ARTIFICIAL SELECTION FOR RECOVERY FROM CONSUMMATORI
SUCCESSIVE NEGATIVE CONTRAST

by

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Submitted to the Graduate Faculty of the
College of Science and engineering
Texas Christian University
In partial fulfillment of the requirements
for the degree of

Master of Science
I would like to acknowledge Mauricio R. Papini for his insightful mentorship. I would also like to thank Gary Boehm, David Cross, and Grace Rowan-Szal for contributing their expertise as members of my dissertation committee. In addition, I am grateful for the continuing support outside of the laboratory from my parents, brother, and Carolina.
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Introduction

During a consummatory successive negative contrast (cSNC) procedure, rats given free access to a 32% solution of sucrose show a sharp suppression of drinking behavior after a downshift to a 4% solution, compared to rats previously receiving 4% solution. Usually, such suppression is followed by a gradual recovery of drinking behavior to the control level. The possibility that a genetic factor is involved in this recovery process is suggested by individual differences for the recovery from reward downshift (Pellegrini, Wood, Daniel, & Papini, 2005), and by the response to artificial selection based on high and low cSNC effects in rats (Flaherty, Krauss, Rowan, & Grigson, 1994). The criterion used in the Flaherty et al. study was the degree of decrement in lick frequency following the downshift, measured as a ratio of the first downshift trial to the last preshift trial. The current proposal will evaluate the response to selective breeding for fast vs. slow recovery from cSNC.

The artificial selection methodology is sensitive to a multiplicity of causes including genetic, developmental, physiological, and environmental. Moreover, the artificial selection methodology may be thought of as involving a microevolutionary comparison concerning the contribution of both phylogeny and ecology to behavior (Timberlake, 1993). Given the heuristic power of evolutionary principles to deal with the issue of diverse biological causes and their application to behavioral theories, a behavioral framework based on such principles will be used to describe the relevant literature. The present introduction approaches the experimental problem by discussing:

1. The sources of behavioral variation, from an epigenetic point of view, that are related to artificial selection procedures.
(2) Previous reports on artificial selection based on traits related to emotion.

(3) Experimental findings suggesting a genetic basis for recovery from cSNC.

**Behavioral Epigenetics**

Phenotypic variation and its underlying genetic variation are the raw material for evolution by natural selection. Individual differences within a species are the basis for speciation following the action of selection pressures. Analogously, behavioral variation is the basis for the artificial selection of a particular behavior. From a classic Darwinian point of view (Darwin, 1872; Gould, 2002; Mayr, 1997a), selection processes mainly act over the phenotypic traits of individual organisms, including morphological and behavioral characteristics. The Darwinian rationale for biological evolution involves three general features: variation, selection, and inheritance (Darwin, 1872; Mayr, 1997a). This apparently simple three-process model has proven to be a strong heuristic tool in the study of life in general. Behavioral phenomena are natural events, so analogous explanations for behavioral events have been proposed (Skinner, 1981; Staddon & Simmelhag, 1971). In this case, it is suggested that the variation of behavioral traits is analogous to variation of phenotypes, the role of selection is accomplished by reinforcement dynamics, and retention is related to the recurrence of behaviors in a given situation. Interestingly, in evolutionary biology and behavioral research similar discussions have been raised about how to understand the sources of variation or, in other words, what is actually selected in natural or artificial selection. One way to approach this problem has been through the understanding of the relationship between genes and phenotypes, either morphological or behavioral.
Comparable answers have been developed to explain gene-phenotype relationships in both evolutionary and psychological theories. In evolutionary biology, one approach was based on the idea of phenotypic traits as direct outcomes of the developmental unfolding of one or more genes. From this point of view, the important topic for study was genetics and not the phenotypic traits, considering the last ones as a genetic by-product. As Rutter (2007) commented, the assumption was that gene-environment interactions were usually of little importance and they could be safely ignored. Examples of this approach are the majority of the works in early population genetics (Mayr, 1997b), and the gene-selection ideas of Dawkins (1976). In the behavioral sciences, the idea of a gene-phenotype direct relationship was defended in the context of the nature-nurture distinction. The nature approach was originally embraced by some authors to explain instincts, which were considered an outcome of the unfolding of a genetic program present in the fertilized egg (Lewontin, 2000; Lewontin, Rose, & Kamin, 1984; Oyama, 2000). Later versions assuming direct gene-behavior dynamics were centered on the study of mathematical genetic models for the transmission of traits, emphasizing single-gene analysis and interactions between single genes as the preferred approach to behavioral genetics. For instance, although McLearn and DeFries (1973) proposed a model for variation within a population that included environmental interactions, the emphasis was on single-gene interactions as causes of variation, such as dominance, epistasis, pleiotropy, and additive genetic effects.

Even in the case of single genes, the gene-phenotype relationship is not straightforward. As noted by Mayr (1997b), single or “naked genes” are not independent entities and their selection is related to the biological mechanisms that relate them to
phenotypic outcomes. Some examples of such biological mechanisms related to behavior are the specific expression of genes depending upon the paternal or maternal origin, or genetic imprinting (Allen, Norris, & Surani, 1990), posttranslational mechanisms and chromatin modulation (Weaver et al., 2004), and maternal and social effects during sensitive developmental periods (Gottlieb, 1991). Therefore, a proper explanation of the relationship between a phenotypic trait and its genetic basis requires taking into account a multiplicity of variables, such as the epigenetic path that includes the genetic basis and the development of the trait. A model that involves a direct relationship between phenotype and genotype is insufficient to explain the majority of genotype-phenotype interactions (Gottlieb, 1997; Lewontin, Rose, & Kamin, 1985; Oyama, 2000). For example, a polymorphism in the serotonin (5-HT) transporter gene regulatory region (rh5-HTTLPR) that results in allelic variation in 5-HT transporter (5-HTT) expression in rhesus monkeys is related to differential behavioral outcomes depending of two circumstances, mothering and allelic organization (Champoux et al., 2002). When the rh5-HTTLPR variants were homozygous, the monkeys performed in a similar fashion in a battery of tests targeting orienting behavior, motor maturity, reflex functioning, and temperament, given two mothering conditions during development, mother- or nursery-reared. However, when the rh5-HTTLPR variants were heterozygous, the monkeys under the nursery-reared condition showed lower orientation scores when compared with mother-reared monkeys.

In recent years, a new understanding of the processes underlying the development of phenotypic traits has revolutionized the biological sciences (Arthur, 2002; Goodman & Coughlin, 2000; Gould, 2002), although the details of the theoretical basis for a
developmental model of the phenotypes are still debated (Griffiths & Gray, 2004). In general, the epigenetic approach makes emphasis on the dynamic nature of genetic and nongenetic factors involved in the development of phenotypic traits (Oyama, 2000). For example, Ho & Saunders (1979, p. 589) proposed that “large evolutionary changes could be the result of the canalization of novel developmental responses to environmental challenges under conditions of relaxed natural selection.” Lewontin (2000) explained organisms as particular outcomes of an ontogenetic process conditional on the consecutive environments in which the development occurs. From this point of view, genes are necessary, but not sufficient to develop a phenotype. This approach was also incorporated in behavioral research in the early years, following on Kuo (1921), who argued against instinct-related ideas as psychological explanations in the nature-nurture debate and pointed out the importance of development. Kuo stated (1921, p. 650), “To call an acquired trend of action an instinct is simply to confess our ignorance of the history of its development.”

The epigenetic rationale has been used successfully in the study of various behavioral processes since then. Several sources of evidence support the importance of influences during development over behavioral mechanisms. Interesting studies have been done focusing on emotional-related behaviors (see Table 1). An example of epigenetic-mediated transgenerational effects is provided by the work done with high and low licking/grooming (LG)-arched-back nursing (ABN) rat mothers (Meaney, 2001). Individual differences in the LG-ABN behavior of rat mothers are highly correlated across generations (Francis, Diorio, Liu, & Meaney, 1999) and are related to both stress reactivity of the pups and the future parental behavior of the female pups. Caldji et al.
(1998) found that in adulthood the offspring of high LG-ABN mothers showed increased startle responses, higher open-field exploration, and shorter times to eat food in a novel environment, than low LG-ABN mothers. Meaney (2001) suggested that these differences in maternal behavior are related to biological changes reported by Liu et al. (1997), such as high LG-ABN adult offspring showing reduced plasma adrenocorticotropic hormone and corticosterone responses to acute stress, when compared to low LG-ABN mothers. The high LG-ABN offspring also showed higher levels of mRNA expression of the glucocorticoid receptor gene in the hippocampus, enhanced glucocorticoid negative feedback sensitivity, and lower mRNA expression of the corticotropin releasing factor gene in the hypothalamus.

Epigenetic inter generational effects on abusive parental behavior have also been reported. In a cross-fostering study using either biological mothers or adoptive mothers, Maestripieri (2005) reported that more than 50% of rhesus monkey females that suffered early abuse by their mothers, regardless of having a biological or adoptive mother, showed abusive parenting behavior with their own offspring, while none of the females reared by nonabusive mothers showed abusive behavior toward their offspring. In an additional study, Maestripieri et al. (2005) tested maternal behavior of female monkeys with previous abusive and nonabusive histories of maternal behavior for corticotropin-releasing hormone and the serotonin metabolite 5-hydroxyindoleacetic acid during the pregnancy and postpartum periods, and carried out observations of social and maternal behavior. Abusive mothers had significantly higher concentrations of corticotropin-releasing hormone and 5-hydroxyindoleacetic acid than nonabusive mothers.
Table 1.
*Overview of Studies Linking Emotional-related Behavioral Outcomes with Epigenetic Effects.*

<table>
<thead>
<tr>
<th>Behavioral or biological measure</th>
<th>Epigenetic modulation</th>
<th>Proposed biological mechanism</th>
<th>Animal model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress reactivity</td>
<td>-Mother licking</td>
<td>Decreased CRF mRNA expression in the PVNh and the central nucleus of the amygdala</td>
<td>Rat</td>
<td>Meaney (2001); Francis, Diorio, Liu, and Meaney (1999); Caldji et al. (1998); Liu et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>-Arched-back nursing</td>
<td></td>
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<td></td>
<td>-Handling</td>
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<td></td>
<td>-Maternal separation</td>
<td></td>
<td></td>
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<tr>
<td>Maternal behavior</td>
<td>-Abusive and non-abusive rearing styles</td>
<td>Higher CSF concentrations of CRH and 5-HIAA</td>
<td>Rhesus monkeys</td>
<td>Maestripieri (2005); Maestripieri et al. (2005)</td>
</tr>
<tr>
<td>Orientation scores</td>
<td>-Rearing</td>
<td>rh5-HTTLPR variants</td>
<td>Rhesus monkeys</td>
<td>Champoux et al. (2002)</td>
</tr>
<tr>
<td>5-HT regulation</td>
<td>-Parental separation</td>
<td></td>
<td>Octodon degus</td>
<td>Jeziernsky, Braun, and Gruss (2006)</td>
</tr>
<tr>
<td>Play behavior</td>
<td>-Ratio of females-males in the litter</td>
<td>HPA axis responsiveness</td>
<td>Mice</td>
<td>Laviola and Terranova (1998); Laviola and Alleva (1995); Terranova and Laviola (1995)</td>
</tr>
<tr>
<td></td>
<td>-Time of weaning</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasonic vocalizations</td>
<td>-Handling</td>
<td>Increased neurotrophin levels</td>
<td>Mice</td>
<td>Cirulli et al. (2007)</td>
</tr>
<tr>
<td>Elevated plus-maze</td>
<td>-Environmental enrichment</td>
<td></td>
<td>Mice</td>
<td>Friske and Gammie (2005)</td>
</tr>
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<td>Open field activity, social interaction</td>
<td>-Environmental enrichment and prenatal cocaine</td>
<td></td>
<td>Rat</td>
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</tr>
</tbody>
</table>

Parental separation, another parenting-related effect, is related to 5-HT (serotonin) modulation. Jeziernsky, Braun, and Gruss (2006) reported that, following acute separations from the mother, a rodent called degu (*Octodon degus*) showed a decrease of 5-HT at postnatal days (PNDs) 3, 8, and 14 in males, and at PNDs 3 and 8 in females. Moreover, repeated parental separation during the first 21 days or just at PND 21 was also related to 5-HT modulation. On the other hand, females repeatedly separated showed attenuated responsiveness of the serotonergic system in the frontal cortex when compared with the females just separated at PND 21. Lastly, repeated separation was related with an up-
regulation of the basal levels of 5-HIAA in the caudal cortex in males, compared to socially reared males, and in females.

Early social stimulation is an important determinant of subsequent behavior in developing rodents (Laviola & Terranova, 1998), as suggested by the effects of early gender composition in the litter on variations in adult exploratory patterns. For instance, when the gender composition is manipulated, female mice born in litters with 50% of males and females showed higher levels of early exploratory behavior and lower levels of playful behavior, whereas females born in litters with 100% females showed higher levels of social play, when compared with other female-male litter ratios (Laviola & Alleva, 1995). Moreover, Terranova and Laviola (1995) found that early weaning was related to increases in eating behavior and in the levels of an exploratory-escape jump activity. Interestingly, the biological mechanism suggested by Laviola and Terranova (1998) for these behavioral effects involves differences in the hypothalamic-pituitary-adrenal axis (HPA axis) responsiveness related to the configuration of the litters.

Another example of an epigenetic effect on development was reported by Cirulli et al. (2007). They used a three-group design, with mouse litters exposed to 15 min of neonatal handling, to an unfamiliar male intruder from PNDs 2 to 14, or to no manipulation (control group). Ultrasonic vocalizations were compared following administration of the anxiolytic chlordiazepoxide on PND 10. The handled group showed no reduction of the calling rate following the drug treatment; in contrast, the other two groups showed a reduction in calling rate. When a maternal separation procedure was administered on PND 8, hippocampal levels of nerve growth factor increased only in the handled group. According to the authors, these results suggest a relationship between
handling, increases in neurotrophin levels, and neural plasticity in mice, although the details of such relationship need to be clarified.

The conditions of the home cage during development provide further examples of environmental modulation of behavior. Friske and Gammie (2005) tested the effects of home cage enrichment from PND 21 to postpartum day 2 (approximately 6 weeks) on the elevated plus-maze and maternal defensive behavior of lactating female mice. They found a higher number of entries to the open arms for the enriched group when they were tested at PNDs 3 and 6, compared with a nonenriched home cage group. Interestingly, when the offspring reared in nonenriched environments were tested around PND 90, the offspring of enriched mothers showed a similar pattern of results, with a higher number of entries to the open arms than the offspring of nonenriched mothers.

Lastly, Neugebauer et al. (2004) tested the behavioral interactions between cocaine prenatally administered, environmental enrichment using open field activity and social interaction. Rats in the prenatal, nonenriched condition showed less grid entries in the activity test at PND 120 than rats treated prenatally with cocaine and rats treated with saline in enriched and nonenriched conditions. For social interaction scores, the prenatal cocaine nonenriched group showed an altered behavioral profile when compared with the other groups. Together, these results suggested that environmental enrichment attenuates the behavioral effects of prenatal cocaine.

The mechanisms underlying the variation of particular behaviors seem to be a set of genetic and epigenetic possibilities. Additionally, in the majority of cases individual differences at the behavioral level are probably related to more than one biological system. Thus, a behavior artificially selected may be seen as the result of modifications
on one or more genetic or epigenetic systems, which in turn may affect other behaviors. A model describing a time scale of effects related to genetic and epigenetic mechanisms is provided by Rando and Verstrepen (2007; see Figure 1). Thus, a top-down approach like that used in artificial selection experiments is convenient for understanding behaviors resulting from complex developmental interactions.

![Epigenetic inheritance](image)

Figure 1. General time scales, in units of cellular generation, for the stability of phenotypes regulated by the indicated mechanisms. From Rando and Verstrepen (2007).

In terms of phenotypic variation, a relative high plasticity and transgenerational consistency would be the preferred characteristics leading to evolutionary novelties. Behavioral traits possess these characteristics and it would not be surprising if a relatively large amount of behavior were environmentally selected. In fact, the artificial selection technique, a form of selection, has been the source of much of the knowledge about the genetic basis of behavior and it proved to be surprisingly powerful in the study of behavior (Papini, 2002). A common sense idea about behavior is its great variability. However, the mechanisms generating behavioral variation have not been studied
extensively despite their importance for an understanding of behavior (Dewitte & Verguts, 1999; Staddon, 1973).

Behavior has been proposed to play a leading role in the evolutionary change (Gottlieb, 2002; Lickliter & Schneider, in press; West-Eberhard, 1989). A possible scenario involving feeding preferences was described by Wcislo (1989). Bees are a monophyletic group that arose from a wasp-like ancestor. Angiosperm plants evolved in the middle-lower Cretaceous, in a flourishing time for sphecid wasps. This environmental change provided the opportunity for variation in feeding preferences for the sphecid wasps, including plants rather than insects as food source. Thus, the original wasps that switched their diet to plants diversified and became ancestors to bees. As suggested by this scenario, the key role of behavior in evolution could be related to the relative flexibility of behavior compared to morphological characteristics and with the consistency of behaviors in similar situations (West-Eberhard, 1989). Consistent with epigenetic approaches, developmental plasticity was proposed as the mechanism underlying the importance of behavior in evolutionary change. The term summarized two evolutionary processes, the developmental reorganization of the ancestral phenotype and the genetic change in the regulation or form of a novel trait (West-Eberhard, 2002). One of the outcomes of developmental plasticity is phenotypic plasticity, defined as the ability of a genotype to exhibit different phenotypes in different environments (West-Eberhard, 1989). At a species level another outcome of development plasticity is the emergence of individual differences.

As noticed before, individual differences on behavior are closely related to epigenetic effects. Additionally, individual differences in a particular behavior could be
related to other behaviors. For instance, Dantzer et al. (1998) found that the susceptibility to show schedule-induced polydipsia with intermittent delivery of food pellets is related to avoidance behavior and defensive responses. Rats that exhibit higher levels of schedule-induced polydipsia display faster active avoidance learning in a 2-way shuttle-box and show less freezing behavior when confronted with an aggressive resident male. Additionally, Cordero, Kruyt, and Sandi (2003) reported that locomotor reactivity levels, measured in an open field test, and chronic stress by restraint are related to contextual fear conditioning. Specifically, fear conditioning was found to be enhanced in low reactive and chronically stressed animals, when compared to low reactive nonstressed rats. Thus, observed individual differences are a function of genetics, epigenetics, and other behaviors. As stated earlier, the artificial selection methodology is a convenient way to study these types of relationships.

**Artificial Selection for Emotional Traits**

Artificial selection involves the measurement of a particular phenotypic trait or combination of traits across generations followed by the selection of some percentage of individuals with bottom and top scores, and the breeding of animals with similar scores (Garland, 2003). In many studies, a control breeding line of randomly mated individuals is also maintained. Some advantages of this technique were already mentioned, such as the higher probability for the artificial selection behaviors related to individual differences, the multicausal approach, and the emphasis on the variation of behavior. However, some caveats should be considered when using this technique (Papini, 2002). First, some behaviors do not respond to selective breeding and such negative outcomes are additionally difficult to interpret. Second, artificial selection sometimes works for just
one of the breeding lines. Third, the interpretation of some artificially selected behaviors is not always easy given that the outcome may not be the one originally assumed for the selection procedure. Fourth, relaxing of the selection pressure could lead to a regression to the original level of behavior typically of the parental population. Fifth, there is a high probability that inbreeding would lead to deleterious effects, especially with small parental populations. A final caveat is related with the interpretation of the genetic basis for a behavior artificially selected. Some epigenetic mechanisms may still determine the outcome of artificial selection, as suggested by Meaney’s (2001) inter generational epigenetic effects on mothering styles discussed above.

The selection of varieties of animals for human use served as an analogy for evolutionary thinking. Early in Darwin’s theory (Darwin, 1872, 1883a, 1883b), a noteworthy importance was given to the role of behavioral traits on artificial selection procedures. For example, Darwin (1883a) suggested that the original fearless behavior of certain wild wolf-like dogs was a condition for the human domestication of dogs. This example is also interesting because it involves an emotional trait with clear consequences for social behavior.

Among the great variety of traits artificially selected, emotional traits are especially relevant to this proposal. Emotional traits can involve diverse configurations of behaviors related to aggression, anxiety, fear, mood, or stress, each one involving diverse biological mechanisms. Similar to simple morphological traits, emotional traits can be considered as inherited characteristics. However, an interpretation of emotionality as a complex set of factors, instead of a single generalized one, is suggested by the analysis of the relationship between different emotional behaviors (Archer, 1973). For example, the
interpretation of defecation as an exclusively emotional factor is problematic because competing responses, strain differences, the possibility of being used as a form of scent marking, and early stimulation can affect the behavioral outcome. As will be described next, complex behavioral and physiological configurations are involved in the artificial selection of a given emotional trait. Moreover, emotional components can be selected in selection procedures not originally thought to involved emotions.

A number of selective breeding studies have targeted emotional behaviors. Emotional behaviors can be organized in a continuum from conditioned to unconditioned emotional behaviors, although all emotional behaviors probably involve a mixture of both. According to this categorization, artificial selection for conditioned emotional behaviors will be described next, followed by unconditioned emotional behaviors, and by emotional outcomes resulting from artificial selection not directly aimed at emotional traits (see Table 2 for an overview of the strains reviewed).

**Artificial selection for conditioned behaviors**

Three emotional strains have been selected using conditioned behavior as the unit for selection. Interestingly, the tree strains are centered on aversive learning in the form of avoidance behavior or learned helplessness. One extensively studied emotional strain is the so-called Roman rats. In the original study, Bignami (1965) selected two strains of rats during five generations according to the number of avoidance responses, developing Roman high avoidance (RHA) and Roman low-avoidance (RLA) strains. Light was used as the discriminative stimulus and shock as the reinforcing stimulus. Bignami found a steady increase of the avoidance response over the first 4 generations in the RHA strain and a decrease, characterized by some fluctuations, in the RLA strain. Additionally,
crossfoster and reciprocal cross experiments were made on the 5th selected generation (S5). For crossfostering manipulations, offspring are removed from their biological parents at birth and raised by surrogates. For reciprocal cross manipulations, a male expressing the trait of interest is crossed with a female not expressing the trait and a female expressing the trait of interest is crossed with a male not expressing the trait.

Bignami found no differences between strains in the crossfoster study, and differences in the reciprocal comparison. These results suggested the possibility of prematernal, but not postmaternal effects.

<table>
<thead>
<tr>
<th>Strain denomination</th>
<th>Trait selected</th>
<th>Animal model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHA, RLA</td>
<td>Avoidance responses</td>
<td>Rat</td>
<td>Bignami (1965); Chamove and Sanders (1980); Gentsch et al. (1982); Guenaire et al. (1986)</td>
</tr>
<tr>
<td>Syracuse</td>
<td>Active two-way shuttle-box avoidance</td>
<td>Rat</td>
<td>Brush (2003); Brush et al. (1985); Brush et al. (1988)</td>
</tr>
<tr>
<td>Congenitally helpless</td>
<td>Susceptibility to learned helplessness</td>
<td>Rat</td>
<td>Shumake et al. (2004); Shumake et al. (2005); Vollmayr et al. (2004)</td>
</tr>
<tr>
<td>Maudsley</td>
<td>Open-field defecation</td>
<td>Rat</td>
<td>Abel (1991); Blizzard and Adams (2002); Broadhurst (1960); Overstreet, Rezvani, and Janowsky (1992); Powell and North-Jones (1973)</td>
</tr>
<tr>
<td>HAB, LAB</td>
<td>Time spent in the open arm of the elevated plus-maze</td>
<td>Rat</td>
<td>Henniger et al. (2000); Liebsch et al. (1998); Ohl et al. (2001)</td>
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<tr>
<td>High, low-USV</td>
<td>Rates of USVs in response to separation from their mother and littermates</td>
<td>Rat</td>
<td>Brunelli et al. (1997); Brunelli (2005a); Brunelli (2005b); Brunelli et al. (2006); Dichter et al. (1996); Zimmerberg et al. (2005)</td>
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<tr>
<td>50-kHz USV</td>
<td>Rates of 50kHz USVs in response to manual tickling</td>
<td>Rat</td>
<td>Burgdof et al. (2005); Pankseep and Burgdof (2000)</td>
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<td>NC-100, 900</td>
<td>Aggressive behavior in a dyadic test</td>
<td>Mice</td>
<td>Cairns et al. (1983); Gariety et al. (2001)</td>
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<td>SwS, Swr</td>
<td>Swim test after an uncontrollable electric tail-shock</td>
<td>Rat</td>
<td>Scott et al. (1996)</td>
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<tr>
<td>SwHi, SwLo</td>
<td>Activity in a swim test</td>
<td>Rat</td>
<td>Weiss, Cierpial, and West (1998)</td>
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<tr>
<td>HOFT, LOFT</td>
<td>Open field thigmotaxis</td>
<td>Mice</td>
<td>Leppi nen et al. (2006)</td>
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<td>Floripa</td>
<td>Locomotion in the central area of the open field test</td>
<td>Rat</td>
<td>Ramos et al. (2003)</td>
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<td>Tsukuba</td>
<td>Runway ambulation</td>
<td>Rat</td>
<td>Fujii et al. (1989); Iso et al. (1998); Wada and Makino (1997)</td>
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<tr>
<td>SHR, WKY</td>
<td>Blood pressure</td>
<td>Rat</td>
<td>Courvoisier et al. (1996); Gentsch et al. (1987); Kurtz and Morris (1987); Pare (1992)</td>
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</table>

Moreover, HRA and RLA strains showed differences in several behavioral tests.

Chamove and Sanders (1980) found faster acquisition of RHA in active avoidance and
active approach tasks, but lower in passive avoidance and the active approach extinction than in RLA rats. Moreover, more activity was found in an open field test in RHA than RLA rats. RHA rats showed less reactivity when they were crossed with Maudsley non reactive (MNR) rats, compared to RHA crossed with MNR rats. Additionally, Guenaire, Feghali, Senault, and Delacour (1986) reported lower behavioral performances for RLA rats than for RHA rats on acquisition of lever pressing using food as reinforcer, locomotor activity, and delayed reinforced alternation, whereas RLA rats showed a higher number of spontaneous alternations in a conditioned suppression task. These results suggest differences in acquisition, but not in conditionability, and a strong emotional component related in a complex way to activity.

Depending on the experimental situation, physiological differences have been reported by Gentsch, Lichtsteiner, Driscollt, and Feer (1982). RLA rats showed attenuated physiological reactivity to novelty, measured by plasma corticosterone, ACTH, prolactin, glucose, and by defecation scores. On the other hand, there were no differences in an unstressed situation and in three stressful situations (ether stress, inescapable foot shock, and immobilization).

An additional artificial selection study focused on avoidance behavior gave rise to the so-called Syracuse rat strains. The avoidance behavior was tested in two-way shuttle boxes, using electric shock as the reinforcer and a sound-light compound as the discriminative stimulus. Brush (2003) described the use of two criteria in order to select the Syracuse high- and low-avoidance strains (SHA/Bru and SLA/Bru, respectively). First, during a 10-trial pretest presenting just the discriminative stimulus, fewer than five short-latency (≤ 5 s) responses to the discriminative stimulus and three short-latency
responses during the last 5 trials of the pretest. Second, during the 60 trials training, either higher or lower avoidance responses in comparison to their generation mean. In the original study, clear differences in the median of avoidance responses between strains were reached by S5, with SHA/Bru rats showing higher scores than SLA/Bru rats. Because of a disease suffered by the rats, it was necessary to perform a procedure of fostering Caesarean-delivered litters, passing litters from the infected mothers to non infected new mothers. After this, artificial selection has continued until the present time.

The Syracuse strains have been tested in several behavioral measures. Brush et al. (1985) reported that SLA/Bru rats of both sexes showed higher defecation scores in an open field task than SHA/Bru rats, while their ambulatory performance was similar. Additionally, there were no differences in speed of escape or avoidance responding, and the two strains did not differ in absolute sensitivity to electric shock. Brush et al. (1988) reported that, in a conditioned suppression of bar pressing task with paired and unpaired CS and US presentations, SLA/Bru rats acquired conditioned suppression faster than SHA/Bru rats. Also, SLA/Bru rats showed better learning in a passive-avoidance task compared to SHA/Bru rats. Finally, SLA/Bru rats showed greater stress-induced suppression of drinking a weak quinine solution than SHA/Bru rats.

Brush (2003) also described other studies reporting additional behavioral differences, unrelated to the selection criterion, between the Syracuse strains. Two behavioral differences are related to SHA/Bru rats consuming more ethanol than SLA/Bru rats, and SLA/Bru rats showed stronger flavor, taste, and odor conditioned aversions than SHA/Bru rats.
A third artificial selection study targeting a learned behavior used rats that were selected in a learned helplessness paradigm, and the strain was called the congenitally helpless line (Vollmayr et al., 2004). One day after exposure to uncontrollable footshocks, rats were tested in an escape task in which a single lever press was enough to eliminate a footshock. In a total of 15 trials, the rats with more than 10 errors were considered helpless and rats with less than 5 errors were considered nonhelpless. The rats were crossed within the same range of performance and two lines emerged, the congenitally helpless line (cLH), which showed helpless behavior without experience with inescapable shocks, and the congenitally nonhelpless line (cNLH), which was resistant to the development of learned helplessness. Various behavioral and physiological tests have been performed to describe these particular strains. Vollmayr et al. (2004) found that cLH rats responded less to 7% sucrose than cNLH rats during operant training, as measured in terms of the finally achieved ratio at which the rats quit responding in a progressive ratio schedule. Moreover, cLH rats showed higher locomotor activity during the first 5-min interval in an open field test, while the lines did not showed differences in a water maze task. These results suggested that the differences were related to the emotional interpretation of the tasks and not to deficits in learning abilities.

Shumake, Barrett, and Gonzalez-Lima (2005) reported that cLH rats, when compared with a commercial strain, showed decreased drinking of a 5% sucrose solution, increased exploratory behavior, and decreased of fearfulness in a open field test, measured in terms of rearing, ambulatory distance, and thigmotaxis. Additionally, cLH rats were notoriously persistent during multiple extinction trials in freezing scores after the presentation of a loud tone in acquisition. Interestingly, in another study with
newborn rats, Shumake, Conejo-Jimenez, Gonzalez-Pardo, and Gonzalez-Lima (2004) reported differences in metabolism, using cytochrome oxidase histochemistry, in various brain regions including hypothalamus, hippocampus, anterior cingulate cortex, and medial orbitofrontal cortex. According to the authors, this particular pattern of metabolism suggests an altered control of the hypothalamic-pituitary-adrenal axis. Moreover, the possibility of epigenetic effects, such as maternal care and postnatal stress, could also explain these alterations.

**Artificial selection for unconditioned behaviors and physiological variables**

Several artificial selection studies have targeted unconditioned behaviors as the selection unit. A classic example of an extensive study of an emotional strain is the so-called Maudsley rats. The original study was developed by Broadhurst (1960), who selected the rats according to their defecation scores in the open-field test, yielding Maudsley reactive (MR) and Maudsley nonreactive (MNR) strains. The effect of selection was already clear on $S_2$. A difference in defecation scores was found, with higher scores for the MR rats than for the MNR rats. The selection effect was asymmetrical, with rats in the MR line performing in a similar way along generations, while MNR rats showed a progressive decline in their defecation scores. Another uneven effect was that the selection had a stronger effect in males than females in the MNR. Other measures were taken in Broadhurst’s study. For example, MNR rats showed higher ambulation scores than MR rats. Finally, in order to control for post- and prenatal maternal effects in the behavior selected, a crossfostering study and a cross breeding studies were performed. The results suggested that post- and prenatal effects were not important for the selection of defecation in the open-field test. Moreover, Powell and
North-Jones (1974) reported no differential effects of early handling over an avoidance behavior task.

Since the original study, various experiments have focused on the description of the behavioral and physiological profile of the Maudsley rats. Abel (1991) reported less activity for the MR rats in the open field, but more immobility in a forced swimming test, when compared to MNR rats. Abel also measured corticosteroid responses in the two tests, but found no strain differences. Overstreet, Rezvani, and Janowsky (1992) used three behavioral tasks to compare the Maudsley lines to Wistar controls. They found that the MR rats were more immobile in the forced swim test and spent less time in the open arms of an elevated-plus maze than MRN and Wistar rats. However, the three lines showed a similar performance in a two-way avoidance task. The differential performance of these strains could relate to various levels of emotionality or to selection for phenotypic traits unrelated to emotionality. For example, in a recent review, Blizard and Adams (2002) described that it has been difficult to demonstrate stable differences between the strains. Moreover, they described consistent differences in the ethanol consumption between the MR/Har and MNR/Har strains (the Har make reference to specific sublines of the strains brought to North America, maintained as separate strains thereafter).

In an interesting study, Imada (1972) compared the Roman and the Maudsley lines using the rate of water-drinking suppression by unsignaled and the signaled electric shocks. Compared to the average of the last two acquisition trials for each strain, the MNR and RHA strains showed, in general, a similar performance and less suppression of drinking behavior by unsignaled shocks than the MR and RLA strains, which behave in a
similar fashion as well. In spite of the higher suppression of drinking of the RLA line when compared to the RHA, no differences were found in the defecation scores between these lines. Finally, the RLA strain had a lower acquisition when the shock was signaled, suggesting poorer conditionability of the RLA strain.

Because the elevated plus-maze is a widely used test for unconditioned anxiety and rats tend to show clear individual differences in this task (Liebsch, Montkowski, Holsboer, & Landgraf, 1998), it was thought as a potential model for trait anxiety (Landgraf, 2003). Liebsch et al. (1998) developed two breeding lines for 6 generations, high anxiety-related behavior (HAB) and low anxiety-related (LAB) lines, selecting from extreme behavioral performance in the elevated plus-maze task, particularly maximum and minimum time spent in the open arms of the maze. In general, they found that LAB rats of both sexes showed higher open-arm and closed-arm entries than HAB rats, while the latency to the first open-arm entry did not show differences. Also, other anxiety-related tasks were compared between the lines. In the open field test, LAB rats showed a higher performance in the parameters indicating anxiety, such as number of entries, time spent, and time traveled on the central zone of the field; moreover, LAB rats showed a significantly higher total distance traveled in the field, a measure related to locomotor activity. In a forced-swim test differences in performance were found between the LAB and HAB lines in struggling, swimming, and floating. Finally, differences in performance were not found in a social discrimination task.

Additional studies (Henniger et al., 2000) reported differential performance in conflict situations. The LAB line, compared with the HAB line, showed higher number of entries to the white side of a black-white box test, while the females of the HAB line
showed less time in active social interaction than LAB females. Moreover, the
performance of the breeding lines in a modified hole-board task was different, with HAB
rats spending less time in the unprotected area of the experimental box, the hole, than
LAB rats (Ohl et al., 2001).

Ultrasonic vocalizations (USVs) in young rats have been widely used in artificial
selection studies. Rats show different USVs during positive and negative emotional
situations, such as tickling and isolation from the mother respectively (Burdgof et al.,
2005). For instance, a negative emotional situation such as isolation of rat pups for 2 min
in the form of separation from the mother and littermates is followed by 22-kHz USVs.
Differences in USVs between rat strains selected for an emotional behavior together with
intra- and interlitter individual differences in rat pups suggested the possibility of a
genetic component for USV behavior following isolation (Brunelli, 2005a). Brunelli,
Vinocur, Soo-Hoo, and Hofer (1997) selectively bred rats using their rates of USV in
response to separation from their mother and littermates at PND 10. They artificially
selected three lines of high, low, and random USV response rates that consistently
showed differential rates of USV by S5.

Selection for different rates of juvenile USV was related to differences in some
emotional behaviors in adulthood. Dichter, Brunelli, and Hofer (1996) tested the S3 of the
USV lines in the elevated plus-maze test. The low line spent more time in the open arms
than the random and high lines and produced a lower rate of head dips from a protected
arm. Moreover, the high USV line showed an increase in heart rate in reaction to stress
(Brunelli, 2005a). These results suggested that the low USV line showed decreased
anxiety compared to the high or random lines. Additionally, the low line showed a less
reactive style of behavior when it was tested in behavioral tasks such as the open field and the forced swimming task (Zimmerberg, Brunelli, Fluty, & Frye, 2005).

Interestingly, the selection for USV lines seemed to be related to developmental effects. Brunelli (2005b) reported regulation of postnatal development of USV in S15. The high USV line showed higher USV scores at PNDs 3, 7, 10, 14, and 18 than the low and random USV lines, and the low USV line showed constant lower scores in the same days than the high and random lines. Additionally, both high and low USV lines showed disrupted juvenile play behavior, when compared to the random line (Brunelli et al., 2006).

A second example of USV strain involves a positive emotional condition. Pankseep and Burgdof (2000) showed that manual tickling of isolated rats has rewarding properties in instrumental, Pavlovian, and avoidance tasks. Moreover, they bred in 4 generations two lines of rats that exhibited, respectively, higher and lower rates of 50 kHz USVs in response to manual tickling than the random line. Interestingly, the high 50 kHz line also showed a higher preference for the tickling compared to the other two lines. Finally, Burgdof et al. (2005) partially replicated the breeding lines, finding differences between the high 50-kHz USV line vs. the low and random lines, but similar performance between the low vs. random lines. The low 50-kHz USV line also showed a higher number of distress calls than the high and random lines.

Focusing on another kind of emotion, Cairns, MacCombie, and Hood (1983) selectively bred mice according to their aggressive behavior at PNDs 45 to 70. They selected three breeding lines, low (NC-100), control (NC-500), and high (NC-900), using a dyadic test measure of frequency and latency of attacks. By the S3, NC-100 mice
showed lower scores in frequency attacks compared to both NC-500 and NC-900 mice, while NC-500 and NC-900 mice showed no differences. Moreover, NC-900 mice did not show differences when compared to the S₀ (i.e., the parental population). Some mice in the S₃ were tested in a crossfostering study in which no differences were found between the breeding lines. These results supported the proposal of genetic differences related with this artificial selection outcome. Interestingly, the authors performed developmental and repeated dyadic tests, allowing the analysis of the interaction between genetic and developmental factors. When mice were exposed to a dyadic test measure repeatedly, the scores of NC-100 and NC-900 lines converged in the second test at PNDs 72 and 235. In another test, mouse pairs were tested for the first time at PNDs 72 and 235, and differences between the NC-100 and NC-900 lines were reported. Finally, dyadic tests at PND 28 showed no differences between the lines. These results suggested an interaction between genetic, developmental, and experience factors in aggressive behavior. Experience with the test plus the developmental profile of the lines were sufficient to eliminate the effects of selective breeding.

In an additional study, Gariepy, Bauer, and Cairns (2001) tested the hypothesis of heterochrony in aggressive behavior for the NC lines. Heterochrony refers to a change in the timing of developmental events in descendants, relative to their ancestors, that can lead to changes in phenotypic characteristics. They used mice from the S₁, S₄, and S₁₃ selected according to the frequency of attacks initiated by males at PND 45 and found that the NC-100 line showed lower attack frequency in the S₁₃ than the NC-900 line. Additionally, the level of attacks of the NC-100 was similar to the level shown by young rats of earlier generations. A second experiment showed that the low level of response
presented by the NC-100 mice was preserved despite repeated testing. These results suggest a developmental change in the time of emergence of attack frequencies for NC-100 mice across generations.

Activity in a swimming test is another behavior susceptible to be selectively bred. Scott, Cierpial, Kilts, and Weiss (1996) exposed rats to a 15-min swimming test 90 min after an uncontrollable electric tail-shock procedure. Using this procedure during 5 generations, they were able to select two lines in which the males differed in swimming activity. The swimming-test-susceptible (SwS) line showed lower scores in struggling when compared to both the S0 and the swimming-test-resistant (SwR) line. However, the SwR did not show differences when compared to the S0. Additionally, SwR rats showed a decrease in home cage ambulatory activity and water intake after exposure to shock, compared to the behavior of SwS rats. Additionally, differential physiological activity in some noradrenergic and dopaminergic areas was found.

Another artificial selection study on swimming activity was published by Weiss, Cierpial, and West (1998). They selected two lines according to their high (SwHi) or low (SwLo) activity in a 15-min swimming test and an additional random-bred line. The SwHi line was selected by a combination of high struggling and low floating times, while the SwLo line was selected by a combination of low struggling and high floating times. The rats in each generation were tested around PNDs 90-120. In a fast and robust selection process, SwHi rats showed a higher floating time and a lower struggling time than both the LwHi and random lines since the S1 and thereafter until the S18. Additionally, by the S18 the struggling and floating performance of the SwHi and LwHi lines did not overlap. Additional behavioral tests were performed for different
generations. In the S_{13}, SwHi rats showed more ambulatory activity for the first 20 min of a 60-min test in a novel situation similar to the home cage. SwHi rats of the S_{14} showed higher daily ambulatory activity scores in the home cage and higher defecation scores in the activity test than SwLo rats, while SwLo rats scored higher in rearing and visited squares in the activity test than SwHi rats.

In addition to the Maudsley rats, the open field test has been used for various artificial selection studies aiming at different behaviors. Leppänen et al. (2006) artificially selected mice for thigmotaxis in the open field procedure. The breeding lines were selected according to higher (HOFT) or lower (LOFT) open field thigmotactic ratios. These ratios were calculated by dividing the number of inner partitions entered by the total sum of units visited by the mice. Exposure to the open field was given in a 2-min daily trial during 5 days and the selection was based on the thigmotactic ratio of the 5^{th} day. All testing took place around the second month of age across all 23 generations. Differences in the thigmotactic ratio between the HOFT and LOFT breeding lines were found since the S_{3}. LOFT mice showed a lower tendency to keep close to the open field wall than HOFT mice. Those differences were maintained during all generations, except S_{12}. Other behaviors were also measured in the study. Differences in rearing scores of the lines were found after the S_{12}, as LOFT mice showed a higher tendency to rear. Finally, the ambulation scores did not showed differences between lines.

Another selection study using the open field was reported by Ramos et al. (2003). In this study, the S_{0} was the product of the cross between various commercial bred lines in order to maximize genetic variability. Rats from this generation were tested once in an open field procedure, selecting the animals with the higher and lower scores for central
locomotion. In the S2 and S4, the two selected lines showed differences in central locomotion in the open field test. The Floripa H line showed higher levels of central locomotion than the Floripa L. In addition, differences between lines were found in peripheral locomotion in the S4 but not in defecation. Additionally, rats in the Floripa H line showed more time spent and higher number of entries in the open arms of the elevated-plus maze. Finally, Floripa H rats spent more time in the white compartment of a black and white box.

The Tsukuba strains were developed according to the ambulation scores of rats traveling across a brightly lit runway, leading to a strain with low ambulation scores, the Tsukuba high emotional (THE) line, and a strain with high ambulation scores, the Tsukuba low emotional (TLE) line (Fujii et al., 1989). Iso, Brush, Fujii, and Shimazaki (1988) compared various strains, including the THE and TLE lines. They reported a higher response level of TLE rats in a multiple active-passive avoidance schedule, compared to THE rats. Moreover, Wada and Makino (1997) reported that THE rats showed higher defensive burying and more immobility than TLE rats. However, Fujii, Asada, Takata, Yamano, and Imada (1989) found no differences between the two strains using either a suppression of licking or suppression of lever pressing by unsignaled shocks.

The next strain was selected using a physiological variable as the criterion for selection. Spontaneously hypertensive rats (SHR) were a breed line originally created as an animal model for hypertension and Wistar-Kyoto rats (WKY), with normal ranges of blood pressure, were bred as a control line for the SHR (Kurtz & Morris, 1987). Interestingly, changes in some emotional-related behaviors have been reported as a by-
product of the breeding for hypertension. For example, Gentsch, Lichtsteiner, and Feer (1987) reported various behavioral differences in the open field and elevated plus-maze tests. In relation with the open field test, SHR rats presented a higher number of crossings and rearings, than WKY rats, and a lower number of defecations. Behavior in the elevated plus-maze was characterized by higher numbers of rearings and enters in the open arms (measured as a ratio between open entries over total number of entries in open and close arms) in SHR rats than in WKY. These interstrain comparisons suggested a higher reactivity of WKY rats to aversive environments. Such suggestion is also supported by studies on other emotional tasks such as the defensive withdrawal test and the conditioned defensive burying test comparing the performance of WKY and Fisher-344 rats (Pare, 1992).

Centering on the behavioral profile of WKY rats, the strain was compared to a strain developed from the crossing of WKY and SHR. The new strain was called Wistar-Kyoto-Hyperactive (WKHA), selected to have a normal blood pressure line of rats combined with the hyperactivity of SHR rats (Courvoisier et al., 1996). Various behavioral and physiological measures were obtained in WKY and WKHA rats. Concerning the behavioral measures, WKHA rats were more active than WKY rats in a novel open field environment; WKHA rats were more active in the central area of the open field and defecated less than WKY rats. Additionally, WKHA rats made more entries and spent more time in the open arms of the elevated plus-maze test than WKY rats. After a 10-min exposure to a novel environment, WKHA rats displayed lower levels of corticosterone, prolactin, and rennin than WKY rats. Once again, WKY rats seem to show a strong reactivity when they are exposed to stressful situations.
Individual Differences in Recovery from cSNC

An additional emotional phenomenon studied through various levels of analysis, including artificial selection studies, involves the consequences of surprising reward loss. cSNC is a model of loss-induced anxiety that involves the direct consumption of an appetitive reinforcer (Papini, Wood, Daniel, & Norris, 2006). As described above, the cSNC procedure implies the use of different sucrose solutions for two groups. During the preshift phase, the unshifted group receives a 4% sucrose solution during 10 daily trials, each 5-min long, and the downshifted group receives a similar training except with a 32% sucrose solution. During the postshift phase, the unshifted group keeps receiving the 4% solution, but the downshifted receives a 4% sucrose solution during 5 trials. A suppression of consummatory behavior by the downshifted group below the levels shown by the unshifted group is seen following the first downshift trial, trial 11. During trials 12 to 15, a gradual recovery of consummatory behavior in the downshifted animals brings behavior to the levels of the unshifted controls.

The suppression of behavior and subsequent recovery during the postshift phase can be explained following Flaherty’s (1996) multistage model of cSNC (Figure 2). According to this explanation, two general behavioral stages are related to reward downshift. The first stage, which happens mainly during trial 11 during the cSNC situation, includes the detection of the discrepancy between the expected and received rewards, which in turn triggers the rejection of the received reward (4% sucrose) and the search for the previous reward (32% sucrose). The second stage, from trial 12, includes a
behavioral conflict between approaching the new reward, but avoiding the downshifted solution, and also a subsequent recovery of consummatory behavior.

Interestingly, Amsel’s (1992) frustration theory can be thought of as supplementary to Flaherty’s multistage model, making some of the components of the model more explicit. The rejection of the new reward can be seen as an emotional reaction following the violation of reward expectancy by the presentation of a smaller reward than expected. Thus, reward loss would elicit an aversive internal emotional state, called primary frustration, leading to the suppression of consummatory behavior on trial 11. During subsequent trials, the behavioral conflict emerges because stimuli present in the rejection situation are paired with the aversive internal state, allowing the formation of a memory of primary frustration that results in an aversive anticipatory state, called secondary frustration, that comes to control behavior. However, secondary frustration coexists with the tendency to approach and consume the 4% solution. This generates an approach-avoidance conflict that results in response competition between the tendency to approach and the tendency to avoid the sipper tube. After trial 12, during the recovery stage, secondary frustration begins to be counterconditioned by its pairings with the 4% sucrose solution reward, resulting in the recovery to levels similar to the preshift of the consummatory behavior.

Support for the distinction between these mechanisms has been provided by the manipulation of selective neurochemical systems. For instance, benzodiazepines affect behavior selectively on trial 12 (Flaherty, Clark, & Coppotelli, 1996; Flaherty, Grigson, & Rowan, 1986). Additionally, corticosterone administered after trial 11 enhanced the cSNC on the next trial (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006). On the other
hand, differential opioid modulation has been reported for trials 11 and 12. Morphine and
naloxone, nonspecific opioid receptor agonist and antagonist, respectively, affect both
However, DPDPE and naltrindole, agonist and antagonist specific to the δ-opioid
receptor, respectively, affect trial 11, but not 12. Lastly, U-50,488H, a specific κ-opioid
receptor agonist, affects trial 12, but not 11 (Wood, Norris, Daniel, & Papini, in
preparation).

\[ RI = \frac{\text{Trial 12 - Trial 11}}{\text{Trial 15 - Trial 11}} \]

*Figure 2.* Scheme of the multistage hypothesis for cSNC proposed by Flaherty.

Of special interest in the framework of this proposal is information on individual
differences for recovery from cSNC. From data recollected from the control groups of
various cSNC experiments, a rate was calculated individually according to the following
recovery index (RI):

\[ RI = \frac{\text{Trial 12 - Trial 11}}{\text{Trial 15 - Trial 11}} \]
Each term in this ratio refers to the cumulative contact time (s) for the appropriate trial. Thus, this ratio represents the proportion of total amount of recovery (trial 15-trial 11) that corresponds to the change in consummatory behavior observed between trials 11 and 12, the initial two trials after the downshift. A RI=1 would indicate that all the recovery occurred between trials 11 and 12. When 0<RI<1, then the animal shows partial recovery from the downshift during the initial two trials. This is the usual pattern. Finally, when RI<0 (i.e., negative) the behavior shows deterioration after de downshift. As seen in Figure 3, individual variation ranging from -3 to 3 in the RI was observed in a sample of 90 rats from several experiments (n=90), which suggests that there is enough room for selection for rats with high or low RI.

Figure 3. Distribution of the percentage of the frequencies of RIs for males and females. The absolute scores are goal tracking times (s). Each value in the abcissa makes a boundary for a RI score. (Data from control groups of various cSNC experiments)

Variability in recovery from incentive downshift is related to opioid sensitivity. In one study, Pellegrini, Wood, Daniel, and Papini (2005), calculated the difference scores for the rats after downshift training (i.e., goal-tracking times on trial 12 minus trial 11).
Then, they segregated the rats according to the higher and lower difference scores, and culled the highest score for the low group and the lowest score for the high groups. Following this, the rats were assigned to one of four conditions: high recovery/naloxone, high recovery/vehicle, low recovery/naloxone, and low recovery/vehicle. Ten days after the ending of downshift training and under free food, rats received a single activity test trial for 15 min, after naloxone or vehicle administration 15 min prior to testing. Interestingly, the decrease in activity was enhanced by naloxone in the low recovery group, but not in the high recovery group. The former result supports the hypothesis that individual differences in recovery from incentive downshift reflect differences in opioid function.

As suggested before, genetic and epigenetic mechanisms can underlie individual differences within a particular species. Then, a question can be raised as to what are the bases for suggesting that the individual differences found in the recovery from incentive downshift are trans-generational? There are four sources of evidence for trans-generational effects, genetic and epigenetic, on recovery from reward loss. Two of them were already described and the remaining ones will be addressed next.

1. Individual differences.
2. Artificial selection for emotional-related traits.
3. Differences between rat strains in reward loss.
4. A genetic component suggested for cSNC.

**Strain differences in reward loss and artificial selection for cSNC**

A number of performance differences between rat strains have been reported in the cSNC paradigm. Flaherty and Rowan (1991) tested MR/Har and MNR/Har rats in
various contrast procedures. In the cSNC procedure, they found that the two strains did not differ in the sucrose drinking behavior in the last preshift trials. However, during the postshift phase, MNR/Har rats showed a larger contrast effect in the postshift trial 11 than MR/Har rats. However, the administration of the benzodiazepine anxiolytic CDP 30 min before postshift trial 12 had no effect on cSNC.

In another cSNC strain comparison, Flaherty and Rowan (1989) compared the performance of the Syracuse strains. During the last preshift trial, trial 10, there was a difference between the strains: SLA rats drank more for the 4% sucrose solution than SHA rats, while the two strains drank similar amounts of the 32% sucrose solution. In the first postshift trial, trial 11, the SLA rats showed a larger contrast effect than the SHA rats, and when CDP was administered on trial 12 there was an alleviation of cSNC for SLA rats, but not for the SHA rats.

Freet et al. (2006) compared Lewis and Fisher rats in cSNC. Lewis rats showed a higher rate of drinking during the preshift phase when compared to Fischer rats. During the postshift phase, both strains showed a decrease in responding after the sucrose shift during the postshift trial 11, but Lewis rats showed a greater contrast effect than Fischer rats. Before the postshift trial 12 the anxiolytic CDP was administered, attenuating contrast in the Fischer rats, but failing to attenuate contrast in the Lewis rats. Interestingly, CDP had different effects on the recovery from reward. Lewis rats in the downshifted group showed an extended contrast effect when CDP was administered, compared to the Lewis saline group. For Fischer rats in the downshifted group, CDP administration was related to a transient recovery from contrast. Additionally, in a second
experiment, Fischer, but not Lewis, rats showed CDP-induced appetitive stimulating effects on drinking behavior of a sucrose solution.

Finally, in a different SNC procedure involving a downshift from a 30 s to 1 s of time in the safe compartment in a one-way avoidance procedure, Torres et al. (2005) reported similar acquisition during the preshift phase for both strains of Roman female rats, but a greater impairment of the avoidance responses for RLA female rats than for RHA female rats after the postshift from 30 to 1 s at the safe compartment.

The fourth source of evidence for transgenerational effects for recovery from incentive downshift comes from the already introduced artificial selection for cSNC study in which the selection criterion was the ratio of lick frequency in the first postshift trial divided by the lick frequency in the last preshift trial (Flaherty et al., 1994). This ratio was proposed to capture the intensity of the initial reaction to the 32% to 4% sucrose downshift. The study was followed until the S7. By the end of this study, there were significant differences in the size of the cSNC effect across the H and L contrast strains. An assessment of the response to selection, however, indicated that whereas there was a significant change in the selection for a small cSNC effect, selection for a large effect was not evident. Moreover, the two lines did not differ in open-field defecation, anticipatory contrast, or in the reward value of sucrose or cocaine. Because the distinction between the three frustration mechanisms involved in the cSNC situation, these results provide a different kind of information from that sought in the present proposal, which focuses on the recovery from reward loss theoretically related to the counterconditioning mechanism.
Methods

The artificial selection study was completed for three selected generations (S₁ – S₃). As described in Table 3, three measures were taken during all generations, cSNC, body weight, and water consumption during three days prior to testing. Supplementary behavioral tests, including sucrose sensitivity, activity, and autoshaping, were applied only to the last generation, S₃.

Subjects. Sixteen Long-Evans rats served as the parental population. Twelve rats from this group, 6 males and 6 females, were chosen as the starting population (S₀). The animals were derived from the TCU vivarium. The rearing protocol used for the starting population was used with all following generations (see Table 3). Following this protocol, after 2 weeks of mating, the male is separated and the female is left alone. Females are maintained in polycarbonate tubs. All pups born to each female are maintained. The pups are weaned around PND 21, and placed in groups of 2-3 individuals in polycarbonate tubs for about 10 more days, receiving food and water ad libitum. Around PND 40, juveniles were switched to a new room and individually housed in metal wire-bottom cages with food, water ad libitum, and a rodent retreat as an enrichment device. These retreats were 15 cm long, 9 cm high, and 9 cm wide, made of dark red plexigas sheets, and placed inside the home cage. They provided a smooth surface and cover. cSNC testing started when the younger litter of the generation reached PND 90. During all phases, the animals were under a 12 h light/12 h dark schedule, in noiseless rooms with constant room temperature (72-74 °F) and humidity between 50 and 65%. They were fed with standard laboratory food.
Apparatus. cSNC training was conducted in 4 conditioning boxes (MED
Associates, VT) made of aluminum and Plexiglas (29.3 cm × 21.3 cm × 26.8 cm,
L×H×W). The floor consists of steel rods running parallel to the feeder wall. A tray with
corncob bedding is placed below the floor to collect feces and urine. In the feeder wall is
a hole 1 cm wide, 2 cm high, and 4 cm from the floor through which a sipper tube, 1 cm
in diameter, is inserted. When fully inserted, the sipper tube is flush against the wall.
Diffuse light is provided by a house light located in the center of the box’s ceiling.
Finally, a computer located in an adjacent room controls the presentation and retraction
of the sipper tube. When the rats make contact with the sipper tube, a circuit involving
the steel rods in the floor is closed and the signal is recorded by the computer. This
provides a measure of cumulative contact called goal-tracking time (in 0.05-s units). Each
conditioning box was placed in a sound-attenuating chamber that contained a speaker to
deliver white noise and a fan for ventilation. Together, the speaker and fan produced
noise with an intensity of 80.1 dB.

Table 3.
Schedule Detailing the Rearing and Behavioral Testing Specific to the Generations to be
Artificially Selected.

<table>
<thead>
<tr>
<th>Test/Activity</th>
<th>PND (Approx.)</th>
<th>Duration/ frequency</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning and group</td>
<td>21</td>
<td></td>
<td>S₀ – S₃</td>
</tr>
<tr>
<td>housing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individually housing</td>
<td>40</td>
<td></td>
<td>S₀ – S₃</td>
</tr>
<tr>
<td>Body weight</td>
<td>40</td>
<td>Every 3 days</td>
<td>S₀ – S₃</td>
</tr>
<tr>
<td>Daily water intake</td>
<td>60</td>
<td>3 days</td>
<td>S₁ – S₃</td>
</tr>
<tr>
<td>Food deprivation</td>
<td>90</td>
<td>7 days</td>
<td>S₀ – S₃</td>
</tr>
<tr>
<td>cSNC</td>
<td>98</td>
<td>15 days</td>
<td>S₀ – S₃</td>
</tr>
<tr>
<td>Sucrose sensitivity</td>
<td>115</td>
<td>3 days</td>
<td>S₁</td>
</tr>
<tr>
<td>Open-field activity</td>
<td>120</td>
<td>1 day</td>
<td>S₁</td>
</tr>
<tr>
<td>Autosholping</td>
<td>124</td>
<td>16 days</td>
<td>S₁</td>
</tr>
</tbody>
</table>
**Procedures.**

*Selective Breeding.* Three generations ($S_1 - S_3$) were selected according to the rate of recovery from incentive downshift. This rate was calculated individually according to the RI described above. This measure describes the rate of recovery in terms of the proportion of recovery in trial 12 compared to trial 11, when an important part of the recovery occurs, relative to the proportion of recovery in trial 15 compared to trial 11, when usually consummatory behavior reaches the level of unshifted controls.

Starting with $S_0$ rats (the parental population), two pairs of rats were chosen randomly to develop the random recovery (R) line. After those rats were selected, the rats with the highest scores in the R will be assigned to the high recovery (H) line, while the rats with the lowest R will be assigned to the low recovery (L) line. The pairing of breeders for subsequent generations followed a similar procedure, using the extreme low values for rats in the L line, and the extreme high values for the H line. Breeders in the R line will be chosen randomly. The pairing was done trying to avoid inbreeding as much as possible; however, inbreeding effects are possible given the low number of animals in the parental population $S_0$. Therefore, this proposal must be considered as a preliminary study to determine the viability of the selection protocol.

*csNc testing.* Rats were randomly assigned to two groups, matched by sex, weight, and water consumption. The downshifted group received 5 min of daily access to a 32% sucrose solution, prepared (w/w) by mixing 32 g of commercial sugar for every 68 g of distilled water. The unshifted group received a similar treatment, except that they had access to the 4% sucrose solution throughout training. Trials 11 to 15 were the
postshift trials, which consisted of 5 min daily access to 4% sucrose for both the unshifted and the shifted groups. Rats were trained in squads of four, which remained constant during the experiment. The order of squads varied across days.

A battery of different behavioral and physiological tests were used in order to address the issues about locus of action of the selection and potential pleiotropic effects related to the artificial selection procedure (see Table 3). These tests assessed the potential of correlated changes in behaviors not directly related to cSNC. Correlated phenotypic changes occur because of pleiotropy, a process observed when a gene affects the development of multiple phenotypic traits. In addition, allelic frequencies change across generations, new combinations of genes can potentially affect nonselected traits.

**Body Weight.** Rats were weighed 3 times/week starting at about PND 40. This assessed potential strain differences in growth rates.

**Water Consumption.** During 3 days, from PND 60-63, the total amount of water consumed by each rat during the entire day was measured. This assessed possible changes in water consumption resulting from the selective breeding.

**Sensitivity for Sucrose Solutions.** After cSNC testing, during 3 days, a 24-h, two bottle tests were administered using 0.125, 0.5, and 1.0 g/ml sucrose solutions (the second bottle will be distilled water). The order of the sucrose concentrations administered across the three days was counterbalanced across subjects. Bottles were be presented in the home cage. This test was scheduled after cSNC testing to avoid any potential interactions between the concentrations of sucrose used here and those used in the main consummatory testing. (For a full description of the procedure, see Dess, 2000.) This test assessed the extent of changes on sucrose sensitivity across strains.
Activity. Differences in activity behavior may emerge, as suggested by other selective breeding studies (Stohr et al., 1998). To test for this possibility, four open field chambers were used (Med Associates, Georgia, VT). The dimensions of each chamber are 43 cm wide, 43 cm long, and 30 cm high. General locomotor activity was automatically recorded in 3-min bins during one 20-min trial in all rats. At the start of the trial, each rat was placed in the center of the chamber. The chamber was cleaned immediately after each trial.

Autoshaping. Four standard conditioned boxes were used, each enclosed in a sound-attenuating cubicle. The dimensions of each chamber are 20.1 cm wide, 28 cm long, and 20.5 cm high. The floor consists 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 the pellets (45-mg Noyes 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 of distance to the left side of the magazine. Insertion or retraction of the lever took 0.2 s. A light bulb (GE 1820) attached to the ceiling of the chamber was positioned at the opposite side of the magazine, and it provided diffuse illumination to the chamber. Each conditioning box was placed in a sound-attenuating chamber that contained a speaker to deliver white noise and a fan for ventilation.

Training took place in two phases, acquisition and extinction. The acquisition phase consisted of 10 daily sessions. Each session started with the onset of the house light and ended when the house light is turned off. Within each session 10 training trials were presented, separated by a variable intertrial interval with a mean of 90 s (range: 60-120
s). Each trial began with the insertion of the retractable lever for 10 s. A computer recorded lever-contact responses while the level is inserted in the chamber. The lever was then retracted at the end of the 10 s, and 5 pellets were delivered on the magazine cup at a rate of one pellet per 0.2 s. Each rat consumed a total of 50 45-mg food pellets per session. In the extinction phase the training conditions were the same for 5 additional sessions, except that food delivery was withheld.

Results

cSNC within generations by strain.

cSNC performance was evaluated for each generation and strain independently. Sex was incorporated as a factor in the analyses of variance (ANOVAs), but the data was represented pooling males and females in the figures. This was done to increase the sample size and because in many cases there were nonsignificant sex differences. For the data reported in this section, acquisition of drinking behavior during the preshift phase was evaluated using a 2 X 2 X 10 (Contrast X Sex X Trial) ANOVA. Similarly, drinking behavior during the postshift phase was evaluated using a 2 X 2 X 5 (Contrast X Sex X Trial) ANOVA. In addition, the number of trials without recovery from cSNC during the postshift phase was calculated using independent one-way ANOVA for each of the postshift trials followed by post-hoc LSD pairwise tests. Differences in these post-hoc tests with a statistical p value smaller than 0.05 were marked with an asterisk in the figures.

$S_0$. As shown in Figure 4, rats in the parental generation showed a pattern of acquisition characterized by an increase in consummatory behavior, a similar preshift
performance for unshifted and downshifted groups, and differences between males and females. This is shown by the main effect of trial (F(9, 261) = 49.24, p < 0.01), a sex by trial interaction (F(9, 261) = 2.45, p < 0.01), a nonsignificant interaction of trial by contrast (F < 1), and a nonsignificant interaction of trial by sex by contrast (F(9, 261) = 1.70, p > 0.09). Additionally, a significant sex effect (F(1, 29) = 4.78, p > 0.04), and nonsignificant group effect (F < 1) and sex by contrast interaction (F < 1). In this and other cases, the nonsignificance of sex-related effects is probable related to sexual dimorphism and its interaction with fluid consumption.

![Figure 4](image_url)  
**Figure 4.** Mean (± SEM) goal-tracking time for S0. Significant differences in independent one-way ANOVA analysis during recovery from cSNC are marked with asterisks.

During the postshift phase, the downshifted group showed significant cSNC relative to the unshifted group, as shown by a main effect of trial (F(4, 116) = 13.18, p < 0.01), a nonsignificant sex effect (F(4, 116) = 1.123, p > 0.349), a significant interaction of trial by contrast (F(4, 116) = 11.38, p < 0.01), and a significant interaction of trial by sex by contrast (F(4, 116) = 3.92, p < 0.01). Moreover, there was a nonsignificant sex
effect (F(1, 29) = 1.307, p > 0.262), a significant group effect (F(1, 29) = 9.87, p < 0.01),
and a nonsignificant sex by contrast interaction (F < 1). Further independent one-way
ANOVA of the recovery phase revealed significant group effects on trials 11 (F(1, 32) =
35.79, p < 0.01), 12 (F(1, 32) = 7.85, p < 0.01), and 13 (F(1, 32) = 7.65, p < 0.01), but
nonsignificant effects for trials 13 to 15 (Fs < 3.32, ps > 0.08).

In short, acquisition performance was similar for unshifted and downshifted
groups, but differences between males and females emerged. Also, males and females
showed comparable cSNC effects, as shown by the suppression of drinking behavior after
the sucrose downshift. Finally, the suppression of consummatory behavior lasted three
trials, as measured in terms of trial-by-trial comparisons.

S1. The cSNC performance for all strains in this generation is shown in Figure 5. Two pairs of rats from the S0 per strain were paired, with all of them producing litters. The number of rats for the S1 was: 6 males and 12 females for the L strain, 8 males and 6 females for the H strain, and 6 males and 5 females for the R strain.

Low strain. Drinking behavior in terms of goal-tracking times for rats in the L
strain is shown in Figure 5a. Unshifted and downshifted males and females showed
similar levels of acquisition of consummatory behavior during the preshift phase, as
shown by a main effect of trial (F(9, 126) = 30.39, p < 0.01), a nonsignificant interaction
of trial by sex (F(9, 126) = 1.28, p > 0.26), a significant trial by contrast interaction (F(9,
126) = 2.028, p < 0.04), a nonsignificant trial by sex by contrast (F(9, 126) = 1.09, p >
0.38), and nonsignificant group effects and group interactions (Fs < 1.75, ps > 0.21).

The downshifted group showed a significant cSNC effect relative to the unshifted
group, as supported by a significant trial effect (F(4, 56) = 5.76, p < 0.01), nonsignificant
interactions for trial by sex, trial by contrast, trial by sex by contrast (Fs < 2.38, ps > 0.06), but significant group effects for trial (F(1, 14) = 15.37, p < 0.01), sex (F(1, 14) = 6.95, p < 0.02), and a nonsignificant trial by sex interaction (F < 1).

*High strain.* Figure 5b shows the acquisition of consummatory behavior for this strain. A similar preshift performance for unshifted and downshifted groups is shown by the main effect of trial (F(9, 99) = 14.69, p < 0.01), nonsignificant interactions for sex by contrast, trial by contrast, and trial by sex by contrast (Fs < 1.03, ps > 0.42), and additional nonsignificant group effects for trial, sex and sex by trial interaction (Fs < 1.29, ps > 0.28).

There was a significant cSNC effect of the downshifted group during the postshift phase, relative to the unshifted group, as supported by a trial effect (F(4, 44) = 7.66, p < 0.05), and a trial by contrast interaction (F(4, 44) = 5.38, p < 0.01), but nonsignificant interactions of trial by sex, and trial by sex by contrast (Fs < 1.43, ps > 0.24). Moreover, a significant group effect (F(1, 11) = 12.54, p < 0.01), but no sex effect or group by sex interactions were found (Fs < 1).

*Random strain.* Acquisition during the preshift is shown in Figure 5c. Drinking behavior during the preshift was similar for unshifted and downshifted groups, but no for males and females, as portrayed by the significant main effect of trial (F(9, 63)= 18.24, p < 0.01) and the trial by sex interaction (F(9, 63)= 2.28, p < 0.03), but nonsignificant interactions of trial by contrast and trial by sex by contrast (Fs < 1.26, ps > 0.28). There were also nonsignificant group effects of trial, sex, and trial by sex interaction (Fs < 4.20, ps > 0.08).
Figure 5. Mean (± SEM) goal-tracking time for S1, cSNC acquisition, trials 1-10, and recovery, trials 11-15, is shown for all strains. (a) L strain. (b) H strain. (c) R strain. Significant differences in independent LSD pairwise analysis during recovery from cSNC are marked with asterisks.
During the postshift phase, there was a significant effect of trials ($F(4, 28) = 6.94$, $p < 0.01$), but there was no evidence for cSNC, as shown by the nonsignificant interactions of trial by contrast, trial by sex, trial by sex by contrast ($Fs < 1$). In addition, a significant group effect of sex ($F(1, 7) = 7.20$, $p < 0.03$), but nonsignificant group effect for trial and trial by sex interaction were found ($Fs < 4.05$, $ps > 0.08$).

Further independent analysis trial by trial of the recovery during the postshift phase provided the following results. For trial 11, a group effect ($F(5, 43) = 10.28$, $p < 0.01$) and significant LSD comparisons between downshifted and unshifted rats in the L ($p < 0.01$), H ($p < 0.01$), and R ($p < 0.01$) strains were found. For trial 12, there was a group effect ($F(5, 43) = 7.21$, $p < 0.01$) and significant pairwise comparisons between downshifted and unshifted rats in the L ($p < 0.01$) and H strains ($p < 0.01$), but nonsignificant effects rats in the R strain ($p > 0.29$). For trial 13, a group effect ($F(5, 43) = 4.04$, $p < 0.01$) and significant pairwise comparisons between downshifted and unshifted rats in the L ($p < 0.01$) and H ($p < 0.03$) strains, but no differences for the R strain ($p > 0.10$). Finally, there were no group differences for trials 14 and 15 ($Fs < 1.94$, $ps > 0.11$).

In brief, there were no differences in acquisition between strains. Additionally, rats in the R strain failed to show evidence for cSNC in the analysis by repeated measures. Finally, downshifted rats in the L and H strains spent similar amount of trials to reach the behavior level of the unshifted rats after reward loss.

S2. The cSNC performance for all strains is shown in Figure 6. Three pairs of rats from the S1 per strain were paired for this generation. However, just one pair from the H strain produced pups, while all pairs in the L and R strains produced litters.
**Figure 6.** Mean (± SEM) goal-tracking time for S2, cSNC acquisition, trials 1-10, and recovery, trials 11-15, is shown for all strains. (a) L strain. (b) H strain. (c) R strain. Significant differences in independent LSD pairwise analysis during recovery from cSNC are marked with asterisks.
The number of rats for the S2 was: 14 males and 18 females for the L strain, 6 males and 6 females for the H strain, and 11 males and 15 females for the R strain.

**Low strain.** Figure 6a shows the acquisition of consummatory behavior for rats in this strain. A similar preshift performance for unshifted and downshifted groups is showed by the main effect of trial (F(9, 243) = 72.10, p < 0.01), the nonsignificant interactions of trial by contrast, trial by sex, trial by sex by contrast (Fs < 1.24, ps > 0.27), and the significant group effect for sex (F(1, 27) = 5.30, p < 0.03), but no for contrast and the interaction of sex by contrast (Fs < 1.99, ps > 0.17).

The downshifted group showed significant cSNC during the postshift phase relative to the unshifted group, as supported by a trial effect (F(4, 108) = 21.30, p < 0.01), a trial by contrast interaction (F(4, 108) = 20.59, p < 0.01), and nonsignificant contrast by sex and trial by sex by contrast interactions (Fs < 1.05, ps > 0.39). A group trial significant effect was also found (F(1, 27) = 13.98, p < 0.01), and nonsignificant sex group effects and trial by sex interaction (Fs < 2.84, ps > 0.10).

**High strain.** Acquisition of consummatory behavior for the H strain is shown in Figure 6b. Similar levels in the acquisition of consummatory behavior were found for the unshifted and downshifted groups. This was confirmed by the main effect of trial (F(9, 72) = 18.78, p < 0.01), nonsignificant interactions of trial by contrast, trial by sex and trial by sex by contrast (Fs < 1.87, ps > 0.07), and a nonsignificant group effect for contrast, sex, and the contrast by sex interaction (Fs < 4.82, ps > 0.06).

Evidence for a cSNC effect during the postshift phase, when the downshifted group was compared to the unshifted group, is supported by significant effects for trial (F(4, 32) = 7.18, p < 0.01) and the trial by contrast interaction (F(4, 32) = 5.11, p < 0.01),
but nonsignificant interactions of trial by sex and trial by sex by contrast (Fs < 1).
Additionally, there were nonsignificant group effects for contrast, sex, and the contrast by
sex interaction (Fs < 1).

Random strain. Figure 6c shows the acquisition of consummatory behavior.

Differences in preshift acquisition performance for unshifted and downshifted groups is
shown by the significant main effects of trial (F(9, 198) = 73.45, p < 0.01), interaction of
trial by contrast (F(9, 198) = 2.21, p < 0.02), but nonsignificant trial by sex and trial by
sex by contrast interactions (Fs < 1.09, ps > 0.37). In addition, there was a group
significant contrast effect (F(1, 13) = 6.95, p < 0.02), but a nonsignificant group effect for
sex and for the contrast by sex interaction (Fs < 1).

Additional differences were found during the postshift phase. The downshifted
group showed a significant cSNC effect relative to the unshifted group, as supported by a
trial effect (F(4, 88) = 8.43, p < 0.01), a trial by contrast interaction (F(4, 88) = 5.46, p <
0.01), nonsignificant trial by sex and trial by sex by contrast interactions (Fs < 1.39, ps >
0.25), a significant group effect for contrast (F(1, 22) = 9.89, p < 0.01), and
nonsignificant sex effects and sex by contrast interaction (Fs < 2.98, ps > 0.10).

Analyses of the recovery during the postshift with independent LSD pairwise
comparisons provided the following results. For trial 11, a group effect (F(5, 68) = 13.09,
p < 0.01) and significant pairwise comparisons between downshifted and unshifted rats
in the L (p < 0.01) and R (p < 0.01) strains were found. However, the pairwise
comparisons failed to show significant differences rats in the H strain (p < 0.06). For trial
12, a group effect (F(5, 68) = 8.13, p < 0.01) and significant pairwise comparisons
between downshifted and unshifted males in the L (p < 0.01), H (p < 0.03), and R (p <
0.01) strains were found. For trial 13, a group effect (F(5, 68) = 5.64, p < 0.01) and significant pairwise comparisons between downshifted and unshifted rats in the L (p < 0.01) and R (p < 0.03) strains were found. However, there were nonsignificant pairwise comparisons for rats in the H strain males (p > 0.31). For trials 14 and 15, there were no between group differences (Fs < 1.64, ps > 0.16).

The results for the S2, in brief, showed differences in acquisition performance for rats in the R strain, but not for rats in the other strains. Interestingly, the cSNC effect in rats in the H line was weaker than the other two lines. Furthermore, rats in the H strain showed a lower number of trials without recovery than rats in the other strains.

**S3.** The cSNC performances for all strains are shown in Figure 7. Three pairs of rats from the S2 per strain were paired for this generation. One pair from the R strain failed to produce pups. The number of rats for the S3 was: 26 males and 14 females for the L strain, 10 males and 19 females for the H strain, and 9 males and 12 females for the R strain.

**Low strain.** Figure 7a shows the acquisition of consummatory behavior for the L strain. A similar preshift performance for unshifted and downshifted groups is shown by the main effect of trial (F(9, 270) = 36.40, p < 0.01), nonsignificant interactions of trial by contrast, trial by sex and trial by sex by contrast (Fs < 1.89, ps > 0.05), a significant group sex effect (F(1, 30) = 6.88, p < 0.01), and nonsignificant group contrast effect and contrast by sex interaction (Fs < 1).

The downshifted group showed significant cSNC during the postshift phase relative to the unshifted group, as supported by a trial effect (F(4, 120) = 12.76, p < 0.01), a trial by contrast interaction (F(4, 120) = 8.72, p < 0.01), nonsignificant trial by sex and
trial by sex by contrast interactions ($F_s < 1.58$, $p_s > 0.18$), a significant group effect for contrast ($F(1, 30) = 12.03$, $p < 0.01$) and sex ($F(1, 30) = 11.86$, $p < 0.01$), and a nonsignificant contrast by sex interaction ($F < 1$).

*High strain.* As shown in Figure 7b, H rats showed different patterns of acquisition of consummatory behavior for unshifted and downshifted groups, especially at the start and end of the preshift, as suggested by the main effect of trial ($F(9, 225) = 23.01$, $p < 0.01$), a significant interaction of trial by contrast ($F(9, 225) = 3.04$, $p < 0.16$), nonsignificant trial by sex and trial by sex by contrast interactions ($F_s < 1$), and nonsignificant group effects for contrast, sex and contrast by sex interaction ($F_s < 1.93$, $p_s > 0.18$).

During the postshift phase, the downshifted group showed cSNC effects when compared to the unshifted group, as supported by significant effects on trials ($F(4, 100) = 4.25$, $p < 0.01$), trial by contrast interaction ($F(4, 100) = 3.06$, $p > 0.01$), nonsignificant interactions of trial by sex and trial by sex by contrast ($F_s < 1$), and nonsignificant group comparisons for contrast, sex, and contrast by sex interaction ($F_s < 3.96$, $p_s > 0.06$).

*Random strain.* The acquisition of consummatory behavior is shown in Figure 7c. A similar preshift performance for unshifted and downshifted groups is shown by the main effect of trial ($F(9, 126) = 40.31$, $p < 0.01$), a significant interaction of trial by contrast ($F(9, 126) = 2.20$, $p > 0.03$), nonsignificant interactions of trial by sex and trial by sex by contrast ($F_s < 1.50$, $p_s > 0.16$), and nonsignificant group comparisons for contrast, sex, and contrast by sex interaction ($F_s < 1$).

The downshifted group showed significant cSNC effects during the postshift phase relative to the unshifted group, as supported by a trial effect ($F(4, 56) = 4.37$, $p <
0.01), a trial by contrast interaction (F(4, 56) = 6.16, p < 0.01), nonsignificant interactions of trial by sex and trial by sex by contrast (Fs < 1), a significant group effect of contrast (F(1, 14) = 18.12, p < 0.05), and nonsignificant group comparisons for sex and contrast by sex interaction (Fs < 1).

Supplementary analysis of the recovery during the postshift with pairwise analysis provided the following results. For trial 11, a group effect (F(5, 80) = 18.71, p < 0.01) and significant pairwise comparisons between downshifted and unshifted rats in the L (p < 0.01), H (p < 0.01), and R (p < 0.01) strains were found. For trial 12, a group effect (F(5, 80) = 4.04, p < 0.01) and significant pairwise comparisons between downshifted and unshifted males in the R (p < 0.01) strain were found. No differences were found for the L (p < 0.08) and H (p < 0.08) strains. For trial 13, a group effect (F(5, 80) = 5.314, p < 0.01) and significant pairwise comparisons between downshifted and unshifted rats in the L (p < 0.03) and R (p < 0.01) strains were found. However, there were nonsignificant pairwise comparisons for rats in the H strain males (p > 0.49). For trials 14 and 15, there were no between group differences (Fs < 1.12, ps > 0.36).

Briefly, the results for the final generation, S₃, showed differences in acquisition for rats in the H strain, but not the other strains. Rats in the H strain showed once again a fast recovery from cSNC, while rats in the L and R spent more days below the level of the unshifted controls. However, rats in the L strain showed faster recovery levels than in previous generations.
Figure 7. Mean (± SEM) goal-tracking time for S₃, cSNC acquisition, trials 1-10, and recovery, trials 11-15, is shown for all strains. (a) L strain. (b) H strain. (c) R strain. Significant differences in independent LSD pairwise analysis during recovery from cSNC are marked with asterisks.
Body weight.

Body weight was measured for S1- S3 generations. Rats are sexually dimorphic and differences in body weight were expected. However, the pattern of change in body weight for males and females in the present experiments was similar. Thus, data for males and females is presented together. Body weight was evaluated using a 3 X 2 X 17 (Strain X Sex X Age) ANOVA followed by post-hoc LSD pairwise tests. Figures in this section show the average of weights by strain across development from PND40 to PND88.

S1. A steady increase of body weight during development and differences between males and females were found for all strains. Body weights during the S1 were similar for all strains, as shown by the main effect of age (F(16, 608) = 1759.35, p < 0.01), a nonsignificant interaction of age by strain (F(32, 608) = 1.03, p > 0.42), a significant interaction of age by sex (F(16, 608) = 252.08, p < 0.01), a nonsignificant interaction of age by strain by sex (F > 1), and nonsignificant group comparisons of strain and strain by sex interaction (Fs > 2.343, p > 0.11), but significant group effect of sex (F(1, 38) = 165.37, p < 0.01).

S2. A steady increase of body weight during development and differences between males and females were found for all strains. Rats in the H strain showed smaller body sizes in a consistent fashion across development than rats in both the L and the R strains, as confirmed by the main effect of age (F(16, 1040) = 1451.66, p < 0.01), significant interactions of age by strain (F(32, 1040) = 5.64, p < 0.01), and age by sex (F(16, 1040) = 146.48, p < 0.01), and a nonsignificant effect of age by strain by sex (F < 1). Group comparisons portrayed a significant strain (F(2, 64) = 22.34, p < 0.01) and sex (F(1, 65) =
239.12, p < 0.01) effects, and nonsignificant strain by sex interaction (F < 1). In addition, differences were found with pairwise comparisons between the H vs. L (p < 0.01), the R vs. L (p < 0.01), and the H vs. R (p < 0.01) strains.

*S3.* Figure 8 shows the body weights for males and females in the S3. Once again, a steady increase of body weight during development and differences between males and females were found for all strains. Rats in the H strain showed smaller body sizes in a consistent fashion across development than rats in both the L and the R strains, as confirmed by the main effect of age (F(16, 1344) = 4037.08, p < 0.01), significant interactions of age by strain (F(32, 1344) = 10.37, p < 0.01), age by sex (F(16, 1344) = 429.79, p < 0.01), and a nonsignificant effect of age by strain by sex (F(32, 528) = 1.32, p > 0.11). Group comparisons portrayed a significant strain (F(2, 84) = 50.44, p < 0.01) and sex (F(1, 84) = 50.44, p < 0.01) effects, and nonsignificant strain by sex interaction (F(2, 84) = 1.15, p > 0.32). In addition, differences were found in pairwise comparisons between the H vs. L (p < 0.01) and the H vs. R (p < 0.01) strains, but not between L vs. R (p > 0.25).

Developmental body weight measures from S1 to S3 suggest the progressive difference in body size between strains. Rats in the H strain were smaller during all developmental measures since S2. Moreover, further differences were seen in S3 when rats in the R strain were bigger than rats in the other two strains.
Figure 8. Mean (± SEM) body weights across development for S3. Significant differences in LSD pairwise comparisons were found for High vs. Low (p < 0.01), High vs. Random (p < 0.01), and Low vs. Random (p < 0.01) strains.

Water consumption.

Water consumption was measured for S1- S3 generations. Water consumption was evaluated using the averages of drinking behavior for the three days of the test. Statistical analyses were performed with a 3 X 2 X 1 (Strain X Sex X Water consumption average) ANOVA followed by post-hoc LSD pairwise tests.

S1. Similar levels of water consumption were found for males and females during the S1. This was confirmed by a nonsignificant strain comparison (F(2, 44) = 1.61, p > 0.21), a significant sex effect (F(1, 44) = 20.45, p < 0.01), and a nonsignificant strain by sex interaction (F <1).

S2. Comparable to the S1, there were no differences in water consumption for rats in the S2, as confirmed by a nonsignificant strain comparison (F(2, 71) = 2.309, p > 0.11), a significant sex effect (F(1, 71) = 24.54, p < 0.01), and a nonsignificant strain by sex interaction (F <1).
Figure 9 shows water consumption for rats in the S3. The water consumption measures for this generation showed a different profile than previous generations. Rats in the H strain differed in the amount of water consumed, drinking less water than rats in the other two strains. This description is supported by a significant strain comparison ($F(2, 90) = 4.67, p < 0.01$), sex effect ($F(1, 90) = 76.46, p < 0.01$), and a nonsignificant strain by sex interaction ($F < 1$). Further pairwise comparisons pointed differences between the H vs. R ($p < 0.01$) and H vs. L ($p < 0.01$) strains, but not between L vs. R ($p > 0.63$).

*Figure 9. Mean (± SEM) water consumption for S3. Significant differences following LSD pairwise analysis are marked with asterisks.*

**Sensitivity for Sucrose Solutions.**

Sucrose sensitivity and the following tests were measured for rats in the S3 that did not undergo breeding. Averages of drinking behavior for water per day and for the different sucrose concentrations were used for the analysis. Two kinds of data are presented, total sucrose consumption per concentration and a ratio of sucrose and water consumption (sucrose consumption / sucrose consumption + water consumption). Each
statistical analysis was performed with a 3 X 2 X 3 (Strain X Sex X Sucrose concentrations) ANOVA followed by post-hoc LSD pairwise tests.

Figure 10a shows sucrose consumption. Similar sucrose consumption for all solutions was found for all strains as shown by a nonsignificant strain effect for the 0.5 sucrose solution (F(2, 33) = 3.76, p < 0.01), and nonsignificant effects for all other strain and sex effects and interactions (Fs < 1).

Alternatively, Rats in the H strain showed a similar preference for the 1.0 and 0.125 solutions, but a lower preference for the 0.5 solution Figure 10b). This description is confirmed by a significant strain effect for the 0.5 sucrose solution (F(2, 71) = 5.15, p < 0.01), and nonsignificant strain effects for the 1.0 and 0.125 solutions (Fs < 1). Sex comparisons and sex by strain interactions were all nonsignificant (Fs < 1). Further pairwise comparisons showed differences between the H vs. L (p < 0.01) and H vs. R strains (p < 0.01).
Figure 10. Sucrose sensitivity for S3. (a) Mean (± SEM) total sucrose consumption ratio. Statistical analyses failed to show any difference between strains. (b) Mean (± SEM) sucrose consumption ratio. A significant strain effect for the 0.5 concentration (F(2, 71) = 5.15, p < 0.01) and additional pairwise comparisons pointed differences between the H vs. R (p < 0.01) and H vs. L (p < 0.01) strains.

**Activity.**

Activity performance, specifically ambulatory distance, was evaluated for S3 rats. An independent analysis for males and females was also performed. For the reported data in this section, activity was evaluated using a 4 X 2 X 3 (Activity bins X Sex X Strain) ANOVA. Figure 11 shows ambulatory distance performance.
All the strains showed a decreasing level of ambulation along the test, suggesting habituation to the activity chamber. Rats in the H strain showed lower levels of ambulation during the first 5-min bin of the test than the other strains. By the fourth 5-min bin all strains seemed to have a similar performance (Figure 10). This is supported by the main effect of bin ($F(3, 162) = 186.48, p < 0.01$), a significant interaction of bin by contrast. ($F(6, 162) = 6.33, p < 0.01$), nonsignificant bin sex and bin by sex by strain interactions ($Fs < 1.75, p > 0.11$), and nonsignificant group effects on strain, sex, and strain by sex interaction ($Fs < 1.31, p > 0.28$).

![Figure 11](image)

*Figure 11.* Mean (± SEM) ambulatory distance for S3. Significant differences were found. Statistical analyses are described in the text.

**Autoshaping.**

Autoshaping performance in terms of number of responses is shown in Figure 12. This behavioral test was evaluated for S3 rats. For the reported data in this section,
autoshaping acquisition was evaluated using a 10 X 2 X 3 (Trial X Sex X Strain) ANOVA, and autoshaping extinction was evaluated using a 5 X 2 X 3 (Trial X Sex X Strain) ANOVA. Both analyses were followed by post-hoc LSD pairwise tests.

For acquisition, there were differences between the strains, specially the H strain that showed lower performance levels. This was supported by a main effect of trial (F(9, 588) = 39.25, p < 0.01), a significant interaction of trial by contrast (F(18, 588) = 2.31, p < 0.01) and trial by sex by strain interaction (F(18, 588) = 1.81, p < 0.02), nonsignificant sex by trial interaction (F < 1), and significant and group effect for strain (F(1, 652) = 4.45, p < 0.02) but not for sex and the strain by trial interaction (Fs < 1). Pairwise comparisons pointed that the differences were between the H vs. L (p < 0.01) and H vs. R (p < 0.05) strains.

Additional differences were found during the extinction phase, again related to the lower performance of the H strain. This is shown by a significant main effect of trial (F(4, 260) = 40.61, p < 0.01), nonsignificant interactions of trial by contrast, trial by sex, and trial by sex by strain (Fs < 1.80, ps > 0.08), but nonsignificant group effect for strain and the strain by trial interaction (Fs < 1.68, ps > 0.19). Pairwise comparisons pointed that the differences were between the H vs. L (p < 0.01), but not for H vs. R (p > 0.05) strains.
Figure 12. Mean (± SEM) number of responses for S3. The acquisition phase includes trials 1-10, while the extinction phase includes trials 11-15. Significant differences were found. Statistical analyses are described in the text.

Discussion

The present experiments explored the viability of a protocol involving artificial selection for recovery from cSNC across three selected generations. Preliminary evidence for the effects of selective breeding for fast (H line) vs. slow (L line) recovery from cSNC is offered by the profiles of recovery and cSNC performance across generations. Their profile is compared to a randomly bred line (R line). Animals in the H strain seemed to need fewer days to recover in S2 and S3 than both previous generations for the H strain, the L and R strains for the corresponding generations, and the S0 rats. On the other hand, rats in the L strain showed a similar number of days without complete recovery than both rats in the R strain and S0 rats. These between and within generation profiles suggest a stronger response to artificial selection for rats in the H line.

Effects of the artificial selection protocol on other behaviors more than the target one would be expected in the case of a response to the selective breeding. Interestingly, consistent changes in additional behavioral tests were found in the H strain, but no for the
L and R strains. Rats in the H strain showed smaller body size in a consistent fashion since the S2, when the apparent selection effect emerged, when compared to males and females in the L and R strains. S3 rats in the H strain also showed less water consumption and less sensitivity for a 0.5 g/ml sucrose solution than rats in the other two strains. Moreover, animals in the H strain showed a different activity profile, with lower activity levels in the early portion of the session. Finally, autoshaping acquisition and extinction were different for rats in the H strain when compared to rats in the other strains, with slower acquisition of rats in the H line compared to both L and R lines.

Additionally, the fact that all the strains showed cSNC effects in the majority of generations and the small number of differences in acquisition of consummatory behavior during preshift trials suggest that the artificial selection procedure did not affect just cSNC. Nevertheless, until this study’s selective breeding effects for the H line are replicated across generations and with larger stock of animals for the S0, any interpretation in terms of genetic or epigenetic mechanisms on recovery from cSNC is premature.

To interpret the behavioral mechanisms underlying recovery from cSNC, Flaherty’s multistage model was proposed. This model includes five major stages: detection, rejection, search, conflict, and recovery. According to one interpretation of this model, recovery from reward loss is related to the behavioral mechanism of counterconditioning, as used by Amsel (1992) to account for the effects of partial reinforcement on extinction. This interpretation privileged an associative, Pavlovian-based, interpretation of recovery from cSNC. However, the present results suggest
recovery from cSNC may share some of underlying mechanisms of several of the proposed stages, although the details of such relationships were not explored directly.

For instance, differences in sucrose sensitivity may be related to the detection of the sucrose downshift. Rats in the H strain may be less sensitive, in a perceptual sense, to the downshift from 32% to 4% sucrose. Similar effects have been reported for another artificial selection study involving high and low saccharin consumption levels. Dess (2000) found that this selection process resulted on differences in the perception and/or hedonic evaluation of sweet tastes.

Furthermore, differences in rejection, search, and conflict may be related to smaller body size and lower activity performance. Lower levels of activity may result in lower levels of search behavior after the downshift experience, in turn increasing the probability of consummatory behavior, as suggested by the reported increases in ambulation and rearing behaviors after a downshift from 32% to 4% sucrose (Pellegrini & Mustaca, 2000).

Lastly, differences in autoshaping performance may be interpreted as differences in associative mechanisms or as behavioral impulsivity. From the associative point of view, rats in the H strain that failed to acquire the autoshaping response may be under the control of the instrumental contingencies of the test. Given that the food pellets were delivered independently from the lever pressing, there was no room for the establishment of a contingency between the lever pressing response and the food reward. On the other hand, those differences may be related to behavioral impulsivity, as suggested by autoshaping performance differences between two other strains of rats. Kearns, Gomez-Serrano, Weiss, and Riley (2006) reported that Lewis rats showed faster acquisition on an
autoshaping task when compared to Fisher rats, while no differences were found in a subsequent reversal of the task or in an omission procedure. Differences in acquisition but no in the other associative phases, together with a trend of Lewis rats to more readily self-administer drugs of abuse, suggest that Lewis rats behaved more impulsively than Fisher rats. This result points to a motivational mechanism underlying autoshaping performance. Whether the observed differences in autoshaping performance of rats in the H line are related to impulsivity or to the instrumental components of the task merits further research.

Finally, a further distinction of mechanisms underlying contrast may arise comparing the present study to the previous experiment on artificial selection for cSNC. In this study, Flaherty, Krauss, Rowan, and Grigson (1994) found evidence for a stronger artificial selection effect on the High contrast Line than in the Low contrast line. Interestingly, the selective breeding for the degree of contrast was not related to changes in other tests such as open field, anticipatory contrast, radial arm maze contrast, and conditioned place preference. However, the present results suggest that the possibility for selection for fast recovery from cSNC is consistently related to mechanisms that contribute to other behaviors. Thus, a plausible working hypothesis is that recovery from cSNC involves a set of distinct behavioral mechanisms that go beyond those engaged by surprising nonreward per se.
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ABSTRACT

ARTIFICIAL SELECTION FOR RECOVERY FROM CONSUMMATORIY SUCCESSIVE NEGATIVE CONTRAST

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The present experiments explored the viability of a protocol involving artificial selection for recovery from cSNC across three selected generations. Preliminary evidence for the effects of selective breeding for the fast (H line), when compared to the slow (L line) and random (R line) lines, was reported. In addition, consistent changes in other behavioral tests were found exclusively for the H line during the third generation. Rats in the H strain showed smaller body size, lower water consumption, and less sensitivity for different sucrose solutions than rats in the other two strains. Moreover, animals in the H strain showed a different activity profile, with lower activity levels in the early portion of the session. Finally, autoshaping acquisition and extinction were slower for rats in the H line when compared to rats in the L and R lines.