Switching from sucrose to saccharin: Extended successive negative contrast is not maintained by hedonic changes.

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Abstract

Previous experiments found that acceptance of saccharin by rats was reduced if they had prior experience of sucrose or some other highly palatable solution. This reduction in saccharin consumption was particularly extended after a switch from sucrose. On the surface, this seems to correspond to a successive negative contrast (SNC) effect – a term coined by C. F. Flaherty to describe the situation where consumption of a target solution is reduced by prior experience of a more valuable solution (typically a more concentrated version of the target solution). However, SNC effects are typically transient and assessed relative to a non-shifted control. Here, we confirm that the reduction in consumption seen when shifting from sucrose to saccharin is persistent and is seen relative to the traditional unshifted control. In addition, an analysis of licking microstructure showed that the shift from sucrose to saccharin suppressed the hedonic value of saccharin relative to controls, but this effect was far less persistent than consumption suppression. Interestingly, a similar dissociation is observed in extinction of conditioned taste aversion (CTA): suppression of consumption produced by CTA is far more persistent than suppression of hedonic value. The comparison of results across procedures suggests that persistent SNC produced by a qualitative downshift from sucrose to saccharin appears different from quantitative downshifts in the concentration of a single solution, and qualitative downshift effects may be based on CTA.

230 words

Key words: sucrose; saccharin; negative contrast; hedonic value; licking microstructure; rats.

Running head: Successive negative contrast and hedonic value

Rats allowed to consume concentrated sucrose solutions for extended periods show cognitive and metabolic impairments (Kendig, Boakes, Rooney, & Corbit, 2013; Kendig et al., 2018). These impairments decline in severity over time if the rats are switched to a saccharin solution but intriguingly consumption of saccharin remain persistently low and similar to intakes of water (Kendig et al., 2018) even though rats generally prefer saccharin and drink more than water (Mook, 1974). Subsequent experiments designed to investigate this effect confirmed that, following an initial stage in which rats were given access to highly palatable solutions, including glucose and maltodextrin solutions as well as 10% w/v sucrose, acceptance of saccharin was reduced relative to controls (Boakes, Rehn, Badolato, & Rooney, 2020).

Described in these general terms, it seems reasonable to assume that these results might reflect a form of successive negative contrast (SNC). This refers to an abrupt drop in consumption of some normally palatable solution following a 'downshift' from a higher valued one or a drop in performance after a downshift in reward (Flaherty, 1996). For example, in a downshift that was widely used in Flaherty's laboratory and by other researchers, rats were switched from 32% sucrose to 4% sucrose (32-4 condition); this results in lower consumption of the 4% solution than that of an unshifted group (4-4 condition) maintained on 4% sucrose throughout (Flaherty & Largen, 1975; Pellegrini, Muzio, Mustaca, & Papini, 2004). However, the effect of shifting from sucrose to saccharin in the studies noted above (Boakes et al., 2020; Kendig et al., 2013; Kendig et al., 2018) differed in two main respects from the example from Flaherty's laboratory of what we will term a *quantitative downshift*, that is, one involving a reduction in the amount or the concentration

of some incentive[1]. First, the effect of the latter is normally short-lived. Recovery to the level of controls is normally rapid in SNC experiments measuring consumption (Flaherty, 1996), as well as for running speeds in runway experiments such as Crespi's (1942) classic study. The second major difference is that a shift from sucrose to saccharin involves the introduction of a new taste as well as an unexpected decrease in palatability. We refer to these relatively rare examples as *qualitative downshifts.* An example is a shift from a highly palatable mixture of glucose and saccharin to glucose alone; the resultant low acceptance of the glucose solution, compared to controls, persisted over eight post-shift sessions (Mitchell & Flaherty, 2005; see Figure 1).

In addition, our previous studies shared a limitation with many prior investigations of SNC, namely that they relied entirely on endpoint measures of behavior such as the total amount consumed or the total number of licks. While intake measures are certainly informative, it has long been recognized that overall intake reflects the combination of a variety of processes (e.g. Davis, 1998; Dwyer, 2012; Spector, Klumpp, & Kaplan, 1998). Analyzing the microstructure of licking displayed by rats during the consumption of fluids has allowed researchers to assess some of the mechanisms contributing to overall consumption in more detail. A cluster is defined as a series of licks that occur prior to some pause criterion (e.g., a series of licks separated by < 0.5 s). The number of licks per cluster is used as an index of hedonic value (Davis, 1989; Dwyer, 2012). For example, when a

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[1] There are also two potentially important practical differences: our prior studies used long (up to 24 h/day) exposures, while most studies of consumption-based SNC have used relatively short (5-30 min) daily sessions; and the typical controls for SNC studies have been "unshifted" groups receiving the lower-valued solution throughout, while our prior studies have typically used controls exposed to water alone.

palatable solution is consumed, there is a positive monotonic relationship between lick cluster size and solution concentration; and pairing a taste with an aversive outcome such as LiCl has been reliably found to reduce lick cluster size (e.g. Arthurs, Lin, Amodeo, & Reilly, 2012; Baird, St John, & Nguyen, 2005; Dwyer, Boakes, & Hayward, 2008). Thus, higher lick cluster sizes are indicative of greater hedonic value (and vice versa).

Three previous SNC studies used microstructural analysis of licking behavior. Grigson, Spector, and Norgren (1993) reported a SNC effect that persisted throughout the four-day post-shift period in rats shifted from 1.0 M (~ 34.2% w/v) to 0.1 M (~3.42% w/v) sucrose solution. The shift was followed by consistent reductions in the number of licks per cluster (i.e., lick cluster size), relative to unshifted controls, which suggests a reasonably persistent reduction in the hedonic value of the downshifted sucrose solution. Conversely, Mitchell and Flaherty (2005) only found lower lick cluster size on the first post-shift day in rats switched from a 2% glucose+0.15% saccharin mixture to 2% glucose. Lick cluster size did not differ between shifted and unshifted groups in this experiment for the remaining seven post-shift days, although a SNC effect on solution intake persisted. In a study with mice, a reduction in cluster size was found in a 32%-4% sucrose shift group; however, this effect was very transient, in that no differences between this group and an unshifted control were found after the first 2 min of a single 10-min test session and no overall SNC effect was detected in terms of total intake of 4% sucrose during this session (Austen, Strickland, & Sanderson, 2016).

The design of the present experiment involved two stages and is outlined in Table 1. In a 10-session Stage 1 rats received daily 30 min drinking sessions with access to either 10% sucrose, 10% maltodextrin, 0.4% saccharin or water. This was followed by a 12-session Stage 2, during which all rats received 0.4% saccharin, and a final 2-session two-bottle choice test between 0.4% saccharin and 2% sucrose. The inclusion of a group given 10% maltodextrin in Stage 1 (*Malto-Sacch*) was to test whether any effects found in the *Suc-Sacch*

group would also be found when rats were given access to this other highly palatable, energy-dense solution, one far less sweet than either sucrose or saccharin (Davis, 1996; Dwyer, 2008; Sclafani, 2004), prior to the shift to saccharin.

The more general aim of this study is to extend our understanding of *qualitative* contrast effects as opposed to the much more extensively studied *quantitative* effects.

Method

Subjects. Sixty-four female Sprague-Dawley rats were purchased from Envigo (Blackthorn, United Kingdom). On arrival they were 6 weeks old, with an average weight of 187 g (range, 165-213 g). The rats were housed in pairs throughout the experiment. The colony room was maintained on a 12:12 h light/dark cycle with lights turning off at 1900 h. All experimental procedures occurred during the light phase of the cycle and only on weekdays. Rats were handled for three days prior to the start of experimental procedures. They were maintained on *ad libitum* access to chow and water, except on experimental days when water bottles were removed 1 h prior to drinking sessions and replaced after the session. Rats were weighed twice a week. All procedures reported here were conducted in accordance with the Animals Scientific Procedures Act (1986) requirements for animal experimentation in the United Kingdom.

Solutions. All solutions were prepared on a weight/weight (w/w) basis using distilled water on the same day that it was provided to the rats. Sucrose solutions (2%, 10%) were prepared using commercially available white sugar (17 kJ/g, Silver Spoon, UK). A 10% maltodextrin solution was prepared using maltodextrin (16.2 kJ/g, C*Dry MD 01904, Cerestar-UK, Manchester, UK). A 0.4% saccharin solution was prepared using saccharin sodium salt hydrate (S6047, Sigma-Aldrich).

Apparatus. All drinking sessions took place in a separate testing room from the colony room. The testing room contained 16 custom-made automated drinking chambers, measuring 32 × 15 × 12 cm, with acrylic walls, steel mesh flooring and wire mesh lids. Two 50 mL drinking bottles with metal spouts could be inserted 8 cm apart at one end of each box. A contact-sensitive lickometer registered the licks made by rats to the nearest 0.01 s once the bottle was available. MED-PC software (Med Associates, Inc) was used to control the equipment and record the data.

Table 1. Design of the experiment.

Groups (*n* = 16)	**Stage 1** (10 x 30-min sessions)	**Stage 2** (12 x 30-min sessions)	**Preference tests** (2 sessions)
Suc-Sacch	10% sucrose	0.4% saccharin	0.4% saccharin *vs* 2% sucrose
Malto-Sacch	10% maltodextrin		
Sacch-Sacch	0.4% saccharin		
Water-Sacch	Water		

Procedure. The experiment design is outlined in Table 1.

Stage 1 (Sessions 1-10). Rats were allocated to one of four groups (*n* = 16), matched for body weight, that differed only in terms of the solution they received during Stage 1: *Suc-Sacch, Malto-Sacch, Sacch-Sacch,* and *Water-Sacch.* Sessions took place on five days each week. They were conducted in four runs of 16 rats each (*n* = 4/group) starting at 1000 h. Rats were transferred from home cages to individual drinking chambers where they received 30-min access to their respective solutions. There was no pre-training period to acclimatize the rats to drinking in the experimental boxes, and so initially the spouts were inserted to protrude by 1cm into the box. While this facilitated initial drinking, it also allows non-lick contact with the spout which can interfere with lick-recording. Thus, the spouts were

progressively retracted over Sessions 1-4 until presented flush with the front of the cage allowing consumption with minimal lick recording artefacts. The position of the solutions alternated between left (Sessions 1, 3, 5, 7) and right (Sessions 2, 4, 6, 8) to habituate rats to the two positions for future preference tests. Bottles were weighed before and after each run to measure consumption and lick data were recorded in each session throughout the experiment.

Stage 2 (Sessions 11-22). For twelve sessions all groups received 30 min daily access to 0.4% saccharin in the licking chambers. As in Stage 1, solutions were presented on the left (Sessions, 11, 13, 15, 17, 19, 21) and right (Sessions 12, 14, 16, 18, 20, 22) in alternate sessions.

Preference tests (Sessions 23-24). On Session 23 all rats received 15 min access to 0.4% saccharin solution in the left bottle and access to 2% sucrose in the right bottle. On Session 24 rats received a second preference test with the positions of the solutions exchanged.

Data analysis. Lick cluster size, the mean number of licks per cluster, was extracted from MED-PC data. A cluster was defined as a series of licks separated by pauses no longer than 0.5 s, a criterion used by Davis (1989) and in previous studies of licking microstructure and contrast effects (Grigson et al., 1993; Mitchell & Flaherty, 2005). Only cluster size data from Stage 1 Session 5 onwards were analysed, as this was when spouts were positioned for optimal lick recording.

Intake and licking data were excluded for rats on days in which observable bottle malfunctions were noted (e.g., blocked spout, leaking bottle). Licking data were also excluded when malfunctions in licking apparatus or recordings were observed (e.g., no licks, short circuit in licking chamber). Licking and intake data were then averaged across two-session blocks to account for potential position preferences as bottle positions alternated between left and right on consecutive sessions. In the single instance where licking data were

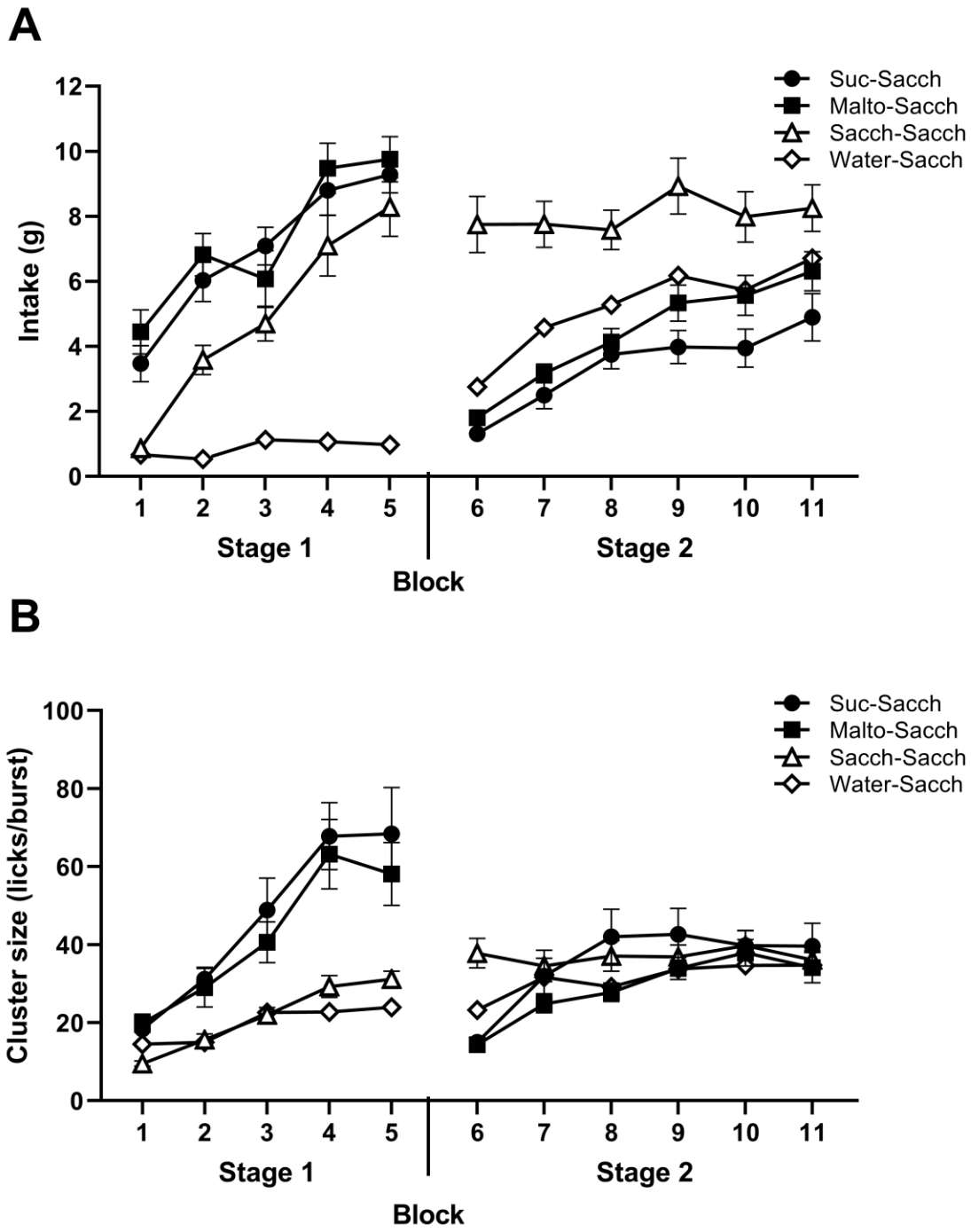
missing on both days in a two-session block for one rat, it was replaced with an average of the previous and following two-session blocks for that rat (note this occurred on training sessions 5 and 6 and so does not affect the critical test-phase data).

Data were analyzed using IBM SPSS (V25) and statistical significance was set at $p < .05$. Intakes and cluster size data were analyzed using separate mixed-ANOVAs in Stages 1 and 2 with Group as the between-subjects variable and two-session Blocks as a repeated measures variable. Significant effects and interactions were followed up using simple main effects with two-tailed pairwise comparisons implemented using the "compare" command in SPSS with a common standard error derived from the relevant ANOVA using Fisher's protected least significant difference methods. Preference for saccharin relative to sucrose was calculated as a percentage for each rat by taking total intakes of saccharin across both preference tests and dividing it by total solution intakes (saccharin + sucrose) across both preference tests. Percentage preference data were then analyzed using one-way ANOVA with Group as the between-subjects variable. Greenhouse-Geisser corrections were applied when Mauchly's test of sphericity was violated in mixed-ANOVAs.

Data Transparency and Openness

This experiment was not pre-registered. The data are available upon request to the corresponding author.

198 **Results**



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200 *Figure 1*. Average (± SEM) (A) intakes and (B) lick cluster size for the *Suc-Sacch*, *Malto-*

201 *Sacch*, *Sacch-Sacch* and *Water-Sacch* groups (*n*=16) during Stage 1 (2-session blocks 1-5),

202 when rats received their respective solutions, and during Stage 2 (2-session blocks 6-11),

when all rats received 0.4% saccharin solution. NB: Error bars for the Water-Sacch group are hidden behind the symbols because they are small.

Stage 1

Intakes. Figure 1A shows the average solution intakes over Stages 1 and 2. In Stage 1 (left hand panel), *Suc-Sacch* and *Malto-Sacch* rats initially had higher solution intakes than *Water-Sacch* or *Sacch-Sacch* rats, indicating some neophobia to saccharin. By the end of Stage 1 intakes remained low in the *Water-Sacch* group compared to all other groups. This description was confirmed by the results of the mixed ANOVA which indicated that there were significant main effects of Block, $F(2.76, 165.7) = 67.81$, $p < .001$, MSE = 4.96, and Group, $F(3, 60) = 49.1$, $p < .001$, $\eta^2_p = .71$, MSE = 14.19, plus a significant Block by Group interaction in solution intakes, $F(8.29, 165.7) = 7.46$, $p < .001$, $\eta^2_p = .27$, MSE = 4.96. Regarding the interaction, in Block 1 intakes were not different between the *Suc-Sacch* and *Malto-Sacch* groups, $t(60) = 1.56$, $p = .125$, Std. Error = 0.62, nor between the *Water-Sacch* and *Sacch-Sacch* groups, $t(60) = 0.32$, $p = .754$, Std. Error = 0.62, while both the *Suc-Sacch* and *Malto-Sacch* groups displayed higher intakes than both the *Water-Sacch* and *Sacch-Sacch* groups, smallest $t(60) = 4.16$, $p < .001$, Std. Error = 0.62 (for the *Suc-Sacch* vs *Sacch-Sacch* comparison). However, by Block 5 the *Water-Sacch* group had lower intakes than all other groups (smallest $t(60) = 8.11$, $p < .001$, Std. Error = 0.90, for the comparison to group *Sacch-Sacch*), and the other three groups did not differ (largest $t(60) = 1.63$, $p = .108$, Std. Error = 0.90, for the *Suc-Sacch* vs *Sacch-Sacch* comparison).

Lick cluster size. Lick cluster size data are shown in Figure 1B. Lick cluster sizes were generally higher in Stage 1 (left hand panel) for groups *Suc-Sacch* and *Malto-Sacch* than groups *Water-Sacch* and *Sacch-Sacch*, with this difference becoming larger along with the general increase in lick cluster sizes across Stage 1. However, because the inferential analysis focused only on blocks 3-5 (due to the fact that Blocks 1 and 2 had the spouts

extending into the cages and thus allowed non-lick contacts to be recorded) the ANOVA analysis revealed only main effects of Block, $F(2, 120) = 9.78$, $p < .001$, $\eta^2_p = .14$, MSE = 317.60, and Group, $F(3, 60) = 12.98$, $p < .001$, $\eta^2_p = .39$, MSE = 1354.61, but no significant interaction between them $F(6, 120) = 1.57$, $p = .163$, $\eta^2_p = .07$, MSE = 317.60. Regarding the group effect, lick cluster sizes were not significantly different between the *Suc-Sacch* and *Malto-Sacch* groups, $t(60) = 1.03$, $p = .308$, Std. Error = 7.51, nor between the *Water-Sacch* and *Sacch-Sacch* groups, $t(60) = 0.58$, $p = .562$, Std. Error = 7.51, while both the *Suc-Sacch* and *Malto-Sacch* groups displayed higher lick cluster sizes than both the *Water-Sacch* and *Sacch-Sacch* groups (smallest $t(60) = 3.53$, $p < .001$, Std. Error = 7.51, for the *Malto-Sacch* vs *Sacch-Sacch* comparison).

Stage 2

Intakes. Inspection of the right-hand panel of Figure 1A shows that intake of saccharin was initially lower, but later recovered, in the *Water-Sacch* group compared to the *Sacch-Sacch* group. This is consistent with the suggestion from Stage 1 of a neophobic reaction to saccharin. Importantly, in the *Suc-Sacch* group intake of saccharin was suppressed relative to the unshifted *Sacch-Sacch* group as well as the *Water-Sacch* group (with the former effect persisting across testing), suggesting that the suppression of intake in the *Sacch-Sacch* group is not simply neophobia. The patterns of intake in group *Malto-Sacch* were similar to those of group *Suc-Sacch* but were numerically smaller.

Consistent with the description above, mixed ANOVA revealed main effects of Block, $F(3.98, 239.0) = 41.24$, $p < .001$, $\eta^2_p = .41$, MSE = 2.61, and Group, $F(3, 60) = 15.13$, $p < .001$, $\eta^2_p = .43$, MSE = 25.33, as well as a Block by Group interaction, $F(11.95, 239.0) = 3.48$, $p < .001$, $\eta^2_p = .15$, MSE = 2.61. Pairwise comparisons revealed that *Suc-Sacch* rats had lower intakes than *Sacch-Sacch* rats in all blocks of Stage 2 (smallest $t(60) = 3.37$, $p < .001$, Std. Error = 1.00, for the final block 6). *Suc-Sacch* rats also had significantly lower

intakes than *Water-Sacch* rats in Blocks 1, 2, and 4 (smallest $t(60) = 2.07$, $p = .043$, Std. Error = 0.69, for block 1), while on the remaining blocks the difference did not reach standard levels of significance on two-tailed tests (smallest $t(60) = 1.80$, $p = .077$, Std. Error = 1.00, for block 5) but would have been significant using one-tailed tests. In addition, *Water-Sacch* rats had lower intakes than *Sacch-Sacch* rats in Blocks 1-5 of Stage 2 (smallest $t(60) = 2.26$, $p = .028$, Std. Error = 1.00, for block 5), but not Block 6 ($t(60) = 1.55$, $p = .127$, Std. Error = 1.00). *Malto-Sacch* rats had lower intakes than *Sacch-Sacch* rats in Blocks 1-5 of Stage 2 (smallest $t(60) = 2.43$, $p = .018$, Std. Error = 1.00, for Block 5), this difference was not significant on a two-tailed test on Block 6 ($t(60) = 1.95$, $p = .056$, Std. Error = 1.00). While intake in *Malto-Sacch* rats was numerically lower than *Water-Sacch* rats in all blocks, this difference was never significant on two-tailed tests (largest $t(60) = 1.90$, $p = .062$, Std. Error = 0.74, for Block 2).

Lick cluster size. Inspection of the right-hand panel of Figure 1B shows that lick cluster sizes in groups *Suc-Sacch* and *Malto-Sacch* were suppressed relative to both *Sacch-Sacch* and *Water-Sacch* on Block 1 but not thereafter. Consistent with this description, mixed ANOVA revealed main effects of Block, $F(3.67, 220.0) = 22.39$, $p < .001$, $\eta^2_p = .27$, MSE = 128.03, and a Block by Group interaction, $F(11.00, 220.0) = 4.36$, $p < .001$, $\eta^2_p = .18$, MSE = 128.03, but no significant effect of Group, $F(3, 60) = 1.39$, $p = .253$, $\eta^2_p = .07$, MSE = 955.21. Pairwise comparisons revealed that in Block 1 lick cluster sizes in group *Suc-Sacch* were smaller than those in group *Sacch-Sacch* ($t(60) = 6.08$, $p < .001$, Std. Error = 3.85) and group *Water-Sacch* ($t(60) = 2.30$, $p = .036$, Std. Error = 3.85), but not in any subsequent block (largest non-significant $t(60) = 1.53$, $p = .131$, Std. Error = 5.83, for the comparison with *Water-Sacch* on block 4; although group *Suc-Sacch* did have significantly *larger* lick cluster sizes than group *Water-Sacch* on block 3 $t(60) = 2.02$, $p = .048$, Std. Error = 6.33). Similarly, in Block 1 lick cluster sizes in group *Malto-Sacch* were smaller than those in group

Sacch-Sacch ($t(60) = 5.93$, $p < .001$, Std. Error = 3.85) and group *Water-Sacch* ($t(60) = 2.30$, $p = .025$, Std. Error = 3.85), but not in any subsequent block (largest $t(60) = 1.70$, $p = .095$, Std. Error = 5.73, for the comparison with *Sacch-Sacch* on Block 2). Group *Suc-Sacch* did have significantly larger lick cluster sizes than group *Malto-Sacch* on Block 3 $t(60) = 2.25$, $p = .028$, Std. Error = 6.33, and there were no other significant differences between groups (largest $t(60) = 1.51$, $p = .137$, Std. Error = 5.83, for the comparison between *Malto-Sacch* and *Suc-Sacch* on Block 4).

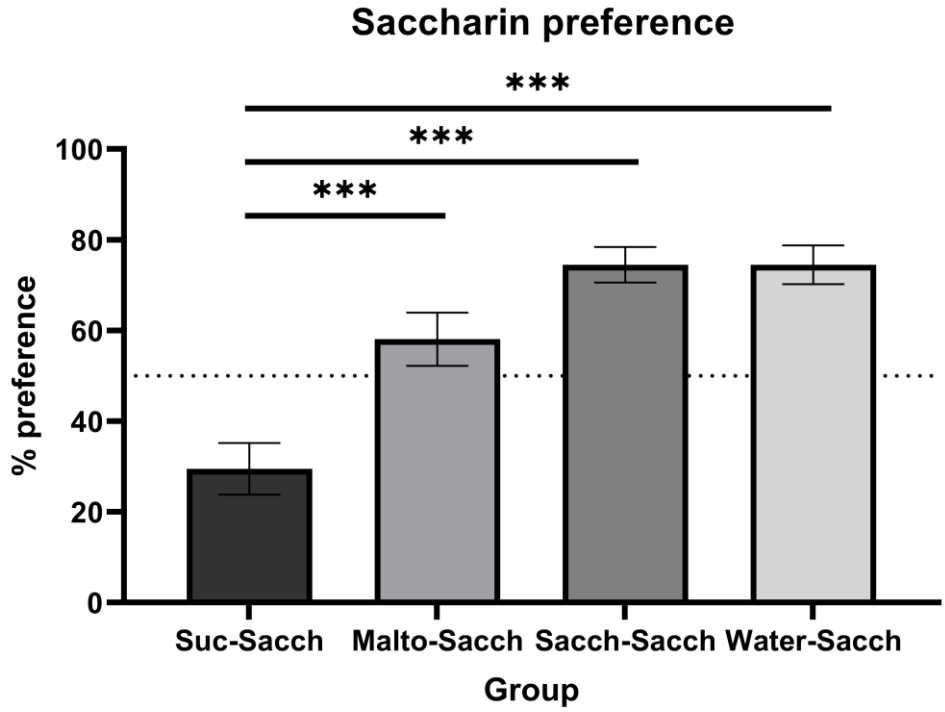

Figure 2. Average (± SEM) preference for 0.4% saccharin relative to 2% sucrose as a percentage of total fluid intake during two-bottle choice tests conducted after the final post-shift session in Stage 2. $n = 16$ for *Suc-Sacch, Malto-Sacch, Sacch-Sacch and Water-Sacch* groups, ***$p < .001$.

Preference tests. Average percentage preference data for 0.4% saccharin over 2% sucrose in two-bottle choice tests are shown in Figure 2. Group *Suc-Sacch* displayed a preference for sucrose, group *Malto-Sacch* appeared indifferent, which groups *Sacch-Sacch* and *Water-Sacch* showed a preference for saccharin. A one-way ANOVA revealed a main effect of Group, $F(3, 60) = 18.0$, $p < .001$, $\eta^2 = .47$, MSE = 0.04. Post-hoc pairwise comparisons revealed that *Suc-Sacch* rats had lower preferences for saccharin relative to sucrose than rats in the other three groups (smallest $t(60) = 4.04$, $p < .001$, Std. Error = 0.071, for the comparison to group *Malto-Sacch*); group *Malto-Sacch* had lower preferences for saccharin relative to sucrose than rats in groups *Sacch-Sacch* and *Water-Sacch* (smallest $t(60) = 2.31$, $p = .024$, Std. Error = 0.071, for the comparison to group *Sacch-Sacch*), which were not significantly different from each other ($t(60) = 0.09$, $p = .992$, Std. Error = 0.071). One-sample t-tests which compared each group's preference against 50% (i.e. indifference) confirmed that *Sacch-Sacch* ($t(15) = 6.27$, $p < .001$, Std. Error = 0.039) and *Water-Sacch* rats ($t(15) = 5.77$, $p < .001$, Std. Error = 0.043) showed a significant preference for saccharin over sucrose. *Suc-Sacch* rats had a significant preference for sucrose over saccharin, $t(15) = 3.61$, $p = .003$, Std. Error = 0.057. *Malto-Sacch* rats did not show a significant preference for either sucrose or saccharin, $t(15) = 1.38$, $p = .19$, Std. Error = 0.058.

Discussion

Following extended exposure to sucrose, switching to saccharin resulted in persistently lower consumption relative to controls receiving prior exposure to either water or saccharin. The switch also produced a reduction in the hedonic value of saccharin indicated by lower lick cluster sizes, but this effect was transient compared to the suppression of consumption. Exposure to maltodextrin had similar effects but to a lesser extent. In addition, following 12 days of post-switch saccharin access, controls showed a preference for saccharin over dilute sucrose that was reversed in animals previously exposed to sucrose and absent in animals previously exposed to maltodextrin. This confirms our prior observation that prior exposure to sucrose (or maltodextrin) results in persistent suppression of consumption after a switch to saccharin and extends this observation to the situation in which exposure is limited to a single 30-min session per day as opposed to the almost 24-h access in earlier studies. The fact that the suppression of consumption and reduction in hedonic value was observed relative to both water-exposed and unshifted saccharin-exposed controls demonstrates that the effects cannot be attributed simply to neophobia. Moreover, the persistence of consumption suppression over extended testing, and the terminal preference test results, are inconsistent with the effect being attributed to the same processes involved in quantitative SNC because that effect is typically short lived.

While the present demonstration that the persistent reduction in saccharin consumption resulting from prior sucrose exposure cannot be explained by a long-term change in the hedonic value of saccharin, neophobia, or "standard" quantitative SNC is informative, it leaves open the question of what mechanism(s) may be responsible. In this light, it is interesting that studies of extinction of conditioned taste aversion display a similar dissociation between long-term changes in consumption and transient effects on hedonic value (e.g. Cantora, López, Aguado, & Rana, 2006; Dwyer, 2009; Dwyer, Gasalla, & López,

2013). For example, when saccharin was paired with lithium chloride-induced malaise, rats subsequently showed suppressed saccharin intakes which did not fully extinguish whereas reductions in lick cluster size did. The similarity in the pattern of results raises the possibility that the downshift from sucrose to saccharin results in an aversion to saccharin. There are multiple possible sources of such an aversion, but perhaps the two most obvious are linked to metabolic effects of extended sucrose exposure and the frustrative effects of reward downshift.

Previous experience with sucrose may establish a conditioned physiological response such as insulin release. When non-caloric saccharin is encountered, its sweet taste may also produce this inappropriate insulin response and cause illness, thus producing an aversion to saccharin which fails to extinguish. In support of this idea, pairing insulin administration with a flavor has been shown to produce an aversion to that flavor in rats (Vanderweele, Deems, & Kanarek, 1990), and we have observed impaired insulin regulation following extended exposure to sucrose in the studies that motivated the current experiment (Kendig et al., 2018; Kendig, Martire, Boakes, & Rooney, 2021). Alternatively, given that SNC is widely assumed to involve a negative emotion – 'disappointment' or frustration (e.g. Papini, 2003) – encountering a novel taste following a qualitative downshift could become associated with frustration to produce an aversion. Although based on simultaneous contrast rather than SNC, there is already evidence for contrast-produced changes in solution value supporting learning (Dwyer, Figueroa, Gasalla, & López, 2018). The fact that extended maltodextrin exposure produces similar metabolic (and cognitive) effects to sucrose (Kendig, Lin, Beilharz, Rooney, & Boakes, 2014) with less apparent consumption suppression effects in the current experiment, may seem to favour the frustration conditioning possibility, but this neglects the possibility that the overlap in sweet taste between sucrose and saccharin might better support conditioned insulin release. Although speculative, the potential link between

fear and frustration (Gray & McNaughton, 2003; Papini, 2003) may also suggest ways to investigate these different putative mechanisms because aversions based on nausea and avoidance based on fear have differing behavioural effects (e.g. Dwyer, Gasalla, Bura, & López, 2017; Parker, 2003).

Setting aside the exact mechanisms involved, the qualitative downshift from sucrose to saccharin examined here had more persistent effects than quantitative shifts that have been the mainstay of SNC research. In turn, this is consistent with the possibility of the effects being driven by the development of an aversion given that quantitative-only shifts would mean that the putative CS (i.e., post-shift solution) would have received extended pre-exposure that would produce latent inhibition to reduce any conditioning effect (Lubow, 1989, 2009).

It will be recalled that a previous quantitative SNC using lick cluster analysis found a relatively persistent effect on cluster size (Grigson & Norgren, 1993). However, in a qualitative SNC study that also employed lick cluster analysis the size effect was short-lived, even though the depression of intakes persisted (Mitchell & Flaherty, 2005). In the light of such apparent inconsistencies and the present results, it would be valuable to carry out a direct comparison between quantitative and qualitative SNC effects.

In summary, shifting rats from sucrose to saccharin exposure results in transient reductions in hedonic value and extended suppression of consumption. The prolonged suppression of saccharin consumption cannot be attributed to either a persistent change in the hedonic value of saccharin or to neophobia, and it also differs from quantitative SNC. The effect may be due to the qualitative downshift from sucrose to saccharin producing (perhaps due to inappropriate conditioned metabolic responses or frustration) an aversion to the saccharin solution.

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