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RAT STRAIN DIFFERENCES IN RESPONSE TO AN UNEXPECTED REDUCTION IN REWARD

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Abstract

Researchers have become increasingly interested in individual variation in genetic expression, a requirement for evolution (Koolhaas et al., 2010). While experimental rodent strains have traditionally been bred for genetic similarity, behavioral variation between these strains does exist (Koolhaas et al., 2010). This thesis project investigated rat strain differences in frustration. Rats of two strains commonly employed for behavioral tasks, Long-Evans and Sprague-Dawley, were evaluated with a common behavioral task in use for several decades, a successive negative contrast (SNC) paradigm (Flaherty, Troncoso, & Deschu, 1979). In SNC, response to a high value reward is contrasted with response to a lower value reward (Flaherty, 1996). The reward value is decreased unexpectedly over a period referred to as the "downshift," and is thought to induce frustration through violation of the expectation of a high value reward (Flaherty, Troncoso, & Deschu, 1979). To further explore how a frustration response can be exacerbated by environmental conditions, the effect of loss of environmental enrichment, known to induce a negative affective state on the frustration response was also assessed by strain (Burman et al., 2008). Long-Evans rats differed from Sprague-Dawley rats in preshift (p = 0.026) and postshift lick behavior (p = 0.037) and showed more frustration than Sprague-Dawley rats in response to the downshift in reward (p = 0.012). Environmental enrichment had no effects on preshift, postshift, or downshift licks. This study suggests that strain choice should be taken into consideration when interpreting results from behavioral tests and when selecting strains for an experiment, as preexisting differences in strain may prove more powerful than environmental manipulation.

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Acknowledgements

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Introduction

Much research in the domain of animal behavior and physiology has emphasized within-species similarity (Koolhaas et al., 2010). To increase statistical power in a laboratory setting, rodent strains have been bred for genetic similarity (Koolhaas et al., 2010). A recent movement in animal models research, however, has questioned the ecological validity of such studies and warns that use of experimental animals may constitute selection bias (Koolhaas et al., 2010). Individual variation is required for evolution; consequently, researchers have become increasingly interested in the functions of individual behavioral and physiological variation and the mechanisms behind this variation (Koolhaas et al., 2010).

One mechanism of behavioral and physiological variation is the melanocortin system. This system drives melanin production, which determines hair, skin, and feather coloration in vertebrates and underpins a number of behavioral and physiological traits in a variety of vertebrate species (Ducrest, Keller, & Roulin, 2008). For example, darker pigmented individuals in certain species exhibit more bold and aggressive behavior than lighter pigmented individuals (Ducrest et al., 2008). Male captive eastern Hermann's tortoises (*Eurotestudo boettgeri*) demonstrate this; individuals with dark shells exhibited more boldness and aggressive behavior than those males with light-colored shells (Mafli, Wakamatsu, & Roulin, 2011). This is also true in an avian system: siskin birds (*Carduelis spinus*) with the largest black bibs showed the shortest latency to approach a novel object, a common measure of boldness (Mateos-Gonzalez & Senar, 2012).

These behaviors likely arise from the interaction between the melanocortin system and the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for mounting the vertebrate stress response (Ducrest et al., 2008). Melanocyte stimulating hormones (MSHs) stimulate the

production of melanin by melanocytes in the periphery (Goodman, 2009). Melanin production may also be increased in two additional ways via stimulation of the HPA axis (Goodman, 2009). MSHs and adrenal corticotropic hormone (ACTH) derive from the protein pro-opiomelanocortin (POMC) (Cone, 2005). Consequently, increased production of ACTH is accompanied by increased production of MSHs (Goodman, 2009). Additionally, ACTH contains a sequence of amino acids that stimulate melanocytes to produce more melanin (Goodman, 2009). It follows that individuals who produce more melanin may also produce more ACTH and, consequently, more corticosterone. Studies in birds and mammals have confirmed that pigmented individuals are more resistant to stressors; they have a greater ability to mount a physiological response to cope with a stressor (Ducrest et al., 2008).

It is important to note that neither dark nor light pigmentation inherently results in increased fitness (Roulin & Ducrest, 2011). Rather, each phenotype is suited for different environmental conditions (Roulin & Ducrest, 2011). For example, the dark pigmentation phenotype, which tends to favor energy expenditure through aggression and physical activity, would not be adaptive in an environment with low or variable food resources (Roulin & Ducrest, 2011). Variability in pigment production between individuals may help ensure that if the environment changes, some individuals in the population will be well equipped to cope with the changes, survive, and reproduce (Roulin & Ducrest, 2011). Awareness of individual variation in pigment production and its associated behavioral phenotypes is important to analyzing the behavior of animals both in the wild and in the laboratory setting (Koolhaas et al., 2010).

Although laboratory rodent strains have been bred to reduce trait variability, physiological and behavioral differences do exist between the hundreds of strains available to investigators (Koolhaas et al., 2010). Pigmented Long-Evans (LE) rats (*Rattus norvegicus*), for

example, typically have a black hood around the face and a stripe down the back (Harlan, 2010), while albino Sprague-Dawley (SD) rats (*Rattus norvegicus*) do not have pigment and so have white fur (Harlan, 2008). In accordance with the studies discussed above, we observe many of the same behavioral and physiological differences between these strains differing in pigment production. To further explore the interactions between melanin production and the stress response, this thesis project will investigate how these two rat strains, the albino SD and pigmented LE strains, respond to a specific stressor.

As one would predict for a strain that produces more pigment, previous research has shown that LE rats exhibit more physical activity than SD rats. In a 25 minute open field task, LE rats actively explored; they spent the majority of their time walking and rearing whereas SD rats acted as passive observers, spending the majority of their time sniffing (van Lier et al., 2003). LE rats also covered more area than SD rats during an open field task (Ader, Friedman, & Grota, 1967). These behavioral differences between LE and SD rats are linked to their physiological differences in stress reactivity – rodents that actively engage in exploration tend to have lower circulating corticosterone than individuals that exhibit low exploratory behavior (Kazlauckas et al., 2011). Indeed, LE rats have been shown to have lower circulating corticosterone than SD rats (Faraday et al., 2005).

LE rats, however, tended to be more reactive to stress than SD rats. Female LE rats experienced dramatic weight loss and ate less following 20 minutes/day restraint stress for 13 days, indicating a greater sensitivity to the stressful experience (Faraday et al., 2005). Female SD rats did not exhibit such adverse effects (Faraday et al., 2005). A study by Kazlauckas et al. (2011) reported a similar finding when comparing rodents that engaged in high or low exploratory behavior. Following a schedule of unpredictable subchronic stress, high exploratory

rodents had increased corticosterone levels while low exploratory rodents did not experience such an increase (Kazlauckas et al., 2011).

When repeatedly exposed to inescapable shocks, it is thought that animals learn that they have no control over their environment (Padilla et al., 2009). Consequently, they do not act to change their environment even if offered the opportunity; this phenomenon is known as "learned helplessness." Padilla et al. (2009) also reported a strain difference in susceptibility to learned helplessness. LE and SD rats were exposed to 60 trials of 10 second inescapable shocks, where shocks were separated by a random amount of time (between 10 and 110 seconds). The next day, the rats went through 30 trials of an escapable shock paradigm. To escape the shock, rats were required to cross from one side of the experimental box to the other (5 trials) or were required to cross twice (25 trials). LE rats had significantly longer latencies than SD rats to escape the shock, which led the authors to conclude that LE rats are more susceptible to learned helplessness and to the stress of inescapable shocks.

The studies discussed above demonstrate that the LE and SD rat strains differ in activity patterns and stress reactivity; these differences may be explained by the strain differences in melanin production. Namely, pigmented LE rats are more active, have lower circulating corticosterone, and mount a stronger stress response than albino SD rats. To further explore these differences, my thesis project will investigate whether LE and SD strains differ in their level of frustration. This is measured in terms of their response to an unexpected reward reduction by utilizing a successive negative contrast (SNC) paradigm (Flaherty, 1996). In this paradigm, animals are trained to expect a high value reward which is unexpectedly downshifted (Flaherty, 1996). The contrast between the high reward and the new lower reward is thought to induce frustration (Flaherty, 1996) which is mediated by the stress hormone, corticosterone

(Flaherty, Troncoso, & Deschu, 1979). The sensitivity of the animal to the SNC effect, the behavioral response to the reward reduction, is thought to indicate an emotional response and has been used to assess welfare in animals (Burman et al., 2008).

There are two main experimental methods of producing and measuring an SNC effect. Flaherty, a pioneer of the SNC procedure, originally measured the latency for rats to run down a runway to a bottle of sucrose solution (Flaherty & Caprio, 1976). Rats experiencing a reward reduction from 32% sucrose solution to 4% sucrose solution took longer to reach the bottle than rats who received 4% throughout the duration of the experiment (Flaherty & Caprio, 1976). This was interpreted as the reward reduction inducing frustration in the shifted group; the group that received 4% sucrose solution the entire experiment experienced no frustration because their reward expectations were always met (Flaherty & Caprio, 1976).

The SNC effect, however, emerges more reliably when measuring sucrose solution lick rates rather than latencies to sucrose solution (Flaherty & Caprio, 1976). Following this finding, Flaherty and his colleagues tended to measure the SNC effect via lick rates without requiring rats to traverse a runway (e.g., Rowan & Flaherty, 1987; Grigson & Flaherty, 1991). Rats which are shifted from 32% to 4% sucrose solution tend to lick less for 3 to 5 days following the downshift, at which point they return to their pre-shift lick rates (Flaherty et al., 1985). The frustration response to the decreased reward is manifested by a spike in corticosterone and a decrease in lick rate (Flaherty et al., 1985). Based on the strain differences in stress reactivity, we predict that LE rats will exhibit a stronger frustration response than SD rats.

In addition to examining strain effects on the response to reward reduction, the current study will examine how the stress of environmental enrichment removal affects the response. Environmental enrichment has been found to alleviate stress – rats housed in enriched cages

engage in less aggressive behavior than those housed in unenriched cages (Abou-Ismail, Burman, Nicol, & Mendl, 2010). A review of the environmental enrichment literature revealed that enrichment promotes exploratory behavior on the open field task and the elevated plus maze, suggesting that enriched animals are less anxious in novel environments (Simpson & Kelly, 2011).

Environmental enrichment likely exerts these behavioral effects by acting on the HPA axis; for example nutcracker birds (*Nucifraga columbiana*) housed with enrichment for 92 days had reduced corticosterone concentrations in the final 25 days of enrichment (Fairhurst et al., 2011). In a similar vein, rodent studies have found that enriched rats exhibit a blunted ACTH response to restraint stress compared with unenriched rats (Moncek, Duncko, Johansson, & Jezova, 2004; Schrijver, Bahr, Weiss, & Wurbel, 2002). In a different test of stress reactivity, the forced swim test, enriched rats had higher levels of hippocampal serotonin than controls (Brenes, Rodriguez, & Fornaguera, 2008; Brenes, Padilla, & Fornaguera, 2009). Serotonin levels positively correlated with time spent swimming and negatively correlated with time spent immobile, suggesting that the boost in serotonin helped enriched rats cope with the stress of the forced swim test (Brenes, Padilla, & Fornaguera, 2009). Overall, environmental enrichment reduces the stress response and enables animals to better cope with stressors.

Noting that the loss of environmental enrichment likely induces a negative affective state, Burman et al. (2008) hypothesized that rats which experienced enrichment removal would be more sensitive to reward loss. Rats experiencing enrichment removal exhibited prolonged frustration following the downshift from 12 food pellets to 1; this was evidenced by greater latencies to approach the reward than enriched rats for 3 days following the downshift (Burman et al., 2008). In the current study, we will test whether the stress of enrichment removal

amplifies a strain difference in response to reward reduction.

LE and SD rats will be exposed to 32% sucrose solution which will be unexpectedly downshifted to 4% sucrose solution. Additionally, 6 rats will experience enrichment removal during the exposure to 32% sucrose solution. The number of licks made to obtain sucrose solution will be measured. We hypothesize that pigmented LE rats and albino SD rats will exhibit divergent reactions to the stress of reward reduction because their differing amounts of pigment production leads to differences in stress reactivity. Specifically, we predict that LE rats will lick less than the SD rats on the day of the downshift, that the pigmented LE rats will show a greater response to the reward reduction due to their higher stress reactivity. We also predict that rats experiencing enrichment removal will be more sensitive to the reward reduction and will lick less than the enriched rats on the day of the downshift.

Methods

Animals

Subjects were 6 male LE rats and 6 male SD rats (Harlan Laboratories, 2010). At the beginning of the experiment, LE rats were 239 days old and SD rats were 245 days old. Rats were pair housed by strain in standard cages on a reverse light cycle (lights off from 10 AM until 10 PM) in order to accommodate testing during the dark hours when rats are most active. All rats were housed in the same room allowing rats to hear, see, and smell the other rats and experience the same environmental conditions. Except during the one hour prior to testing, food (LabDiet, 2011) and water were available *ad libitum*. Food was removed one hour prior to testing to increase motivation for sucrose consumption.

Enrichment

All rats were housed with enrichment items for two months prior to the start of the experiment. Rats were housed with two 1 in. x 3 in. wood blocks and one Kong (rubber pet toy) for gnawing. One PVC pipe 3 in. in diameter and 6 in. long hung from the wire cage top at each end of the cage. These pipes provided a shelter for each rat.

Materials

We used a successive negative contrast (SNC) procedure (Flaherty, Troncoso, & Deschu, 1979). Rats were repeatedly exposed to a bottle of high rewarding 32% sucrose solution (Flaherty et al., 1979). This reward is then unexpectedly downshifted to 4% sucrose solution for the remainder of the experiment, resulting in frustration (Flaherty et al., 1979). Figure 1 shows a timeline of the SNC procedure. We conducted the SNC procedure in two 12 in. x 12 in. x 12 in. black boxes, each with a bottle attached to a wall. The testing bottles were identical to the water bottles in the home cages. Bottles had a wire attached to the metal spout; upon contact with the

rat a circuit was closed, enabling a computer program to count each time a rat's tongue licked the spout administering sucrose. Following each trial, the apparatus was wiped with a 70% ethanol solution to remove urinary and fur scent cues. Sucrose solutions were prepared by weight with reverse osmosis water and refined sugar. Solutions were kept refrigerated when not in use; room temperature solution was presented to rats during trials.

Enrichment Manipulation

Six subjects had their enrichment removed after trials on the 5th day of SNC. Two LE rats lost enrichment and 4 SD rats lost enrichment. As these animals were part of a larger, long-term experiment and were pair housed, it was not possible to represent both strains equally across enrichment treatments.

SUCCESSIVE NEGATIVE CONTRAST (SNC) TIMELINE

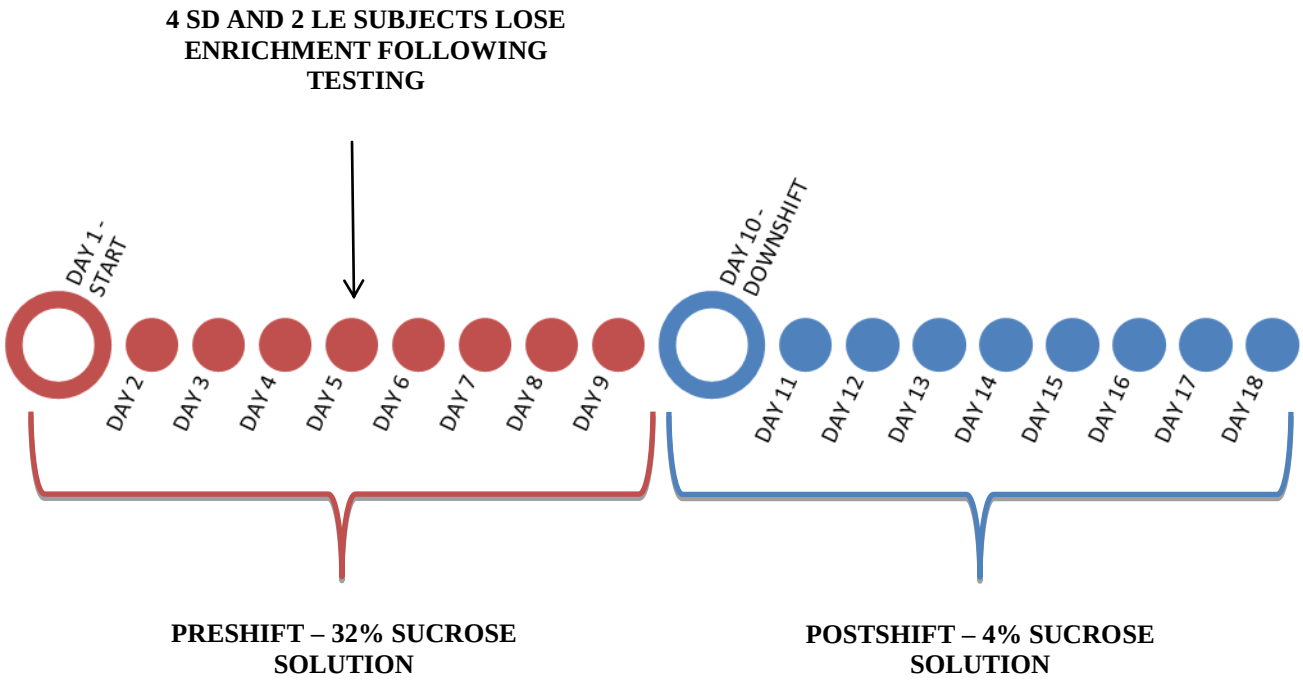

Figure 1. Schematic representation of the successive negative contrast procedure and the sequence of events experienced by both Long-Evans (LE) and Sprague-Dawley (SD) rats during the experiment.

Phase I of SNC – Preshift

One hour prior to testing, feed was removed. Cagemates were run simultaneously, each in a different test chamber; cage order for testing was randomized. Once placed in the box, rats had 5 minutes to lick from a bottle of 32% sucrose solution. A research assistant outside the testing room recorded the number of licks each rat took during the 5 minute session. Data collection ceased after 5 minutes. Following the end of data collection, each rat was removed from the box and placed back into the home cage and feed was replaced. This procedure was repeated for each rat daily for 9 consecutive days to allow rats ample time to habituate to the apparatus.

Phase II of SNC – Postshift

Conditions during Phase II of SNC were the same as during Phase I with the exception of sucrose solution concentration; during Phase II rats were exposed to 4% sucrose solution for 9 consecutive days to allow all rats ample time to return to their preshift lick rates.

Data Analysis

Data were tested for normality and where necessary, data were standardized to meet the assumptions of the analyses of variance (ANOVAs). Repeated measures ANOVAs were performed on the standardized preshift and postshift data to examine the effect of strain on lick rate and the effect of enrichment removal on lick rate. Student's *t*-tests were performed to determine whether lick rate differed between strains and enrichment status on the downshift day.

Results

Preshift

Figures 2 and 3 show mean licks across SNC for strain and enrichment status, respectively. Data from all covariates were standardized to reduce variance. Eleven out of the 12 animals were not reliably responding until the third day of the experiment; therefore, data from the first two days of the preshift phase were excluded from all analyses. The remaining animal, a LE rat in the EN+ group, failed to learn the task and was excluded from all analyses.

To examine the effect of strain (SD, LE) on licks taken across 7 days of the preshift phase, a repeated measures ANOVA was conducted. As Figure 4 shows, a significant difference between the number of SD and LE rat licks emerged during the preshift phase $F(1, 7)= 7.967$, $p = 0.026$. On the last day of the preshift phase, an independent-samples *t*-test revealed that LE (M=153.40) rats licked more than SD rats (M=99.50), $p = 0.045$.

Differences between environmentally enriched (EN+) rats and rats that experienced enrichment loss (EN-) were also examined during 7 days of the preshift phase. A repeated measures ANOVA revealed no significant preshift differences in lick rates between EN+ and EN- rats $F(1, 7)= 0.010$, $p = 0.923$ (see Figure 5).

Response to Shift

Because LE rats tended to lick more frequently than SD rats in the preshift phase, direct comparisons between licks taken on the day of the downshift would not be meaningful. Instead, the degree of change from preshift lick rate to the lick rate for the first 3 days of postshift was computed. Data were transformed using a square root of *x* transformation. Difference scores were created by subtracting each rat's average lick rate for the final 2 days of preshift from each rat's lick rate on the first, second, and third days of the postshift phase. After averaging

difference scores for SD and LE rats, a repeated measures ANOVA was conducted to determine whether the strains deviated at different degrees from their preshift lick rate following the downshift.

The repeated measures ANOVA revealed no significant differences between LE and SD difference scores across the first 3 days of postshift $F(1, 7)= 0.551$, $p = 0.482$. However, an independent samples *t*-test conducted on the first day of the postshift revealed that LE rats (M=146.1) had significantly higher difference scores than SD rats (M=81.667); $p = 0.012$ (see Figure 6).

An independent samples *t*-test was conducted to compare the response of EN+ and EN- rats to the downshift. EN+ rats (M=112.2) did not significantly differ from EN- rats (M=133.833) in response to the downshift; $p = 0.904$.

Postshift

To examine the effect of strain (SD, LE) on lick rate during the first five days of the postshift phase, a repeated measures ANOVA was performed. The repeated measures ANOVA revealed significant differences between SD and LE rat lick rates during the postshift phase $F(1, 7)= 6.572$, $p = 0.037$ (see Figure 7).

A repeated measures ANOVA was also performed to examine differences in postshift lick rate between EN+ and EN- rats. No significant differences between EN+ and EN- rats emerged in the postshift phase $F(1, 7) =0.395$, $p = 0.550$ (see Figure 8).

Graphs

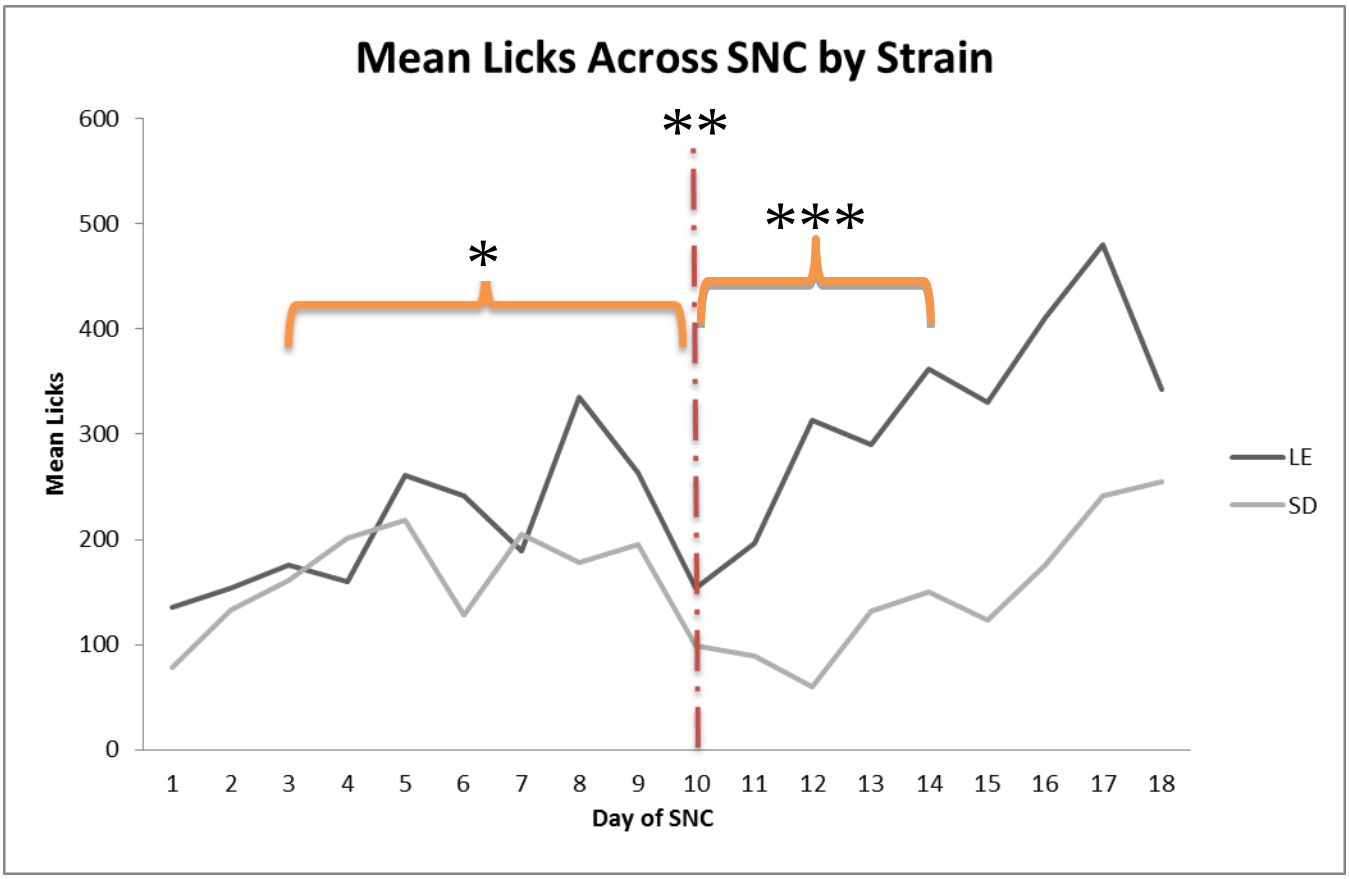

Figure 2. Mean licks of Long-Evans (LE) and Sprague-Dawley (SD) rats across 18 days of the successive negative contrast (SNC) experiment. The vertical dashed line indicates the first day of the downshift from 32% sucrose solution to 4% sucrose solution on day 10 of the experiment, or day 1 of the postshift phase. Significant differences ($p < 0.05$) were observed between LE and SD licks on days 3-9, day 10, and days 10-14.

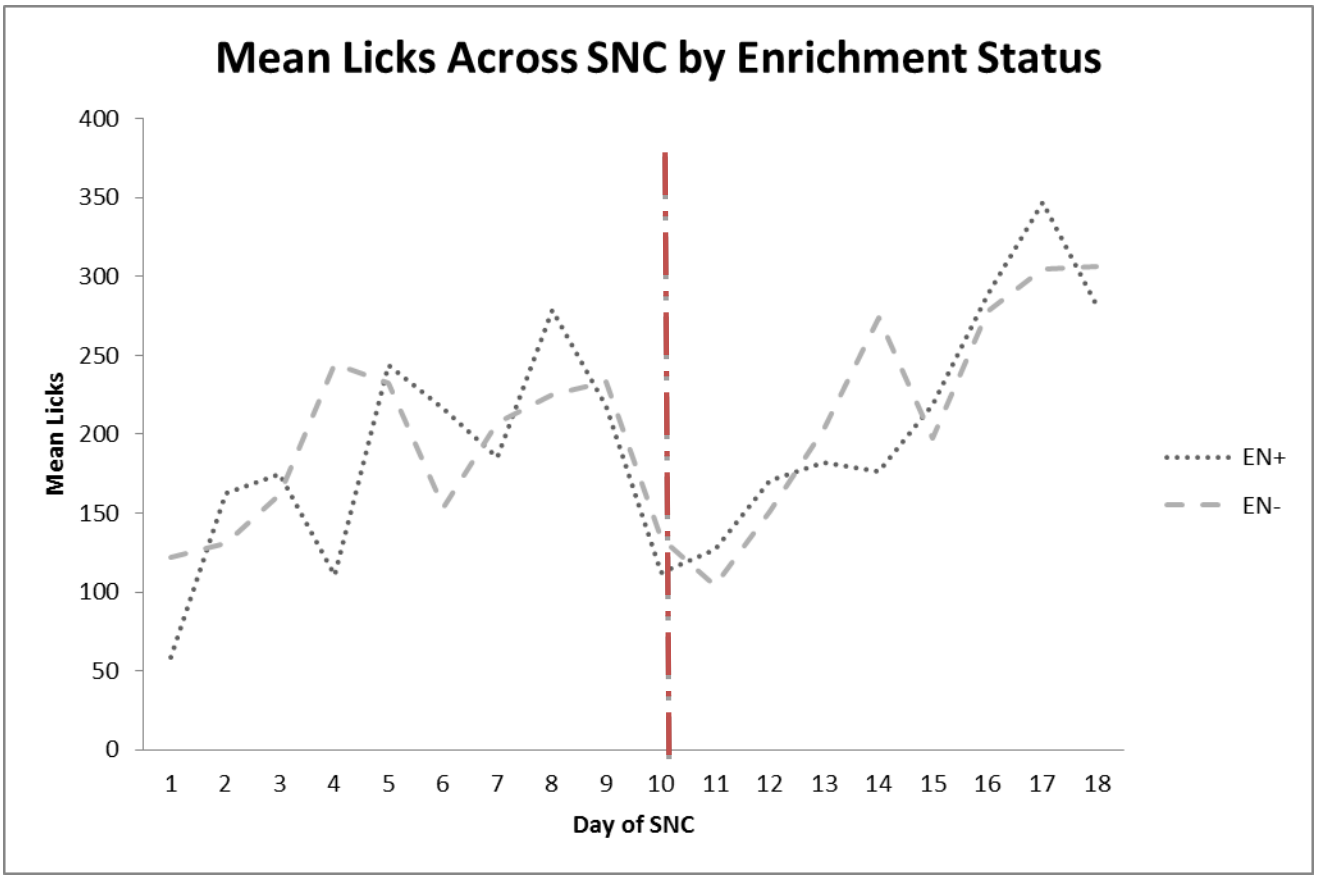

Figure 3. Mean licks of enriched (EN+) and unenriched (EN-) rats across 18 days of the SNC experiment. The vertical dashed line indicates the first day of the downshift from 32% sucrose solution to 4% sucrose solution on day 10 of the experiment, or day 1 of the postshift phase. No significant differences were observed between EN+ and EN- licks on days 3-9, day 10, and days 10-14.

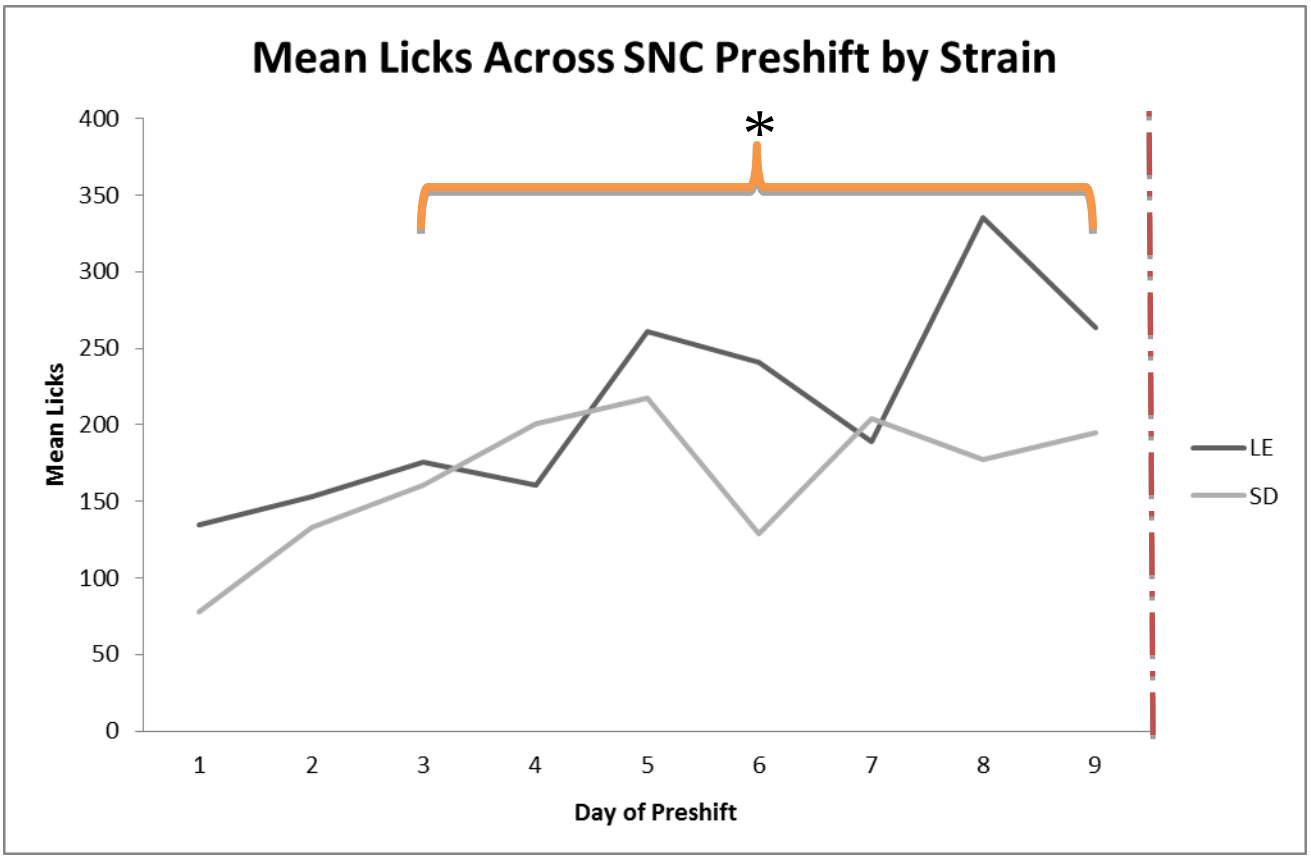

Figure 4. Mean licks of LE and SD rats across 9 days of the preshift phase of the SNC experiment. The vertical dashed line indicates the first day of the downshift from 32% sucrose solution to 4% sucrose solution on day 10 of the experiment, or day 1 of the postshift phase. Significant differences between LE and SD rats emerged during the preshift; $F(1, 7)= 7.967$, $p = 0.026$.

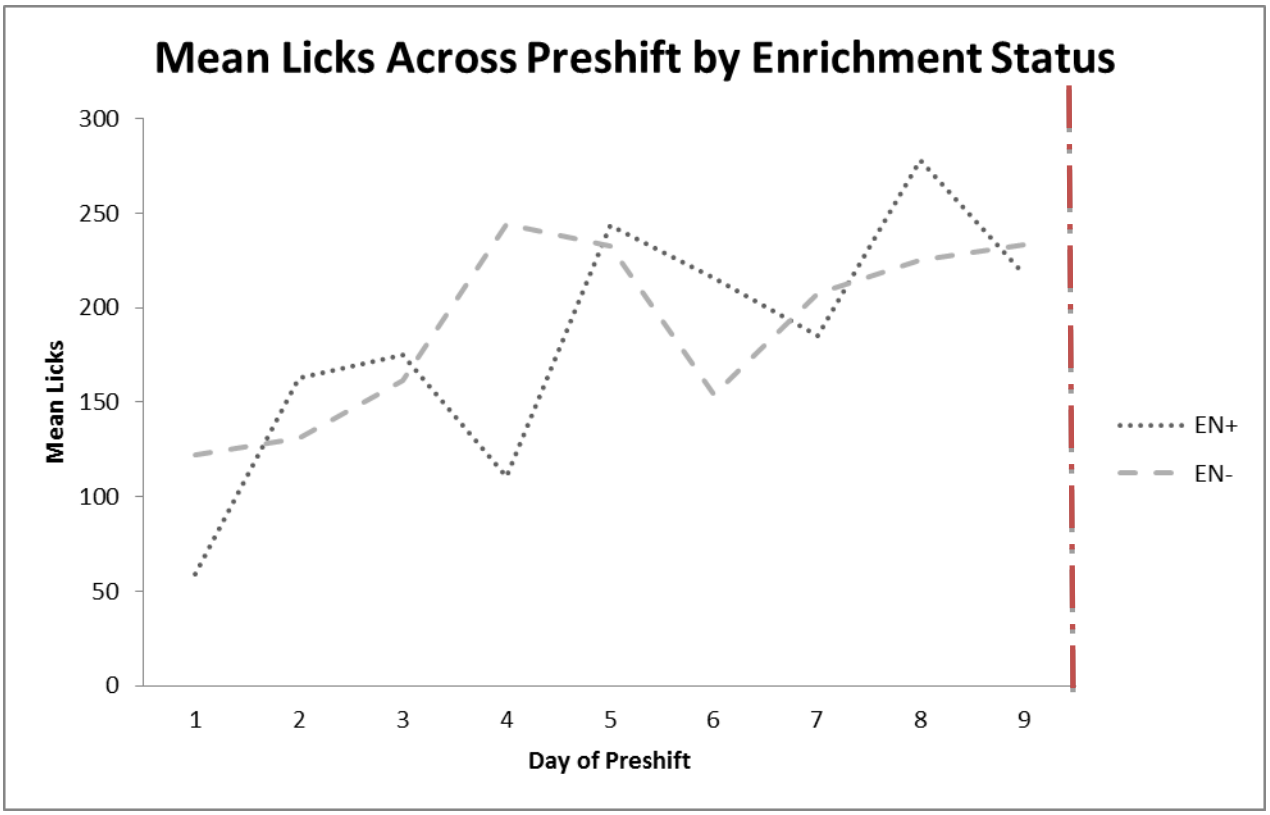

Figure 5. Mean licks of EN+ and EN- rats across 9 days of the preshift phase of the SNC experiment. The vertical dashed line indicates the first day of the downshift from 32% sucrose solution to 4% sucrose solution on day 10 of the experiment, or day 1 of the postshift phase. No significant differences were observed between EN+ and EN- licks during the preshift phase.

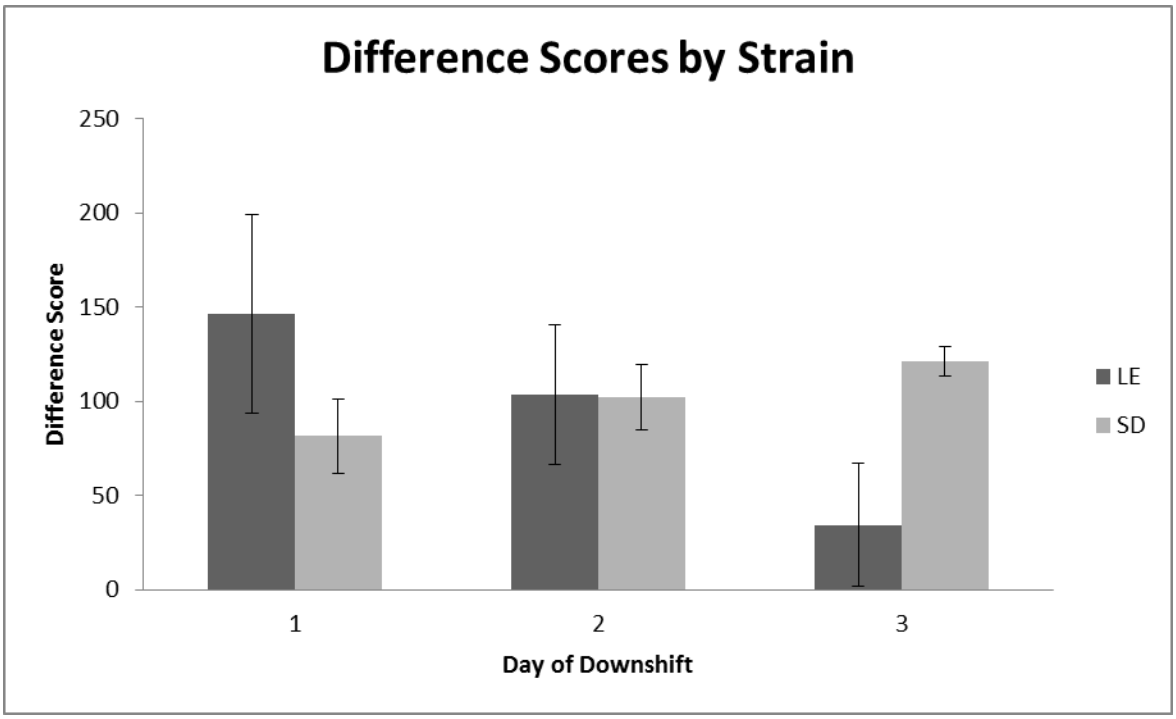

Figure 6. Difference scores for LE and SD rats on the first, second, and third days of downshift. Scores were calculated by subtracting the lick rate for the postshift day from the average lick rate for the last three days of the preshift phase. On the first day of the downshift, LE rats (M=146.1) had significantly higher difference scores than SD rats (M=81.667); p = 0.012.

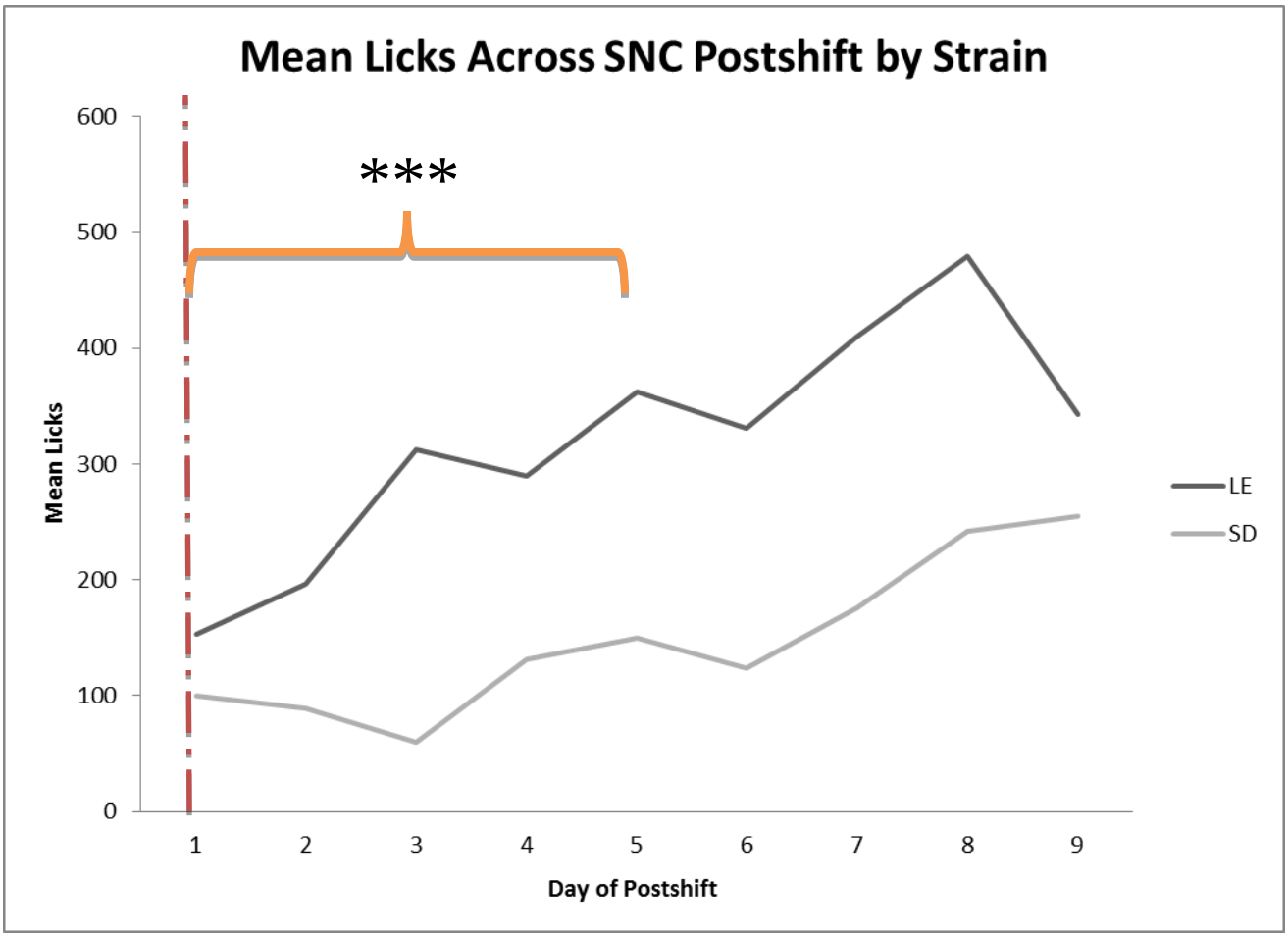

Figure 7. Mean licks of LE and SD rats across 9 days of the postshift phase of the SNC experiment. The vertical dashed line indicates the first day of the downshift from 32% sucrose solution to 4% sucrose solution on day 10 of the experiment, or day 1 of the postshift phase. Significant differences emerged between LE and SD licks during the postshift; $F(1, 7)= 6.572$, $p = 0.037$.

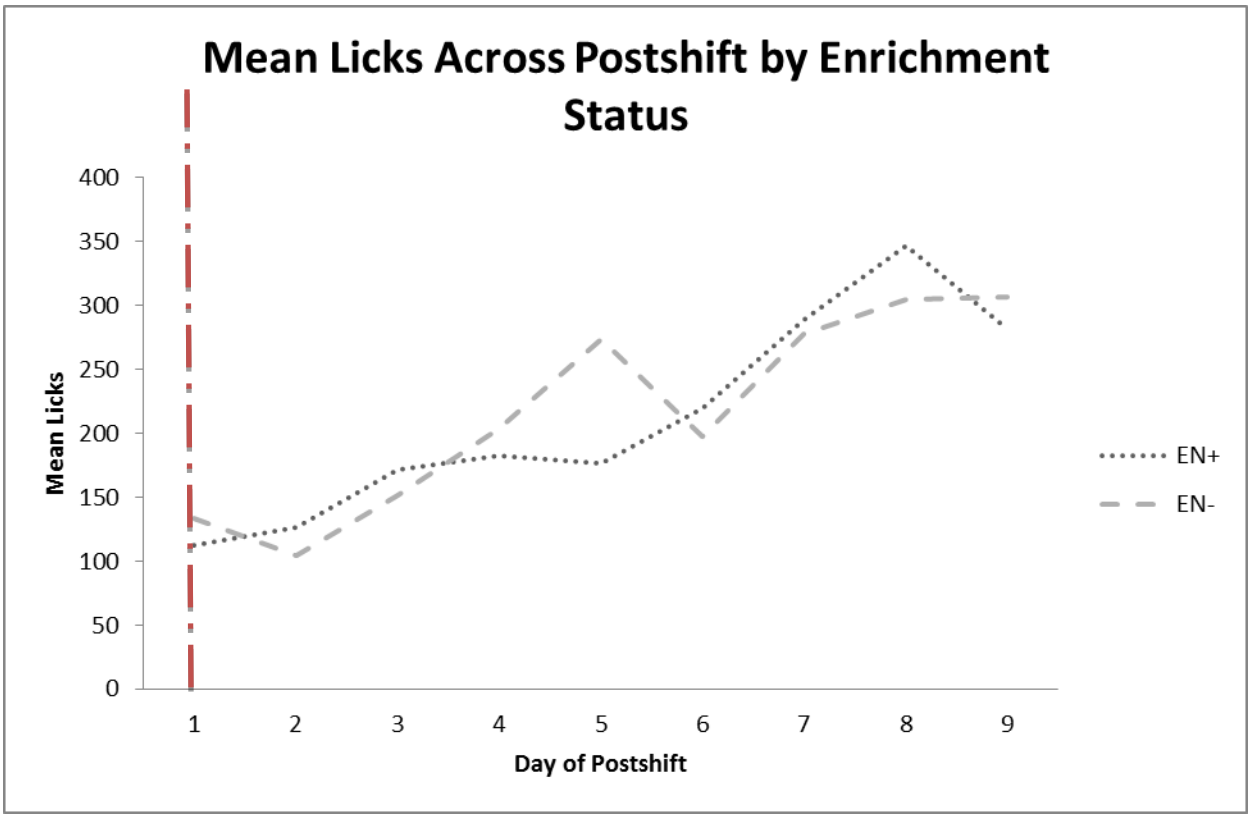

Figure 8. Mean licks of EN+ and EN- rats across 9 days of the postshift phase of the SNC experiment. The vertical dashed line indicates the first day of the downshift from 32% sucrose solution to 4% sucrose solution on day 10 of the experiment, or day 1 of the postshift phase. No significant differences were observed between EN+ and EN- licks during the postshift phase.

Discussion

Individual variation, the raw material for evolution, has received increasing attention from researchers in recent years (Koolhaas et al., 2010). Increasing genetic similarity in experimental rodent strains has increased statistical power and reduced sample sizes but has come at a cost (Koolhaas et al., 2010). Studies using lab strains, such as the Long-Evans (LE) and Sprague-Dawley (SD) rat strains, may yield results that cannot be applied to wild-type animals (Koolhaas et al., 2010). Investigating the underpinnings of individual differences is crucial to interpreting behavioral results (Koolhaas et al., 2010); the current study investigated whether two rat strains differed in response to a reward reduction.

Pigmented LE and albino SD rats were run through a successive negative contrast (SNC) paradigm (Flaherty et al., 1985). In addition to comparing strain differences, there was a manipulation of cage enrichment. Two LE rats and 4 SD rats lost enrichment after the 5th day of SNC; those rats which lost enrichment were in the EN- group while rats which had enrichment through the entire SNC paradigm were in the EN+ group. The unequal representation of the strains across enrichment groups was a consequence of pair housing the animals and the fact that the current experiment used 8 LE and 8 SD rats.

In the current study, a strain effect emerged in the preshift lick patterns; on the last day of the preshift phase, LE rats licked more than SD rats. The strains also responded differently to the downshift; on the first day of the downshift, LE rats deviated more from their preshift lick behavior than SD rats. This suggests that LE rats were more frustrated by the reward reduction than SD rats. Furthermore, during the postshift phase, the strains recovered their preshift lick patterns at different rates. These results are supported by evidence from an earlier selective breeding study performed by Flaherty et al. (1994). They found that the 6th and 7th generations

of SD rats bred for large SNC effects covered more area in the open field task than those generations bred for small SNC effects (Flaherty et al., 1994). Other studies have demonstrated that LE rats exhibit more exploratory behavior than SD rats (Ader, Friedman, & Grota, 1967; van Lier et al., 2003). Therefore, we would expect that high-exploratory LE rats exhibit a larger SNC effect than low-exploratory SD rats; this is what the data indicate.

The strain differences observed in the degree of frustration response on the SNC may have resulted from strain differences in stress reactivity. Flaherty et al. (1985) demonstrated that corticosterone mediates the response to the downshift in reward. SD rats have higher circulating cortiosterone than LE rats (Faraday et al., 2005) but LE rats mount stronger stress responses (Faraday et al., 2005; Padilla et al., 2009). These behavioral phenotypes likely arise from the interaction of the melanocortin system and hypothalamic-pituitary-adrenal (HPA) axis (Ducrest et al., 2008). High HPA axis activity is accompanied by increased pigment production (Goodman, 2009). Consequently, individuals that produce large amounts of pigment are also likely to be highly reactive to stressors.

The current study also investigated the effect of enrichment loss on the response to reward reduction. EN+ and EN- rats did not differ in mean licks taken during the preshift or postshift phases. The enrichment groups also did not respond differently to the reward reduction across the last two days of the preshift phase and the first three days of the postshift phase. These results are not consistent with the results reported by Burman et al. (2008). They found that rats experiencing enrichment loss took longer to recover their preshift lick behavior than those rats which did not experience enrichment loss; they concluded that unenriched rats were in a negative affective state and were consequently more sensitive to the negative effects of the reward reduction. A study by Latham and Mason (2010) compared mice housed without

enrichment and mice housed with enrichment for 90 days before enrichment removal. Mice experiencing enrichment removal engaged in more stereotypic behavior and were willing to push higher weights in order to access an area with enrichment objects than unenriched mice. Researchers inferred that enrichment removal induced frustration in mice. As the downshift in reward and enrichment removal would be expected to induce increased frustration, it is unexpected that no differences between EN+ and EN- rats were observed.

The current study may have failed to uncover any differences in the response to reward reduction from the enrichment comparison because the strains responded differently to the effects of enrichment and enrichment loss and the comparison here pooled across two strains. LE rats are more responsive to stressors; they eat less than SD rats following repeated exposure to restraint stress (Faraday et al., 2005) and demonstrate more persistent learned helplessness than SD rats following exposure to inescapable shocks (Padilla et al., 2009). For these reasons, LE rats may have been more likely to react strongly to the loss of enrichment. This may have been compounded with the fact that the strains were not represented equally across enrichment treatments. Alternatively, the effects of strain may have overridden the effect of environmental enrichment.

The current study faced several problems. The 6 LE rats and 6 SD rats in this study were part of a larger study. Since animals were pair housed, it was impossible to evenly represent LE and SD rats across enrichment groups. Therefore, it was difficult to tease out enrichment loss effects from strain effects. In addition, one subject suffered from variable and occasionally missing data; therefore, this subject was excluded from analyses. The device used to count the number of licks taken during the SNC task occasionally malfunctioned. Experimenters had visual confirmation that licks went unrecorded; consequently, some animals were missing a day

of data.

The current study raises several new research questions. LE rats and SD rats differed in response patterns to the reward reduction. A future study could address whether differences in corticosterone response to downshift explain this strain difference. Although no significant differences were found between the EN+ and EN- groups on the SNC task, future studies should also consider strain effects on response to enrichment loss and should consider removing enrichment closer to the downshift.

This study further raises concerns about interpreting behavioral results from lab animal strains. Other studies have demonstrated that the LE and SD rat strains differ in their activity patterns; LE rats exhibit greater activity (van Lier et al., 2003) and cover more distance (Ader, Friedman, & Grota, 1967) in the open field task than SD rats. The current study found that the strains differ on the SNC task, a task which induces frustration (Flaherty et al., 1985) and may serve as an indicator of affective state (Burman et al., 2008). It is clear that variation between laboratory rat strains should be considered when researchers interpret results from behavioral experiments. Further research into the mechanisms of individual variation may also illuminate factors that increase susceptibility to mood disorders and factors that enable individuals to survive and reproduce in varying environments.

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