effect of that protocol on reduced recovery from cSNC. Second, H rats displayed a tendency toward a reduced cSNC effect across generations that ran opposite to that of both L and R rats. Therefore, this trend is attributed to selective breeding for high recovery rates. Interestingly, such selection led to a modified response not only in the ability of H rats to increase responding on Trial 12, but also on Trial 11. Unlike these generational changes, selective breeding for high recovery rates (i.e., RR) actually preserved across generations the level of recovery from cSNC achieved by the parental generation.

Sensitivity for sucrose solutions (S_5). Means of fluid intake for water and sucrose solutions were transformed to an index to assess sucrose sensitivity: sucrose / (sucrose + water). Figure 6 presents the results for each line and sucrose concentration in the last generation, S_5. The data were analyzed with a Line × Sex × Sucrose Concentration model. Despite a tendency for the H line to exhibit somewhat reduced sensitivity to sucrose, none of the main effects or interactions achieved significance, Fs < 2.51, ps > 0.08.

Activity (S_5). Activity assessed in terms of ambulatory distance traveled during the 20-min test, was evaluated for S_5 rats and is presented in Figure 7 for each line and separately for males and females. There was habituation of activity across 5-min blocks, but no clear evidence of differential performance as a function of line. These results were evaluated with a Line × Sex × 5-min Block analysis, with repeated measures for the last factor. There was a main effect of sex, $F(1, 80) = 21.63$, $p < 0.001$, and a significant decrease in activity across 5-min blocks, $F(3, 240) = 225.77$, $p< 0.001$, but all other effects were nonsignificant, Fs < 1.26, ps > 0.28.

Autoshaping (S_5). Autoshaping performance in terms of number of responses per minute for each line and each schedule of reinforcement is shown in Figure 8. Partially reinforced L and R rats produced consistently higher response rates than their continuously reinforced counterparts, an effect known as the PRAE (Goodrich, 1959). Interestingly, the PRAE was reduced in H rats. Moreover, the level of responding for both PR and CR groups in the H line was equivalent to the level of CR rats in the other lines (i.e., relatively lower). Thus, PR increased autoshaping responding in L and R rats, but not in H rats. These results were evaluated with a Line × Schedule × Sex × Session, with repeated measures for the last factor. There were significant effects for the schedule by session interaction, $F(9, 666) = 1.99$, $p < 0.04$, the schedule effect, $F(1, 74) = 8.85$, $p < 0.005$, and the session effect, $F(9, 666) = 65.67$, $p < 0.001$. All other effects were nonsignificant, Fs < 1.

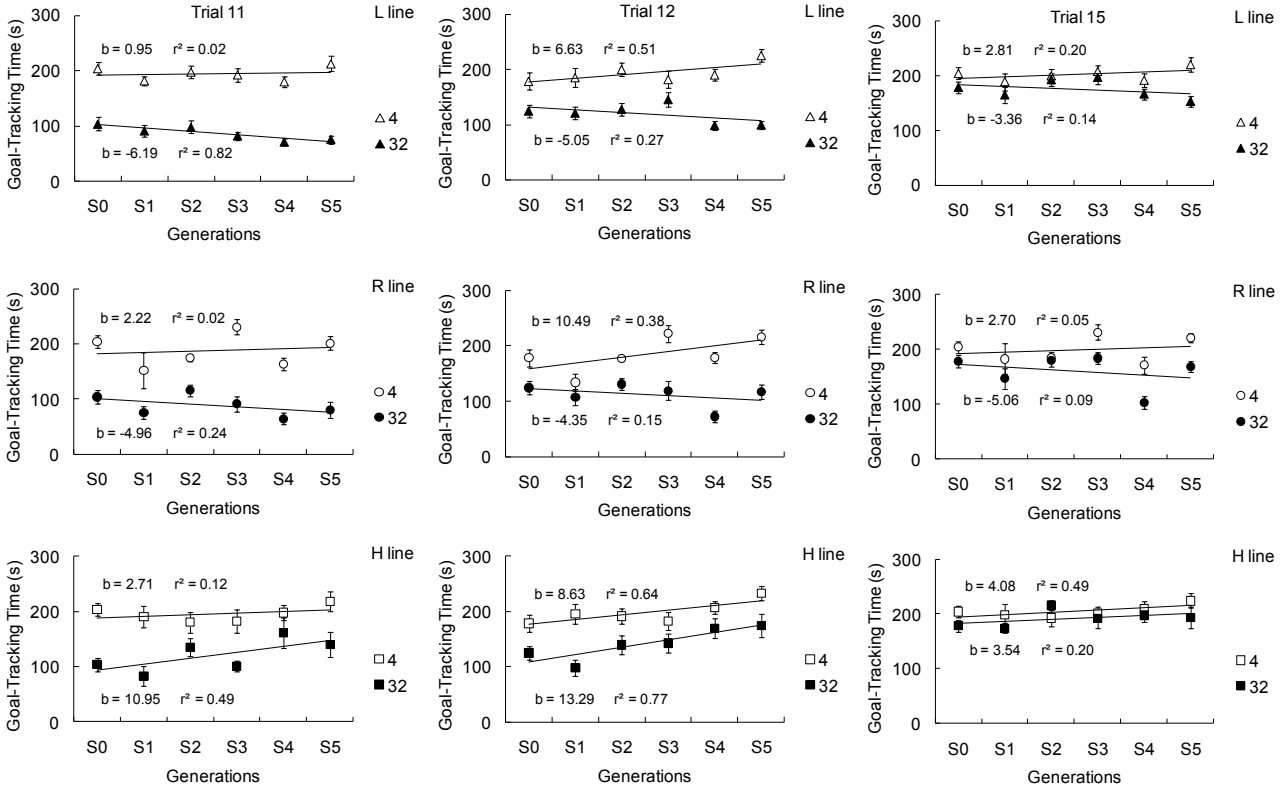

Figure 5. Goal-tracking times (s) for each line (L, R, and H) during the three key postshift trials (Trials 11, 12, and 15), of groups that had access to either 32% or 4% sucrose solution during preshift trials, in each selected generation. See Figure 1 for further details.

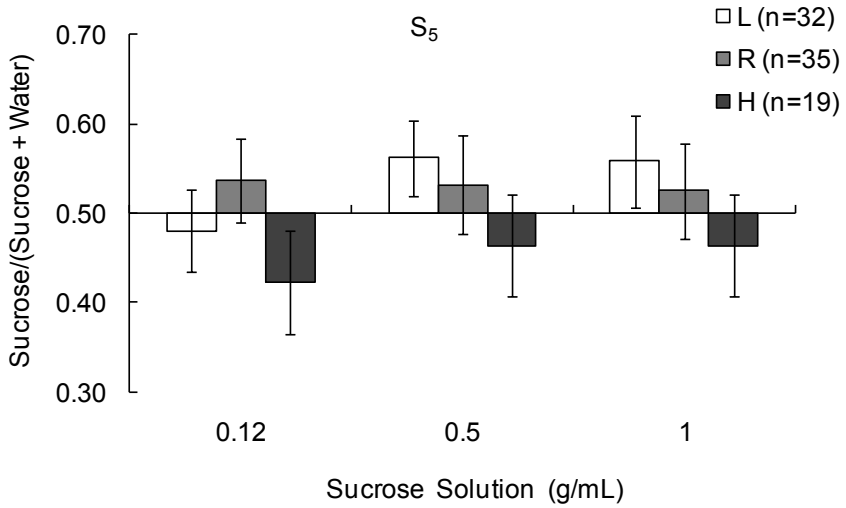

Figure 6. Sucrose preference over water in tests with three different sucrose concentrations. Results from the fifth selected generation in Experiment 1.

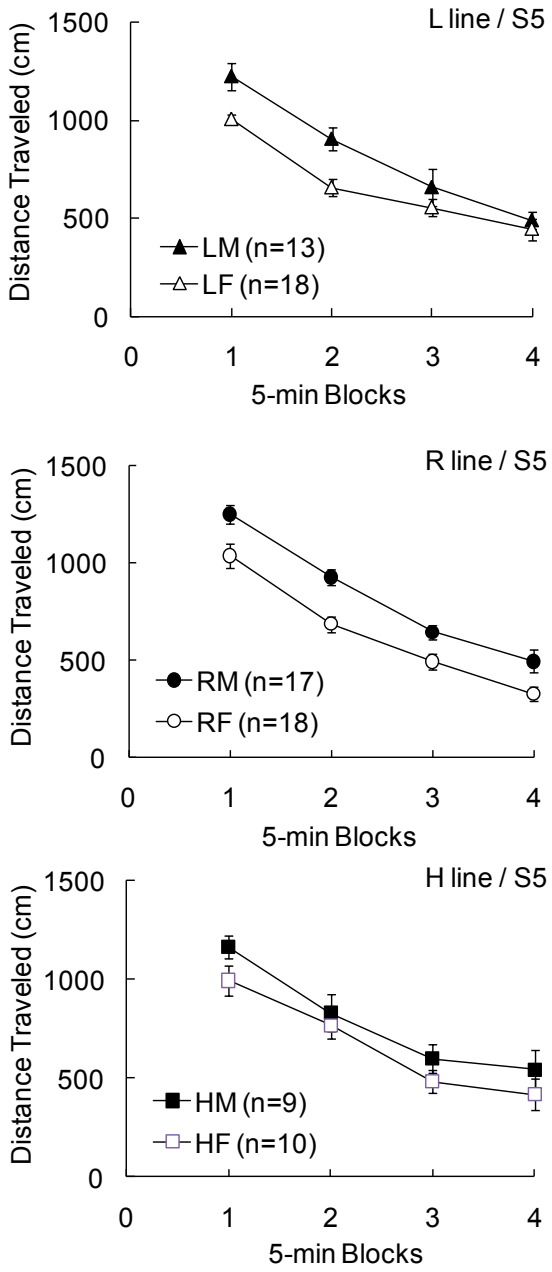

Figure 7. Mean open-field activity (cm) for each line (L, R, and H), and separately for males (M) and females (F). Results from the fifth selected generation in Experiment 1.

To clarify the schedule by session interaction, independent Schedule × Sex × Session analyses were calculated for each line. The results support the conclusions drawn from the figure. The schedule by session effect was significant for L rats, $F(9, 252) = 1.99$, $p < 0.05$, and R rats, $F(9, 279) = 1.97$, $p < 0.05$, but not for H rats, $F < 1$. The same pattern was observed for the main effect of schedule: L rats, $F(1, 28) = 5.36$, $p < 0.03$; R rats, $F(1, 31) = 7.71$, $p < 0.005$; and H rats, $F < 1$. The main effect of session was significant for all lines, Fs > 11.26, ps < 0.001. Other effects were nonsignificant, Fs < 1.04, ps > 0.30.

Because acquisition performance in L and H rats was affected by the schedule of reinforcement, the extinction response rates obtained for each animal, in each session, were divided by the mean response rate of that animal during the last 3 acquisition sessions (sessions 8-10). This transformation is typically used in

similar studies (e.g., Wagner, 1961). Furthermore, because extinction can only be evaluated in animals that exhibited a minimum amount of responding in acquisition, only animals that responded in every one of Sessions 6 to 10 were included in this analysis. Figure 9 shows the extinction ratios for each line and group, and provides the resulting sample size for each group. Ratios cross over for L and R rats, thus yielding a partial reinforcement extinction effect (PREE; Rashotte & Surridge, 1969). However, these functions overlap extensively for H rats. These results were evaluated with a Line × Schedule × Sex × Session, with repeated measures for the last factor. The analysis uncovered a significant schedule by session effect, $F(9, 558) = 2.31$, $p < 0.02$. There was also a significant extinction effect, $F(9, 558) = 57.87$, $p < 0.001$. All other effects failed to reach significance, Fs < 2.04, ps > 0.15.

Individual Schedule × Sex × Session analyses for each line provided the following results. For L rats, the schedule by session interaction was significant, $F(9, 216) = 2.10$, $p < 0.04$. For R rats, the schedule by session interaction fell short of significance, $F(9, 234) = 1.78$, $p < 0.08$, but the interaction between schedule, sex, and session was significant, $F(9, 234) = 2.66$, $p < 0.01$. In contrast, none of the interactions were significant for H rats, Fs < 1. The extinction effect was significant for all lines, Fs > 10.58, ps < 0.001.

Experiment 2

The theoretically interesting result from Experiment 1 was the general agreement between the behavioral response of H rats in the cSNC and partial reinforcement situations. By contrast, L rats, which did not respond to selective breeding in the cSNC situation, produced a profile in the partial reinforcement situation that was indistinguishable from that of R rats. Additional evidence was sought in a different testing situation. If these effects were the result of strain differences in emotional reactivity, then one should see similar strain differences in terms of other situations involving frustration. Infant-mother separation tests show a pattern of results reminiscent of those seen in food-reinforced situations involving incentive downshifts (Papini & Dudley, 1997). The mother of a newborn mammal provides milk, warmth, tactile comfort, and familiar olfactory cues, all potential sources of appetitive reinforcement. Therefore, separating the infant from the mother can be seen as analogous to devaluing or omitting a previously available food reinforcer, as done in incentive contrast and partial reinforcement situations.

Infant rats produce ultrasonic vocalizations (USVs) in response to a variety of situations, including separation from their mother and isolation in an unfamiliar environment (e.g., Hofer & Shair, 1978; Oswalt & Meier, 1975). Infant rats also produce USVs in response to positive social interactions, including play and tickling (Burgdorf, Panksepp, Brudzynski, Kroes, & Moskal, 2005). Separation-induced USVs tend to have a peak frequency around 40 kHz, whereas play-induced USVs tend to have a peak frequency around 50 kHz. The goal of this experiment was to test whether the properties of these two general classes of USVs (40- vs. 50-kHz calls) were different in infants of the three strains involved in the Experiment 1. Infants from S_6 were used as subjects. Given the results of Experiment 1, it was predicted that the USVs of L and R rats would be more similar to each other than the USVs produced by H rats.

Method

Subjects. Infants from S_6 were tested at PND 11. There were 33 pups/3 litters for the L line, 20 pups/3 litters for the R line, and 23 pups/3 litters for the H line.

Apparatus and testing procedure. Ultrasonic vocalizations (USVs) were induced by isolating the pups from the dam (Wohr & Schwarting, 2008). Pups were isolated from the mother and home cage during 10 min, using a testing cage (28 cm long, 17 cm high, and 12 cm wide) made of Plexiglas, under room temperature (22-24 °C). Testing was performed between 08:00 and 17:00 h. The home cage with the dam and litter were transported from the vivarium to a waiting room. Then, pups were individually and gently removed from the home cage in a random fashion and placed in the testing cage. Prior to each test, the testing box was cleaned using water. USVs were recorded using an UltraSoundGate Condenser Microphone (CM 16; Avisoft Bioacoustics, Berlin, Germany), which

records frequencies from 10–150 kHz with a flat frequency response between 15 and 200 kHz. The microphone was 14 cm above the testing cage. The acoustic data were digitized using an Avisoft UltraSoundGate 116 USB Audio device (Avisoft Bioacoustics, Berlin, Germany). Ultrasonic vocalizations were displayed in real time and recorded continuously during the 10-min testing session (Avisoft Bioacoustics, Berlin, Germany; 250 kHz sample rate, 16 bit). Pups from the same litter were tested successively, but litters of the same line were never tested successively. In addition, litters within a line were tested at different times during the day. After testing, pups were placed back in the home cage with dam and litter-mates. Once all pups in a litter were tested, the home cage was transported back to the vivarium.

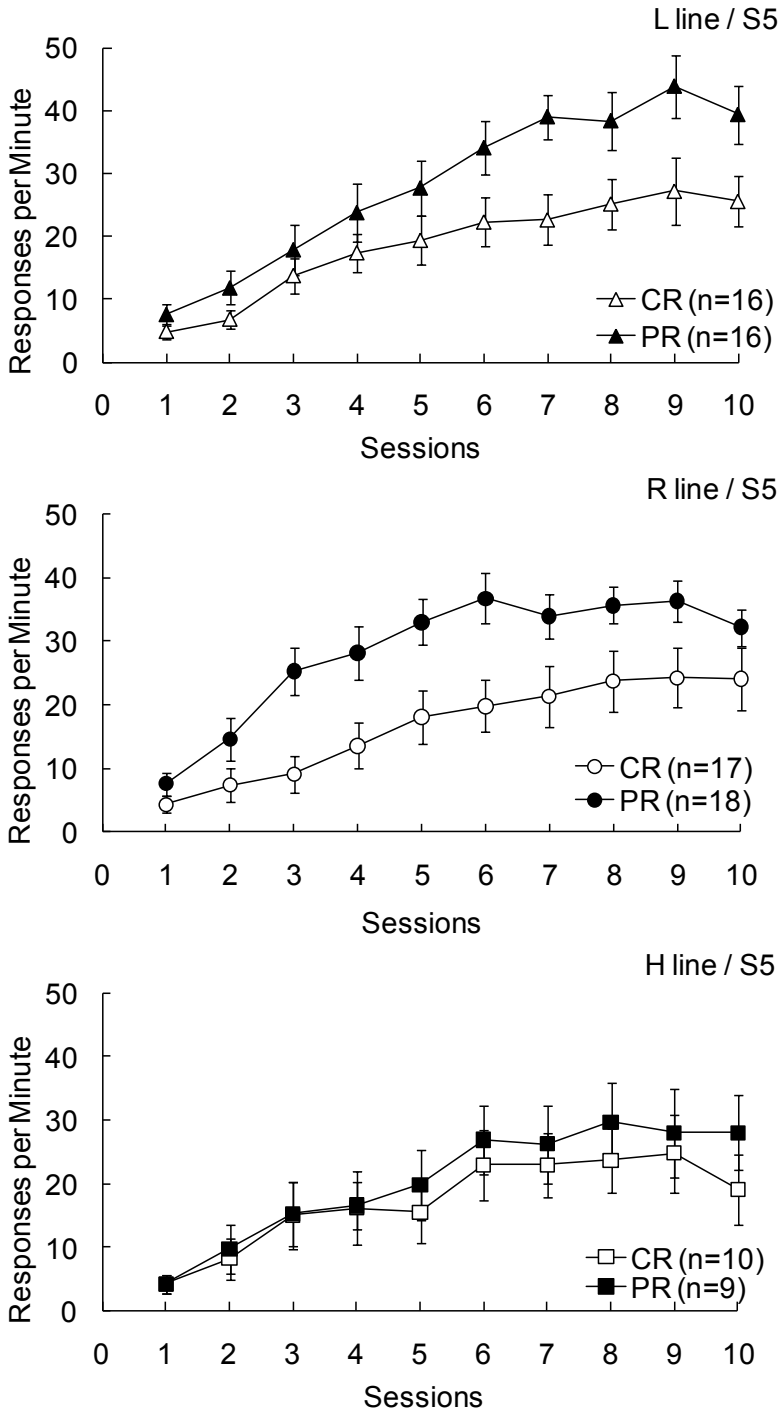

Figure 8. Lever presses per minute in the autoshaping procedure for each line (L, R, and H) and for groups exposed to 50% partial reinforcement (PR) or continuous reinforcement (CR). Results from Experiment 1.

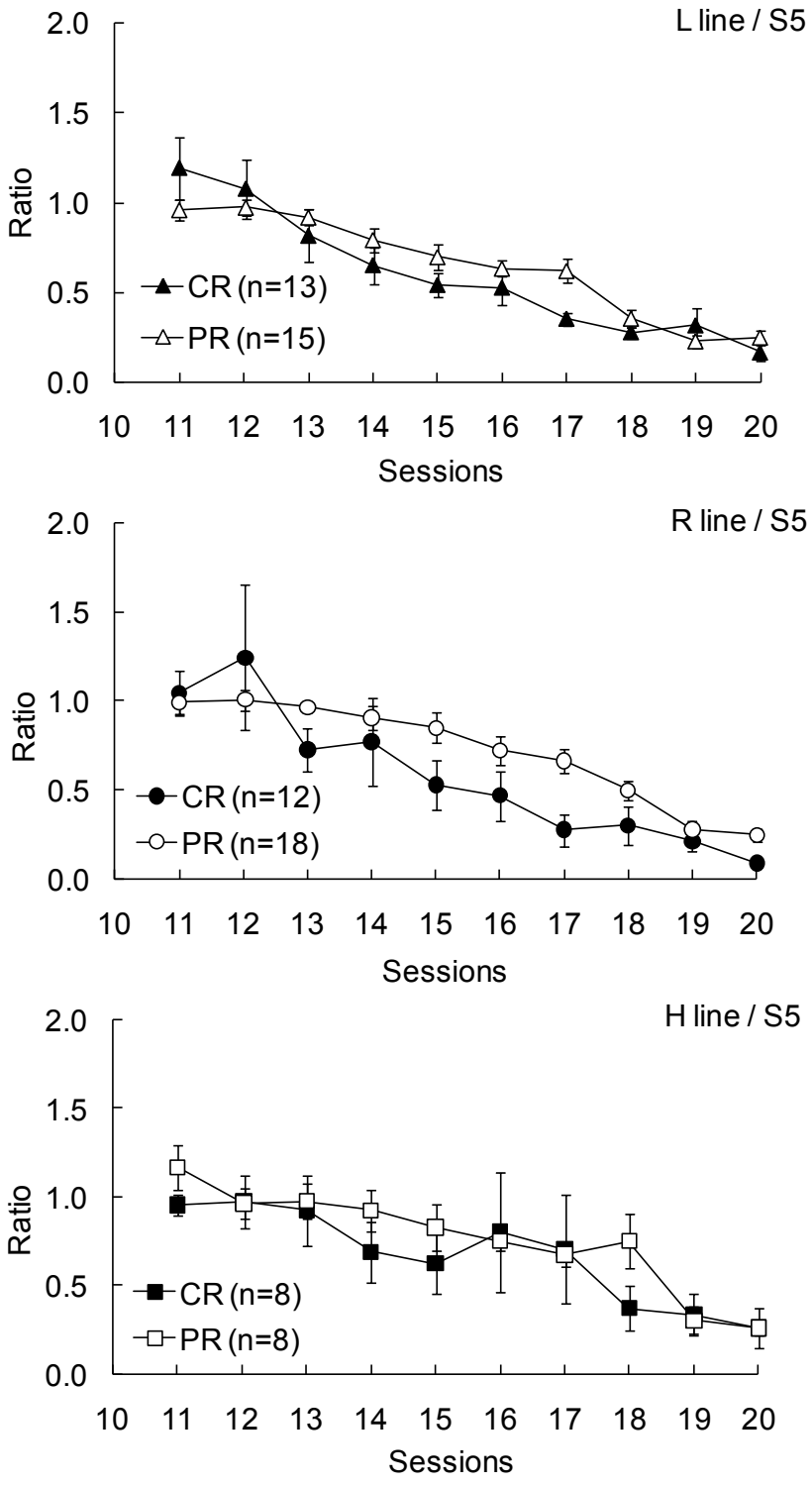

Figure 9. Response rate in each extinction session relative to rates during late acquisition for each line (L, R, and H) in groups previously exposed to 50% partial reinforcement (PR) or continuous reinforcement (CR). Results from Experiment 1.

Data analysis. Spectral and temporal analyses of 40 and 50 kHz calls were performed using SASLab Pro (version 4.38; Avisoft Bioacoustics). Then, spectrograms were calculated with a frequency resolution of 488 Hz and a temporal resolution of 0.512 ms, using a Fourier transformation (1024 FFT-length, 100% frame, Hamming window and 75% time window overlap). The detection of calls was performed adjusting an amplitude threshold for each individual spectrogram with a hold-time of 10 ms. Prior to analysis, acoustic data were filtered (highpass, 10 kHz cut-off) and cage noises were manually removed from the signal. Accuracy of the call detection was verified by an experienced user (agreement rate between data analysts for number of calls: 99.7%). The following parameters were calculated automatically by SASLab for each call: (1) the number of calls; (2) the average duration of calls (seconds); (3) the total amount of time calling during the test (seconds); (4) the frequency with the highest energy in the call, or peak frequency (kHz); and (5) the difference between the highest and the lowest peak frequency within each call, or bandwidth (kHz). Calls in the range of 30-45 kHz were assigned to the 40 kHz category, whereas calls in the range of 50-90 kHz were assigned to the 50 kHz category.

Results

The main results of this experiment are presented in Table 2 in terms of the mean (±SEM) for each variable recorded during the separation test and for 40- and 50-kHz calls separately. One-way analyses were computed for each variable with strain as a factor; when significant results were obtained, LSD pair wise tests were used to determine the source of the effect. As shown in Table 2, only two variables were significant for 40-kHz calls: number of calls, $F(2, 73) = 5.04$, $p < 0.01$, and bandwidth, $F(2, 72) = 4.73$, $p < 0.02$. In terms of the number of calls, L rats vocalized significantly less than R rats, $p < 0.003$; other comparisons were not significant, ps > 0.10. In terms of bandwidth, H rats exhibited a significantly broader range of frequencies than R, $p < 0.009$, and L rats, $p < 0.02$.

Two variables also differentiated strains in terms of 50-kHz calls. Again, strains differed in terms of the number of calls, $F(2, 73) = 6.21$, $p< 0.005$, with L rats vocalizing significantly less than R, $p < 0.006$, or H rats, $p < 0.005$. R and H rats did not differ from each other, $p > 0.99$. Strains also differed in terms of the total call time, $F(2, 73) = 4.50$, $p < 0.02$. L rats differed from H rats, $p < 0.005$, but not from R rats, $p > 0.17$. R and H rats also failed to differ, $p > 0.17$.

Table 2
Mean (±SEM) USV call data recorded during the mother-infant separation test

Variables	40-kHz Calls			
	Low	**Random**	**High**	**p<**
*Number of calls	283.9 (±42.4)	627.1 (±104.4)	454.6 (±97.1)	0.01
Duration (s)	0.089 (±0.009)	0.061(±0.006)	0.089 (±0.011)	0.08
Total call time (s)	35.3 (±7.8)	49.1 (±10.9)	59.4 (±14.1)	0.26
Peak frequency (kHz)	37.5 (±0.4)	36.6 (±0.4)	37.1 (±0.6)	0.42
*Bandwidth (kHz)	31.7 (±3.8)	29.0 (±4.1)	50.3 (±7.1)	0.02
Variables	**50-kHz Calls**			
	Low	**Random**	**High**	**p<**
*Number of calls	57.1 (±10.2)	162.1 (±39.3)	162.1 (±31.9)	0.005
Duration (s)	0.02 (±0.01)	0.01 (±0.00)	0.02 (±0.00)	0.56
*Total call time (s)	0.72 (±0.11)	2.00 (±0.57)	3.38 (±1.13)	0.02
Peak frequency (kHz)	65.7 (±0.8)	64.3 (±1.1)	63.5 (±1.2)	0.28
Bandwidth (kHz)	35.1 (±3.6)	31.9 (±3.6)	43.5 (±4.1)	0.13

These results do not conform precisely to the expected similarity between L and R rats, and dissimilarity between H and R rats, based on the different outcomes of selective breeding described in Experiment 1. Two conclusions stem from these results. First, H rats exhibited a broader bandwidth than any of the other strains in the 40 kHz range. Interestingly, an increase in the frequency range has been suggested to strengthen communication of the infant with its mother, thus inducing maternal care (Brudzynski, Kehoe, & Callahan,

1999). The cSNC effect in adult rats is known to be lengthened by increased neonatal stress (Ruetti, Justel, Mustaca, Torrecilla, & Gonzalez-Jatúff, 2010); although it is not known whether this effect was mediated by maternal behavior, this result indicates that cSNC is sensitive to epigenetic manipulations. Thus, it is tentatively suggested that an epigenetic factor of enhanced maternal care mediates the effects of the current selective breeding protocol on recovery from cSNC in H rats. Second, L rats, which were no different from R rats in any of the behavioral variables or in body size recorded in Experiment 1, produced a lower number of calls than R and H rats, in the two types of calls analyzed here.

Experiment 3

Opioid blockage by the nonselective opioid-receptor antagonist naloxone enhances the cSNC effect (Pellegrini et al., 2005). Such enhancement could rest on at least two mechanisms. First, naloxone could enhance the consolidation of the emotional memory of the incentive downshift—an associative mechanism. Posttrial 11 administration of corticosterone (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006; Ruetti, Justel, Mustaca, & Papini, 2009) and the NMDA-receptor partial agonist D-cycloserine (Norris, Ortega, & Papini, 2011) both enhance the cSNC effect by strengthening the emotional memory of the downshift. However, similar Posttrial 11 administration of naloxone does not affect recovery from cSNC or influence appetitive extinction (Daniel, Ortega, & Papini, 2009). Second, opioid blockage could increase the intensity of the aversive emotional state of frustration induced by incentive downshift—a nonassociative mechanism. In addition to increasing response suppression in the consummatory situation when administered before the trial (Daniel et al., 2009; Pellegrini et al., 2005), pretrial naloxone also facilitates appetitive extinction in an instrumental, lever-pressing task (Norris et al., 2009). Pellegrini et al. (2005) also reported that naloxone had a greater effect on locomotor activity in rats that recovered slowly from incentive downshift than in fast-recovery rats. This result was interpreted as reflecting differential binding effectiveness by endogenous opioid ligands (e.g., Bond et al., 1998; Zimprich, Simon, & Höllt, 1995), with slow-recovery rats having isoforms of the receptor and/or ligand genes that are less effective. Thus, one interpretation of the effect of the current selective breeding protocol is that H rats have especially effective opioid ligand-receptor binding allowing them to recover relatively fast from incentive downshift.

Based on these results, strain differences were expected to occur in the extent to which opioid blockage via naloxone modulates behavior during the reward downshift experience. Specifically, and given the results of the selective breeding study, it was predicted that (1) naloxone would increase consummatory suppression during the initial incentive downshift trials equally in L and R rats (given that L rats showed no response to selective breeding), relative to saline controls, but (2) naloxone would have no effect on consummatory behavior in H rats, relative to saline controls.

Method

Subjects. The same subjects from S_6 used in Experiment 2 served in this experiment. Because they had all been treated alike in Experiment 2, no special assignment to new groups was needed. There was a total of 75 rats; 33 L rats (21 males, 12 females), 20 R rats (11 males, 9 females), and 22 H rats (13 males, 9 females). Animals were deprived and maintained as described in Experiment 1.

Apparatus. The same conditioning boxes described in Experiment 1 for cSNC testing were used in the present experiment.

Procedure. Within each strain, rats were randomly assigned to either a group receiving naloxone (Nlx, 2 mg/kg, ip) or isotonic saline (equal volume). Sample sizes for each group are shown in Figure 10. Only downshifted groups were used in this experiment because the number of available animals was not enough to include unshifted naloxone and saline controls. The design is similar to one used before (Pellegrini et al. 2005, Experiment 2), which yielded evidence of increased suppression of consummatory behavior by naloxone. Food deprivation started around PND 90, but not before this age. Training started once animals reached the target deprivation weight (81-84% of the ad libitum weight). All animals received access to 32% sucrose during Trials 1-10 and then were downshifted to 4% sucrose during Trials 11-20. All aspects of training (including trials and sucrose solution preparation) were as

described in Experiment 1. Naloxone hydrochloride (Sigma-Aldrich, St. Louis, MO) in desiccate form (stored at 2-8 °C) was dissolved in isotonic saline solution within 48 h of use and stored in sealed, air tight containers at the appropriate temperature. Naloxone and saline were administered 15 min before Trials 11, 12, and 13.

Results

The results are shown in Figure 10. As expected, H rats produced higher goal-tracking times than L and R rats throughout the experiment. H rats also exhibited less reduction in goal-tracking times on trials when naloxone was administered (Trials 11-13), compared to L and R rats. Preshift data were analyzed in terms of a Strain (L, R, H) x Naloxone (Nlx, Sal) x Trial (1-10) analysis. Sex was not incorporated because of the relatively small sample sizes that would result, especially for females (female sample sizes would be 4-6 rats per group). As expected, given that no naloxone treatment was administered during these trials, none of the effects involving this factor were significant, Fs < 1. There was a significant difference between strains, $F(2, 69) = 10.83$, $p < 0.001$, and a significant increase across trials, $F(9, 621) = 64.18$, $p < 0.001$. Post hoc LSD pairwise test revealed that H rats were significantly above L and R rats, ps < 0.001, which in turn did not differ from each other, $p > 0.63$.

Naloxone reduced goal-tracking times in all strains, but especially in L and R rats. A Strain x Naloxone x Trial (11-13) analysis yielded a strain by postshift significant interaction, $F(4, 138) = 5.50$, $p < 0.001$, and also significant main effects for strain, $F(2, 69) = 4.64$, $p < 0.02$, and naloxone, $F(1, 69) = 12.01$, $p < 0.002$. None of the other factors were significant, Fs < 1.85, ps > 0.13. To identify the source of the strain by postshift interaction, independent Naloxone x Trial analyses were computed for each strain. For L rats, both the interaction and naloxone effects were significant, Fs > 3.78, ps < 0.03. The trial effect was not significant, $F(2, 62) = 2.06$, $p > 0.13$. For R rats, the naloxone effect was also significant, $F(1, 18) = 5.36$, $p < 0.04$, as was the increase across trials, $F(2, 36) = 8.26$, $p < 0.002$. The interaction was not significant, $F < 1$. Importantly, neither the interaction nor the naloxone effects were significant for H rats, Fs < 1. The trial effect was also nonsignificant for H rats, $F(2, 40) = 2.16$, $p > 0.12$.

Training was continued for an additional 7 trials to determine whether the stability of strain differences in goal-tracking times would remain when rats were consuming 4% sucrose. A Strain x Naloxone x Trial (16-20) analysis was calculated over the last 5 trials of the experiment. Only two significant effects were found: strain, $F(2, 69) = 3.54$, $p < 0.04$, and trial, $F(4, 276) = 4.02$, $p < 0.004$. All other effects were nonsignificant, Fs < 1. Post hoc LSD pair wise tests indicated that H rats scored significantly above L and R rats, ps < 0.03. L and R rats did not differ, $p > 0.86$.

From a small parental population of 16 males and 17 females, three pairs were selectively bred according to their high (H) or low (L) rate of recovery from incentive downshift in a typical cSNC situation. Three additional pairs were randomly chosen, that is, mated independently of their cSNC performance, as a control line. This protocol was repeated in every one of six selected generations and a variety of measurements were taken. Although the recovery ratio used to select individuals did not respond to the selective breeding protocol, orderly changes in cSNC performance were observed across generations. R rats, for example, showed divergent consummatory behavior across generations on Trials 11, 12, and 15 (see Figure 5). Two conclusions can be drawn from this result. First, behavioral change may be the result of the small number of pairs in each generation. Second, without an R line controlling for the nonspecific effects of the selective breeding procedure, the L line would have been concluded to have responded to selective breeding (see also Figure 5). However, R rats indicate that the type of generational change observed in L rats did not exceed that induced by the selective breeding protocol implemented in this experiment. This adds a note of caution on the interpretation of a previous study involving artificial selection for degree of consummatory suppression on Trial 11, relative to Trial 10, in the cSNC situation, which, as noted above, did not include a random control line (Flaherty et al., 1994).

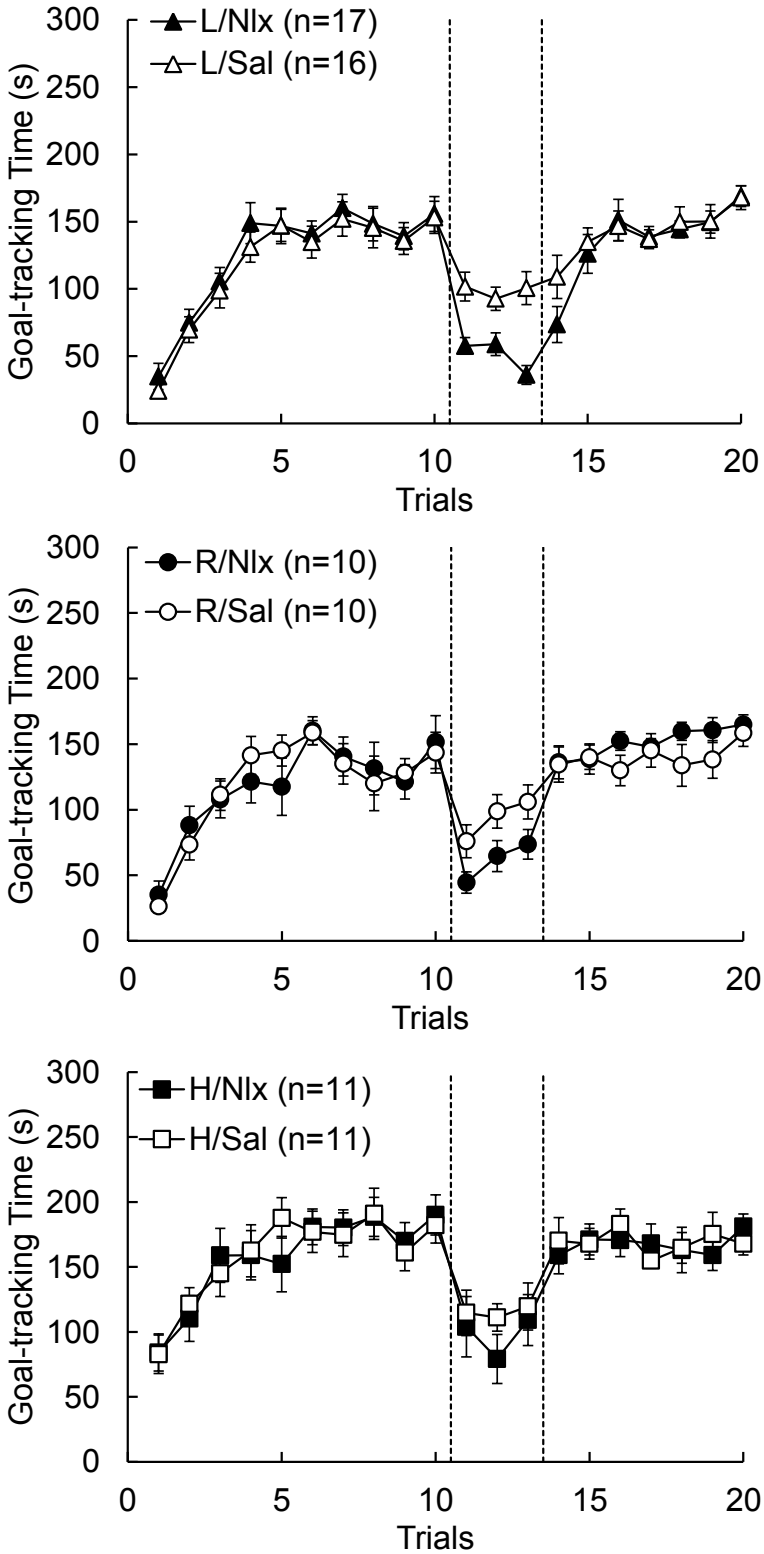

Figure 10. Goal-tracking times (s) for each line (L, R, and H) during preshift trials (Trials 1-10) with access to 32% sucrose for all animals and postshift trials (Trials 11-20) with access to 4% sucrose for all animals. Naloxone (Nlx; 2 mg/kg, ip) or saline (Sal) was administered before Trials 11-13.

General Discussion

Unlike L rats, H rats did respond to the selective breeding procedure. The consummatory behavior of H rats changed across generations in a manner consistent with their selective breeding criterion. Although consummatory suppression after a 32%-to-4% sucrose downshift was still apparent on Trials 11 and 12, after 5 selected generations, the trend was clearly toward reduced suppression (see Figure 5). By Trial 15, although the performance of downshifted L and R rats was still below that of unshifted controls, there was substantial overlap in these two groups of H rats. Therefore, under the present conditions, the response to selective breeding for contrasting recovery rates from cSNC in these lines can be summarized in the following manner: H > (L = R).

Assessments of additional variables discard several alternatives. First, there was no indication that selective breeding for recovery affected the preshift performance of rats given access to 32% vs. 4% sucrose (Figure 4). However, there was a steeper increase in goal-tracking times during preshift in the H line than in either the L or R lines, consistent with the increased in postshift goal-tracking times observed in H rats, especially in downshifted groups (Figure 5). Second, although this may suggest that H rats evolved a tendency to display a higher level of consummatory behavior, this was not supported by the water intake tests. No strain differences were found in the water consumption (Figure 3). Third, perhaps H rats increased their consumption of 4% sucrose after the downshift because selective breeding inadvertently favored increased sensitivity or palatability for low sucrose concentrations. The results of the sucrose sensitivity test did not support this hypothesis; if anything, the nonsignificant trend indicated that H rats were actually less sensitive to low sucrose concentrations than L and R rats. Fourth, it seems plausible that the breeding protocol favored reduced activity in H rats, thus minimizing a source of interference with consummatory behavior (e.g., Pellegrini & Mustaca, 2000). However, open-field tests detected no strain differences either in terms of total activity levels or in terms of habituation of exploratory behavior in the fifth selected generation (Figure 7).

The selective breeding protocol was correlated with changes in weight in both L and H rats, relative to R rats. Such correlational effects with body weight are expected given the polygenic nature of body weight (Chan, Jones, McConnell, Bryk, Bünger, & Tautz, 2012). L and H rats were both significantly smaller than R rats, with H rats being the smallest. Strain differences in ad libitum weight were in place around PND 40 (Figure 2) and clearly present also around PND 90 (Figure 1), but not during the first selected generation. It is expected that smaller rats would display less consummatory behavior, but H rats, which responded to selective breeding, actually displayed higher levels of goal-tracking times in the cSNC situation and across generations than L or R rats (positive vs. negative slopes, see Figure 5). Moreover, whereas some parameters of ultrasonic vocalization in rats vary with body weight, bandwidth is not known to change significantly with changes in weight (Inagaki, Takeuchi, & Mori, 2012).

Integrating the results from the three experiments reported in this article, it may be concluded that resilience in the cSNC situation (i.e., high recovery rates) relates to a relatively small body size, less emotional reactivity under partial reinforcement conditions, infant vocalizations with properties that may enhance maternal care, and relatively more efficient opioid receptors. The theoretical connection between cSNC and partial reinforcement training was made before (Wood et al., 2005) on the basis of Amsel's (1992) frustration theory. Such theory has been suggested to potentially extend to the mother-infant separation situation (Papini & Dudley, 1997) and used to account for pharmacological data (Papini et al., 2006). These results encourage the view that frustration, as conceptualized originally by Amsel (1992), not only plays a significant role in the induction of cSNC and the recovery from response suppression, but that a set of shared and independent psychobiological mechanisms underlie induction and recovery from cSNC. For instance, shared mechanisms are suggested by enhanced cSNC after administration of the nonselective opioid receptor antagonist naloxone before Trials 11 and 12 (Pellegrini et al., 2005) and by the correlated effect of selection of high recovery rates and the decrease of the cSNC initial effect (present results). Independent psychobiological mechanisms underlying cSNC are highlighted by a comparison between the present results with those of a previous

experiment on artificial selection for cSNC. Flaherty et al. (1994) selected animals according to the degree of consummatory suppression on Trial 11, relative to Trial 10 (last preshift trial), and found evidence of a stronger effect in the High line than in the Low line. Interestingly, such selective breeding criterion did not correlate with performance in the open field, anticipatory contrast, radial arm maze contrast, and conditioned place preference. However, the present results suggest that selective breeding for fast recovery from cSNC may be correlated to mechanisms that contribute to other frustration-related behaviors, such as the effects of partial reinforcement in the autoshaping situation. Thus, a plausible working hypothesis would suggest that recovery from cSNC involves a set of distinct behavioral mechanisms that go beyond those engaged by the initial surprising nonreward. This implication is currently being tested in terms of transfer effects from cSNC to partial reinforcement training in the autoshaping situation, and vice versa.

Limitations of the current research suggest some interesting future directions. The procedure used in this study was planned as a preliminary approach in order to evaluate the merits of using a selective breeding procedure with more extensive samples in the future. Thus, these results should be seen as preliminary. Given the characteristics of the present selective breeding protocol, alternative explanations for the pattern of reported data, such as genetic drift or founder effects (random genetic changes with especially strong effects in small populations; Papini, 2008), cannot be discarded. Although the present study provides promising data on the plausibility of selective breeding for recovery from cSNC, two key characteristics of the present protocol should be improved in future studies. First, the parental population and subsequent generations must be larger to avoid inbreeding effects. Second, the selective breeding protocol could have two or more replicate lines in each selection direction. Additionally, disentangling the genetic and epigenetic factors contributing to the effects of selective breeding on incentive contrast will require a better understanding of the role of maternal behavior via direct observations and cross-fostering studies.

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