Assessing the External Validity of Successive Negative Contrast – Implications for Animal Welfare

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ABSTRACT

When unexpectedly switched from a preferred to a less-preferred food reward, non-human animals may decrease consumption below that when only receiving the less-preferred reward – a successive negative contrast (SNC) effect. SNC has been proposed as an animal welfare indicator, however, to be effective it should show external validity; being demonstrable outside of highly standardized laboratory settings. We therefore investigated whether the SNC effect typically shown in laboratory rats was observed in owned (pet) rats from heterogeneous non-laboratory environments. Subjects (N = 14) were tested in a consummatory SNC paradigm with solid food rewards. “Shifted” rats received a high-value reward for 10 days (pre-shift), a low-value reward for six days (post-shift), then one additional day of high-value reward (re-shift). “Unshifted” rats always received the same low-value reward. “Shifted” rats consumed more food during pre-shift and re-shift trials, but ate less of the low-value food than “unshifted” animals in the post-shift trials – a SNC effect. This confirms the external validity of the SNC paradigm, extending reproducibility to outside the laboratory, indicating translatability across contexts, thus enhancing its potential use as a welfare indicator.

KEYWORDS

External validity; Rattus norvegicus; successive negative contrast

Introduction

Many non-human animals show a reduction in instrumental or consummatory responses when an anticipated food reward is unexpectedly reduced in either quality or quantity. If the responses of the “shifted” animals fall below those of animals who have only ever received the less preferred reward (“unshifted” animals), then the phenomenon is known as a successive negative contrast (SNC, see Flaherty, 1999, for a review). The behavioral (Amsel, 1962; Flaherty & Rowan, 1986; Mustaca, Bentosela, & Papini, 2000; Papini, 2003; Papini & Dudley, 1997; Papini & Ramallo, 1990; Rosen & Tessel, 1970) and physiological (Flaherty, Becker, & Pohorecky, 1985) response of subjects to an unexpected reduction in anticipated reward suggests that this downshift elicits a negative affective state in animals, akin to frustration and/or disappointment (Amsel, 1962; Flaherty, 1999; Papini & Dudley, 1997).

This interpretation has resulted in more recent investigations as to whether or not the background affective state of the subjects (when altered via manipulations of the environment, e.g., enriched cages (see Burman, Parker, Paul, & Mendl, 2008; Mitchell, Marston, Nutt, & Robinson, 2012, with opposing results), or treatments imposed directly on subjects, e.g., induction of peripheral pain (Ortega, Daniel, Davis, Fuchs, & Papini, 2011)), influences how animals respond to surprising reward reduction. Initial findings (Burman et al., 2008) suggesting that SNC may have potential as an indicator of animal affective state have contributed to interest in the use of cognitive measures to assess animal welfare (Mendl, Burman, Parker, & Paul, 2009; Paul, Harding, & Mendl, 2005), with
the prediction that animals already experiencing poor welfare would show an enhanced SNC effect, whereas those experiencing good welfare would be more resilient against a SNC effect (Burman et al., 2008, although see Galeano et al., 2011). These preliminary studies on the effects of affective manipulations on SNC, as well as numerous studies investigating different aspects of the SNC paradigm, have utilized the domestic rat (Rattus norvegicus), typically kept under highly standardized laboratory conditions, as subjects.

Standardization, for example with regard to animals’ age, body weight, sex and housing conditions, is common practice, in an attempt to avoid between experiment variation (Beynen, Gartner, & Van Zutphen, 2001; Crabbe, Wahlsten, & Dudek, 1999; Wahlsten, 2001), generate reproducible results, and thereby improve comparability of results within and between laboratories (Beynen et al., 2001; Gärtnner, 1999; Örink & Rehbinder, 2000; van der Staay & Steckler, 2001; Wahlsten, 2001). However, while many authors argue that such standardization is important in terms of demonstrating internal validity of experimental findings (Guala, 2003; Kazdin & Rogers, 1978), others have argued that such studies are limited in terms of generalizability outside of the laboratory and to other conditions, populations and species, resulting in a lack of external validity (Campbell, 1957; Richter, Garner, & Würbel, 2009; van der Staay, Arndt, & Nordquist, 2010; Würbel, 2000, 2002).

Such latter arguments are based on the premise that laboratory conditions render animals within experiments more homogenous which has been postulated to result in pseudo-replication, thus potentially introducing a systematic source of false positive or spurious results which in turn leads to poor reproducibility; the opposite of the goal of standardization (Richter, Garner, Auer, Kunert, & Wurbel, 2010; Richter et al., 2009). As pointed out by Chapanis (1967), laboratory experiments can assess only a tiny proportion of all possible influential variables, and so confounding effects of unsuspected interactions in real-life settings may result in different conclusions than those obtained under laboratory conditions. Controlling other contributing factors to increase precision of the experiment may lead to the discovery of effects that are so small that they have no practical relevance (Chapanis, 1967). Thus, as results from the laboratory are not always necessarily replicated in a natural setting (e.g., Bell, Hankison, & Laskoswki, 2009; Fiore, Dell’Omo, Alleva, & Lipp, 1995), caution should be exerted when generalizing from the results of laboratory experiments (Chapanis, 1967).

Consequently, in order for SNC to truly be a useful and impactful measure of animal welfare that can be reliably utilized outside of the laboratory and with a range of species, a key step is to demonstrate that the paradigm has external validity – the extent to which results can be generalized across varied test situations. One proposed way in which to improve external validity and reproducibility of results while avoiding spurious results is to replace standardization with deliberate environmental heterogenization (Richter et al., 2009; van der Staay et al., 2010). This can be carried out through a number of means including varying environmental conditions such as housing conditions, and conditions related to the experimental subjects, for example, genetic make-up, age and gender (van der Staay et al., 2010). Few studies have investigated SNC in animals outside of a laboratory setting. Evidence for consummatory SNC was found in sheep (Ovis aries, Catanese, Freidin, Cuello, & Distel, 2011; Greiveldinger, Veissier, & Boissy, 2011) and fallow deer (Dama dama, Bergvall, Rautio, Luotola, & Leimari, 2007); however, these subjects were kept under standardized housing conditions, albeit outside of a laboratory. Furthermore, wild-caught opossums (Lutreolina crassicaudata and Didelphis albiventris, Papini, Mustaca, & Bitterman, 1988) and starlings (Sturnus vulgaris, Freidin, Cuello, & Kacelnik, 2009) demonstrated consummatory SNC after an acclimatization period in captivity. SNC has not been studied in a population of laboratory dogs, but results for owned (pet) dogs (Canis familiaris) – using subjects with diverse life histories – are ambiguous. Bentosela, Jakovcevic, Elgier, Mustaca, and Papini (2009) reported higher food rejection rates in a group of dogs following a reward downshift than in unshifted controls, whereas Riemer, Ellis, Ryan, Thompson, and Burman (2016) using the same approach failed to observe SNC for dogs either housed in homes or rescue shelters. There has, however, been no SNC study carried
out in a heterogeneous, non-laboratory, environment using a species that is typically tested under laboratory conditions.

The aim of this study was therefore to assess the external validity of the SNC paradigm by investigating whether the SNC effect commonly shown in rats when tested in highly standardized laboratory conditions was observable if tested in a population of owned (pet) rats living in heterogeneous, non-laboratory environments.

**Methods**

This work followed the Association for the Study of Animal Behaviour (ASAB) guidelines for the use of animals in research and was approved locally by the Research Ethics Committee of the School of Life Sciences at the University of Lincoln. Rat owners gave written consent for their rats to be included in the study.

**Subjects**

Eighteen adult pet rats of indeterminate heritage were recruited for the study. The rats varied from one another in a number of factors including age (range 8–48 months; mean 20.16 months ± 3.43 SEM) and gender (12 female, 6 male). The rats were kept in heterogeneous housing conditions. For example, all rats, with the exception of one individual, were housed socially in groups of different sizes, either as domestic pets in one of two private homes in the local area (Lincolnshire, UK) or as animals kept for student education into animal care at Riseholme College, Lincoln, UK. The rats were housed in a variety of differently sized cages (all larger than standard laboratory cages) containing diverse enrichment items (e.g., hammocks, cage shelves, tubes, ropes). While all rats were fed a mixture of commercially available rat nuggets and rat muesli once daily, they varied considerably in the frequency and range of other food treats to which they were exposed. Food and water was always available ad libitum with the exception of the testing days, where food was temporarily withheld until after the experiment. Although all of the rats were used to daily positive human interaction, this ranged from bouts of handling/tickling to regular positive reinforcement training sessions.

**Procedure**

Subjects were either tested within a testing room at the University of Lincoln (n = 15), or at their home (n = 3), but always in a separate room from where their home cages were kept. Testing was performed in standard rat cages measuring 57L x 30W x 46.5H cm with wire roof and sides and a plastic floor. The cage sides had their visual view blocked by a cardboard barrier placed on the outside of the cage on three of the four sides allowing the experimenter to view the rat from one exposed side while minimizing external visual stimulation from the other three sides. The right lateral wall contained the feeder cup (4 cm high and 8 cm diameter, made of aluminum, placed 4 cm from the front wall). Cages were thoroughly washed and disinfected using “Safe4” disinfectant before each test to prevent cross-contamination of scent. Morning feeds were withheld on testing days to increase feeding motivation, with ad libitum food replaced after testing had finished.

**Pre-testing exposure to food rewards**

The methodology used broadly followed the general approach described in Pellegrini and Mustaca (2000) for using solid foods in a consummatory SNC paradigm. Two types of solid food reward were selected, a high-value mashed sugar coated cereal (“Frosted Shreddies,” c.f. Dachowski & Brazier, 1991; Elliott, 1928) and mashed rabbit food pellets as the low value reward (c.f. Pellegrini & Mustaca, 2000). As both types of food were novel to all the subjects at the start of the study, they received both food stuffs in
their home cages as part of their daily diet during the week before they commenced the experiment (for days 1 to 5). This was a change to the protocol of Pellegrini and Mustaca (2000) and was done in order to give equivalent dietary experience to all rats prior to testing, and to confirm the relative values of the two food types. On day 6, the rats did not receive either of the food types, and on day 7, they received 2g of their allocated pre-shift food in their home cages. Our preliminary observations revealed that when both foods were simultaneously available, all rats chose to eat the high-value food before the low value food, confirming the prescribed qualitative reward values. Both food types were prepared freshly each day and kept at room temperature in sealed containers to prevent evaporation.

**SNC testing**

Subjects were pseudo-randomly assigned to the two treatment groups such that both groups had approximately the same mean weight at the start of testing. The experiment included 17 trials, with one trial taking place per day at 0700–1000 hrs. “Shifted” animals (N = 9) received the high-value food (cereal mash) for the first 10 trials (pre-shift phase), then the low value food (mashed rabbit food) for a further 6 trials (post-shift phase), before returning to the high-value food on the final trial (trial 17, re-shift phase). “Unshifted” animals, (N = 9) received the same low-value food (mashed rabbit food) for all 17 trials. Thus, the testing schedule followed that employed by Pellegrini and Mustaca (2000), with the addition of the 17th (re-shift) trial. This trial was included as an additional control to allow us to determine whether any potential reduction in consumption seen in the “post-shift” trials was due SNC or to fatigue due to repeat testing (e.g., Bentosela et al., 2009).

Rats were tested individually and the order of testing was randomized for the treatment groups. At each trial, 5 g of the allocated food was weighed and placed in the feeder cup in the test cage. The rat to be tested was then placed in the center of the cage with its nose facing the feeder cup. On their first test trial, rats were given time to explore the test cage and the trial only began once the rat had tasted the food in the feeder cup for the first time. The rats were removed from the test cage five minutes after their first feeding bout. All subsequent trials lasted five minutes from when the rat was placed into the test cage, regardless of when it started feeding. After each trial, the feeder cup was removed and the weight of remaining food (g) measured and recorded.

**Statistical analysis**

Statistical analyses were conducted on IBM SPSS version 21.0 with the alpha value set at the 0.05 level. “Pre-shift,” “post-shift” and “re-shift” trials were analyzed separately due to the different predictions at each stage (e.g., during “pre-shift” and “re-shift” trials you would predict the “shifted” rats to consume more than the “unshifted” rats, whereas during the “post-shift” trials you would expect the converse). “Pre-shift” trials were analyzed using repeated measures General Linear Models with Trial (1–10) as a within-subjects factor and Treatment (shifted/unshifted) as the between-subjects factor. The same process was carried out in a separate analysis for the “post-shift” trials (trials 11–16). Models were checked for sphericity, and Huyn-Feldt correction was used if this condition was not met. Normality of the residuals and homoscedasticity were checked and adequate for all models. Treatment differences in the “re-shift” trial 17, where shifted animals once again received the high-value food reward, were analyzed using T-tests with Treatment (shifted/unshifted) as the between-subjects factor (data again met the assumptions of parametric tests).

**Results**

Four rats had to be excluded from the analysis because they did not meet the performance criterion of consuming at least some food during each of the pre-shift trials. As all of these subjects were in the “unshifted” treatment group (i.e., receiving the low value food reward), the final sample included five rats in the “unshifted” group and nine in the “shifted” group.
**Pre-shift trials**

During the “pre-shift” trials (trials 1–10), rats with access to the high-value food (“shifted” individuals) consumed significantly more food than those with access to the low value food (“unshifted” individuals) ($F_{1, 12} = 13.705, p = 0.003, \eta^2 = 0.533$) (see Figure 1). There was also a significant main effect of Trial on food consumption during pre-shift ($F_{4.448, 53.380} = 3.975, p = 0.005, \eta^2 = 0.249$), with increased food consumption, for both food types, as the rats experienced more trials. There was no significant Treatment*Trial interaction ($F_{4.448, 53.380} = 0.526, p = 0.736, \eta^2 = 0.042$).

**Post-shift trials**

In “post-shift” trials (trials 11–16), where all rats received the low value food, there was a significant effect of Treatment ($F_{1, 12} = 5.019, p = 0.045, \eta^2 = 0.295$; see Figure 1), with rats in the “shifted” treatment consuming significantly less food than the “unshifted” rats. There was no significant effect of Trial on food consumption ($F_{5, 60} = 0.666, p = 0.651, \eta^2 = 0.053$) nor a Treatment*Trial interaction ($F_{5, 60} = 0.936, p = 0.465, \eta^2 = 0.072$).

**Re-shift trial (trial 17)**

When the “shifted” rats once again had access to the high-value food in trial 17, they ate significantly more food than “unshifted” rats in the same trial ($t = −2.220, DF = 12, p = 0.046, \text{Cohen’s } d = 1.222$; see Figure 1).

**Discussion**

The rats showed a preference for the high value over the low-value reward, as evidenced by the significantly greater consumption during pre-shift and re-shift trials in the shifted compared to the unshifted treatment group. During post-shift trials, rats exhibited a negative contrast effect, i.e., consumption of the same low-value food reward by the shifted rats dropped significantly below that shown by the unshifted subjects. Thus, this study demonstrates that the SNC effect commonly observed in rats housed in highly standardized laboratory conditions can also be observed in pet rats housed in heterogeneous environments, indicating that the occurrence of SNC is robust for this species across test situations.

![Figure 1](image-url). Mean food consumption (g) ± SEM, dependent on reward shift group and trial. Vertical lines indicate pre-shift, post-shift and re-shift trial phases.
While SNC effects have been extensively investigated in laboratory settings (including in primates, Cowles & Nissen, 1937; Tinklepaugh, 1928; rodents, reviewed in Flaherty, 1982; Pellegrini & Mustaca, 2000; and fish; Cochrane, Scobie, & Fallon, 1973) and recently also in a few non-laboratory – yet standardized – environments (e.g., sheep, Catanese et al., 2011; Greiveldinger et al., 2011; fallow deer, Bergvall et al., 2007), individuals housed in a more diverse range of environmental conditions have been studied far less. For example, in the studies by Bentosela et al. (2009) and Riemer et al. (2016) on dogs, by Papini et al. (1988) on two didelphid marsupial species and by Freidin et al. (2009) on starlings – the latter two studies used wild-caught subjects. This study shows, for the first time, that the SNC paradigm, for a given species, is demonstrable in a heterogeneous environmental setting that contrasts with the highly standardized laboratory environment in which it is typically observed, showing a potential to translate across varied test situations as is the case for other cognitive measures of welfare (e.g., cognitive bias: laboratory dogs, Burman et al., 2011; owned dogs, Karagiannis, Burman, & Mills, 2015; rescue/shelter dogs, Mendl et al., 2010).

Research exploring the potential of SNC as a cognitive indicator of animal welfare has, thus far, focused on standardized laboratory studies in rats, investigating whether housing manipulations, such as the provision of environmental enrichment (e.g., Burman et al., 2008; Mitchell et al., 2012), can influence SNC response. But, to extend its potential application outside of the laboratory it is important to determine if SNC can also be observed in uncontrolled heterogeneous environments. Here, we have shown that, at least for a heterogeneous population of pet rats with a mix of housing and husbandry experiences, SNC can be observed outside the laboratory. However, the pronounced SNC effect observed in our subjects, despite being kept under highly enriched conditions and, presumably, experiencing good welfare compared to laboratory rats kept under standard conditions, raises the question as to whether an enhanced SNC effect reflects poor welfare (Burman et al., 2008) or whether other cognitive mechanisms are involved. Future research should therefore continue to focus on identifying how manipulations in affective state, for example through changes in housing environments and husbandry techniques, can influence SNC, but our findings do suggest that this approach can now be extended to, and explored further in, both laboratory and non-laboratory environments.

An unexpected occurrence during testing was the failure of four of the “unshifted” pet rats to achieve the performance criterion (i.e., the requirement to consume at least some food during each of the pre-shift trials); this suggests a lack of motivation for the low-value food, an interpretation that could have been aided by recording additional behaviors during the study, such as time spent in locomotion. A similar study in laboratory rats (Pellegrini & Mustaca, 2000) tested their subjects at 80–85% of their individual ad libitum weights, whereas we increased the motivation of the rats only by withholding food on test days until after testing had finished. In addition, the rats in our study, whether or not they fed during the 5 mins of testing, received an ad libitum supply of their normal food immediately afterwards. As a result, the subjects in the study by Pellegrini and Mustaca (2000) were likely to be more food motivated, possibly inducing them to value food of any type (even low value) higher than the rats in our own study. This emphasizes the importance of taking into consideration the general level of food motivation (e.g., Cuenya et al., 2015; Riley & Dunlap, 1979), as well as the appropriate choice of high and low value foods, in the implementation of the SNC paradigm. However, the re-shift trial demonstrated that those subjects that had achieved the consumption criteria did not lose motivation with the testing procedure itself, with the “shifted” animals showing a return to their previous higher levels of consumption once re-exposed to the high-value food reward. An additional possibility is that, by giving the “unshifted” animals pre-test experience of the high-value food, this itself resulted in a SNC, which may have led to subsequent demotivation.

**Conclusion**

This study demonstrates the existence of an SNC effect in a varied (age/sex) population of owned, pet, rats housed in heterogeneous environmental conditions, reflecting the same effect commonly observed in standardized laboratory conditions (e.g., Gómez, Escarabajal, de la Torre, Tobena, &
Fernandez-Teruel, 2009; Pellegrini & Mustaca, 2000), thereby demonstrating external validity of the SNC paradigm. This is an important step in the development of this approach for assessing welfare, not only for laboratory and pet rats, but potentially also for a range of mammalian species (although see Freidin et al., 2009) housed across a variety of different housing environments.

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