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Insular Cortex and Consummatory Successive Negative Contrast in the Rat

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Abstract

Rats that are expecting a high value reward (e.g., 1.0 M sucrose) show an exaggerated underresponding when they are instead given a low value reward (e.g., 0.15% saccharin), an effect termed successive negative contrast (SNC). In the present experiment, insular cortex-lesioned (ICX) rats showed normal responsivity to sucrose and saccharin prior to the reward downshift. However, when switched from sucrose to saccharin during the postshift trials these rats displayed no evidence of SNC. Indeed, over the downshift trials these ICX rats consistently drank more saccharin than the ICX rats maintained on saccharin throughout the experiment. Potential interpretations are discussed including a lesion-induced impairment in the ability to accurately recognize the novelty of the postshift saccharin stimulus.

Keywords

insular cortex; incentive relativity; neophobia; rats

Introduction

The ability to accurately predict the nutritive quality of a gustatory stimulus is important to ensure that humans and non-human animals make sound decisions about the foods they ingest. Comparisons between foods are therefore of great consequence, allowing for the optimizing of nutritional gains from a given feeding episode. Rats that are expecting a high-value reward (H; e.g., 1.0 M sucrose) make significantly fewer licks when given a low-value reward (L; e.g., 0.15% saccharin) relative to rats that receive an expected L. This phenomenon is known as consummatory successive negative contrast (SNC; Flaherty & Hamilton, 1971; Vogel, Mikulka & Spear, 1968), and demonstrates that rats respond according to the relative value of the current L as determined by a comparison with the absolute value of an expected but absent H.

Because of the relevance to foraging strategies and food selection, a great deal of information has accrued about the behavioral characteristics of SNC (for a review see Flaherty, 1996). Indeed, based on his exhaustive review of the literature, Flaherty described a multistage model for SNC as follows. Consummatory contact with the unexpected L on the first postshift trial

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triggers a comparison with the neural representation of the memory of the expected H. This comparison generates a mismatch that results in a search for the “missing” H. Continuing for a few minutes, the unsuccessful search precipitates a negative emotional response, due to an approach-avoidance conflict between accepting (because of its absolute reward value) and avoiding (because of its relative reward value) the unexpected L. Indicative of conflict resolution, the rat eventually consumes the postshift reward because of the absolute value of the now expected L.

Relative to the number of behavioral and pharmacological investigations, substantially fewer studies have examined the neural substrates of SNC. One brain area of particular relevance to present purposes is the parvicellular region of the ventral posteromedial nucleus of the thalamus, also known as the gustatory thalamus (GT). Reilly and Trifunovic (1999) and Sastre and Reilly (2006) found that GT-lesioned (GTX) rats that were switched from H to L rapidly (i.e., on the first postshift trial) adjusted their responding to the same level as their unshifted counterparts maintained on L throughout the experiment. Thus, irrespective of whether they had prior experience with H or not, the GTX rats responded according to the absolute value of the current reward. That is, GT lesions prevent the occurrence of SNC. Providing evidence that the absence of SNC in GTX rats was not due to an impairment of gustatory memory, Reilly and Trifunovic (2003) favored an explanation in terms of a lesion-induced disruption of the reward comparison mechanism that underlies the phenomenon. This disruption, it was argued, preempts both the search for the missing H and the ensuing approach-avoidance conflict and consequently the GTX rats respond according to the absolute value of unexpected L. Another form of consummatory contrast, anticipatory negative contrast (ANC), is also dependent upon a determination of the relative values of two rewards. In the ANC task, rats suppress responding to L when it is sequentially paired with H in a one-trial per day procedure (Flaherty & Checke, 1982). Supportive of the view that SNC and ANC each involve a similar reward comparison mechanism, GT lesions also prevent the occurrence of ANC (Reilly, Bornovalova & Trifunovic, 2004; Reilly & Pritchard, 1996; Schroy et al., 2005).

The GT is reciprocally connected with the gustatory area of the insular cortex (IC; Cechetto & Saper, 1987; Kosar, Grill, & Norgren, 1986), which encourages the view that the IC also may be involved in SNC. Indeed, it is possible that the SNC deficit displayed by GTX rats results from a lesion-induced disruption in the transmission of gustatory information to an IC reward comparison mechanism. Using the same behavioral procedure as Reilly and Trifunovic (1999) and Sastre and Reilly (2006), the present experiment examined the influence of bilateral excitotoxic lesions of the IC on consummatory SNC.

Method

Subjects

Forty-one male Sprague-Dawley rats, weighing ~300 g, were obtained from Charles River Laboratories (Wilmington, MA) and individually housed in stainless steel hanging cages in a vivarium maintained on a 12 hr alternating light-dark cycle (lights on at 7:00 AM). All treatments and behavioral testing occurred during the light phase of the cycle. The rats had free access to food and water at all times except during behavioral testing when they were maintained at 80% body weight by a once per day feeding (~15 g lab chow) given a minimum of 30 min after completion of the behavioral testing. The Institutional Animal Care and Use Committee of the University of Illinois at Chicago approved the experimental protocols and the care and use of the rats conformed to the guidelines of the American Psychological Association (1996).

Surgery

Twenty-one rats were given bilateral lesions of the IC (Group ICX). Each rat was anesthetized with sodium pentobarbital (injected i.p. at 50 mg/kg), fixed in a stereotaxic apparatus with blunt ear bars, and the head was shaved and sterilized with Povidone (Qualitest Pharmaceuticals, Huntsville, AL). A midline incision was made to expose the skull surface, and the head was leveled between bregma and lamda by adjusting the level of the bite bar. Body temperature was monitored with a rectal thermometer and maintained at 37°C with a heating pad. Trephine holes were drilled above the IC in each hemisphere. A glass micropipette (~70 μm tip diameter) filled with 0.15 M N-methyl-D-aspartic acid (NMDA; Sigma, St. Louis, MO), was lowered, in turn, to two lesions sites in each hemisphere. At site 1 (AP +1.2 mm; ML ±5.2 mm; DV −5.0 mm) a 10-min iontophoretic current was applied using a Midgard precision current source (Stoelting, Wood Dale, IL); site 2 (AP +1.2 mm; ML ±5.2 mm; DV −4.3 mm) involved a 6-min infusion of NMDA. After the final infusion, the incision was closed with wound clip and the rat was allowed to recover under a heat lamp until it regained consciousness.

Ten rats received surgical treatments that were identical in all respects to the ICX surgeries with the exception that no NMDA infusions took place. An additional 10 rats were anesthetized but did not undergo any further treatment. These two control groups were collapsed together into a single group, Group SHAM. All subjects were given a minimum of 1 week to recover from surgery before undergoing weight reduction in preparation for the behavioral procedure.

Apparatus

Six identical operant chambers (Med Associates Inc., St. Albans, VT), housed inside sound-attenuating cubicles, were used. Each chamber had a stainless steel grid floor, aluminum sidewalls, and the front and back walls were, like the ceiling, made of clear Plexiglas. An oval access hole (1.3 × 2.6 cm; width × height) was centered on the right side wall of each chamber, 6.0 cm above the floor. The stainless steel spout of a retractable stimulus bottle was accessible through this hole. Tongue contact with the spout was monitored using a lickometer circuit. A shaded bulb, which reflected light off the ceiling, was located directly above the cage speaker on the left sidewall. Inside each operant chamber, white noise provided an ambient sound level of 70 dB background. Control of events in the chambers and collection of the data were carried out on-line by a computer running Med-PC software (Med Associates Inc.).

Procedure

Normal rats sometimes show large individual differences in saccharin consumption during the first few days in the test apparatus when they are learning to drink from the retractable spout, an effect we attribute, in part, to taste neophobia. To minimize the occurrence of this disruptive influence on performance in the test apparatus, we followed the procedure of Sastre and Reilly (2006) and gave the randomly assigned rats access to 5 ml of either 0.15% saccharin (Unshifted condition; 10 SHAM, 10 ICX) or 1.0 M sucrose (Shifted condition; 10 SHAM, 11 ICX) in the home cage on the day before the first context habituation session.

All subjects were given 5 min access to the illuminated test chambers on each of the next two days. Beginning the next day, SHAM and ICX rats in the Unshifted control condition were given 5-min per day access to 0.15% saccharin for a total of 20 trials on a one trial per day protocol; the SHAM and ICX rats in the Shifted experimental condition were given 5-min daily access to 1.0 M sucrose on preshift Trials 1-14 and then switched to 0.15% saccharin on postshift Trials 15-20. The number of licks made during each trial was measured as the dependent variable.

Histology

After the conclusion of behavioral testing, the rats were given a lethal injection of sodium pentobarbital (100 mg/kg) and perfused transcardially with 4% buffered formalin. The brains were extracted and stored in 4% buffered formalin for 2 days, followed by two days in 20% sucrose. The brains were then blocked and frozen in a cryostat, and tissue sections were taken through the IC and mounted on slides. These sections were stained with cresyl violet and viewed under a light microscope. Photomicrographs were taken using Q-Capture (Quantitative Imaging Corporation; Burnaby, BC), and serial schematics created with Photoshop (Adobe; Seattle, WA) based upon images from Paxinos and Watson (2005)

Results

Anatomical

The target of the excitotoxic lesions in this experiment was the gustatory region of the IC which extends (Kosar et al., 1986) ~1 mm both anterior and posterior from the intersection of the middle cerebral artery and the rhinal fissure (approximately 0.8 mm anterior to bregma). Serial schematics of the largest and smallest IC lesions that were analyzed and representative lesions are shown in Figure 1A; Figure 1B shows a photomicrograph of an intact IC and Figure 1C displays a representative IC lesion. As shown, NMDA-induced damage was primary located within the IC. Some lesions spread into the somatosensory cortex, the claustrum and the piriform cortex but such damage was not consistently found across subjects. Rats with lesions that were either unilateral (n = 2) or subtotal (n = 4) were excluded and the final sample sizes were: 10 SHAM-Unshifted, 10 SHAM-Shifted, 8 ICX-Unshifted, and 7 ICX-Shifted.

Behavioral

During the preshift phase (Trials 1-14), all groups showed a gradual increase in the number of licks made for either L or H (see Figure 2). This impression of the data was confirmed with an analysis of variance (ANOVA) that found a main effect of Trial, $F(13, 390) = 42.15$, $p < .001$. Additionally, a main effect of Condition (Unshifted, Shifted), $F(1, 31) = 454.84$, $p < .001$, indicated that the Shifted rats made more licks for H than the Unshifted rats made for L. There was, however, no main effect of IC lesions on responding ($F > 1$), and no interaction involving the Lesion factor (*ps* > .25). These results confirm that axon-sparing lesions of the IC have no influence on the detection or responsivity to the absolute value of L (0.15% saccharin) or H (1.0 M sucrose) during the preshift phase.

When the SHAM-Shifted subjects were given L instead of H on Trial 15, they demonstrated SNC by decreasing their consumption below the level of the SHAM-Unshifted subjects. An ANOVA conducted on the six post-shift trials uncovered a Lesion X Condition X Trial interaction, $F(5, 155) = 16.59$, $p < .001$. To detect the source of the significant interaction, planned comparisons (F-test using the adjusted error term taken from the overall ANOVA) of the SHAM data revealed that the SHAM-Shifted rats made significantly fewer licks for L than did the SHAM-Unshifted subjects on Trials 15 and 16 (*ps* > .002), but not on Trials 17-20 (*ps* > .25). The performance of the SHAM-Unshifted subjects and the ICX-Unshifted rats did not differ during the post-shift phase of the experiment ($F < 1$).

While the SHAM-Shifted subjects showed an exaggerated decrease in licks for L, the ICX-Shifted rats failed to show SNC, making more licks for L than the ICX-Unshifted rats on Trials 15-18 and 20 (*ps* < .03). On Trial 19, the performance of the two sets ICX rats was not significantly different ($p = .09$). As is clear from inspection of Figure 2, there was a dramatic difference in licking for L in the SHAM-Shifted and ICX-Shifted groups. That is, the ICX-Shifted rats made significantly more licks for L than the SHAM-Shifted subjects on Trials 15-18 (*ps* > .001), but not on the final two trials (*ps* > .05). So, the SHAM-Shifted and ICX-

Shifted rats eventually made the same number of licks for L, but the SHAM subjects achieved this level of performance by gradually increasing their postshift responding over trials, $F(5, 45) = 17.49$, $p < .001$, whereas the ICX rats showed a gradual decrease of licking to achieve the same baseline, $F(5, 30) = 2.77$, $p < .05$. Overall, the postshift performance indicates the presence of SNC in SHAM subjects and the absence of SNC in ICX rats.

Discussion

As indicated by an exaggerated under-responding to the unexpected L on the initial postshift trials, neurologically intact (SHAM) rats displayed SNC. The ICX rats, however, showed no evidence of SNC. In fact, the ICX-Shifted rats over-responded for L on the postshift trials. Given the anatomical connectivity between the GT and IC, it is tempting to suggest that the IC, like the GT (e.g., Reilly & Trifunovic, 1999, 2003), is a neural component of the comparison mechanism that computes the relative value of gustatory rewards over time. If this is the case, then presumably these two structures are performing interdependent not identical functions because if the latter were true then one might expect that the intact GT would compensate for the absent IC and that ICX rats would show normal SNC. That this does not occur encourages the view that the GT and IC are each involved in different aspects of the reward comparison process.

Beyond the present report, some recent findings shed additional light on the role of the IC in taste-guided behavior and a brief survey of these results provides reason to question the preceding analysis of the role of the IC in SNC. Lin, Roman, St. Andre and Reilly (2009) found that IC lesions disrupted the initial occurrence of, but not the recovery from, taste neophobia. Of further interest, the same ICX rats displayed normal neophobia to both an aqueous odor and an oral trigeminal stimulus. We interpret this pattern of impaired and spared neophobic reactions as a lesion-induced disruption of the perception of taste novelty. Second, this hypothesis anticipates that ICX rats should only show impaired CTA acquisition when the taste CS is novel; when the taste is familiar ICX rats would be expected to acquire the aversion at a similar slow rate as normal rats that have been preexposed to the taste CS. This prediction was confirmed by Roman, Lin and Reilly, 2009 (see also Kiefer & Braun, 1977, Roman & Reilly 2007). The results obtained from neophobia and CTA experiments encourage the view that ICX rats treat novel taste stimuli as though they were familiar (Reilly, 2009).

In the present experiment IC lesions not only disrupted SNC but the pattern of postshift responding in ICX rats was different from that found in rats with GT lesions (Reilly & Trifunovic, 1999; Sastre & Reilly, 2006). Whereas ICX rats showed a gradual reduction of responding for L over the postshift trials relative to the ICX-Unshifted rats, GTX-Shifted rats in our earlier experiments immediately reduced their lick rate to that of the GTX-unshifted rats on the first postshift trial. Although this may represent a quantitative difference between the effects of the two types of lesions, the foregoing analysis encourages the view that an IC lesion-induced disruption of taste novelty is contributing to the pattern of results obtained in the present experiment. That is, the patterns of postshift responding may represent a qualitative difference between ICX and GTX rats. If this analysis is correct, then taste neophobia has a greater role in SNC than previously suspected.

Few studies have examined whether the novelty of the postshift solution influences SNC. Indeed, we are aware of only one such report. Meinrath and Flaherty (1988) approached this issue by exposing subjects to a variety of preshift flavors on the assumption that such experience would reduce the influence of taste neophobia on the postshift L trials and thereby reduce the magnitude of SNC. Although Meinrath and Flaherty found that prior taste experience does influence postshift responding, the result was a reduction, not an elimination of SNC. Perhaps not unsurprisingly, the authors rejected the idea that a neophobic reaction to the postshift L

was responsible for SNC. However, there are reasons to suspect that this rejection may have been premature. First, the preshift and postshift solutions used by Meinrath and Flaherty were different concentrations of the same taste stimulus (32% and 4% sucrose, respectively). Such a "within-stimulus" change might be expected to support less of a postshift neophobic reaction than occurs in our standard procedure which involves a "between-stimulus" switch from sucrose to saccharin, which is over-consumed by ICX rats when it is novel (Lin et al. 2009; Roman et al., 2006). That is, the bittersweet taste of saccharin might engage a more pronounced neophobic response than occurs following a reduction in the sweetness of sucrose. Second, the experimental manipulation used by Meinrath and Flaherty involved seven different flavors (i.e., maple, banana, lemon, chocolate, anise, orange and cherry) of the preshift solution (32% sucrose). It is possible that this procedure may have non-maximally influenced the neophobic reaction to the postshift solution (4% sucrose). Thus, there are reasons to suppose that neophobia has a meaningful, if under-appreciated role in SNC, a role that may be more pronounced when the expectancy violation involves a between-stimulus rather than a within-stimulus downshift in reward value.

In the present experiment, excitotoxic lesions of the IC were found to have no influence on responsivity for sucrose or saccharin during the preshift trials. As noted in the Procedure section, rats were given pretraining access in the home cages to their preshift solution (either sucrose or saccharin) to obviate the problem that neurologically intact animals tend to show large degrees of individual variability in saccharin intake during the first few preshift trials in the test apparatus. As in previous studies (e.g., Sastre & Reilly, 2006), this treatment had the desired effect on the performance of the SHAM rats. Absent this pretraining access to the preshift solutions it would have been difficult, if not impossible, to interpret with any degree of confidence any differences in the performance of the SHAM and ICX rats over the initial preshift trials. The experimental design thus ensured that any lesion-induced deficit in the neophobic reactions to the test solutions would likely not be detected during the preshift trials. It might also be noted that the brief duration of the SNC trials (5 min) relative to the longer duration of trials (15 min) in our neophobia experiments (Lin et al., 2009) would tend, particularly at the outset of training, to minimize the likelihood of finding group differences in saccharin intake. However, once the animals became familiar with the apparatus and the task demands during the preshift trials, these constraints would not mask the occurrence of neophobia during the initial postshift trials. As argued above, although IC lesions, like GT lesions, might disrupt the reward comparison mechanism that governs expression of SNC in normal rats, we propose that the most parsimonious explanation of the present finding is a lesion-induced impairment in the inability to accurately recognize the novelty of the postshift saccharin stimulus. If this analysis is correct, then neophobia has a more significant role in the expression of SNC than previous suspected.

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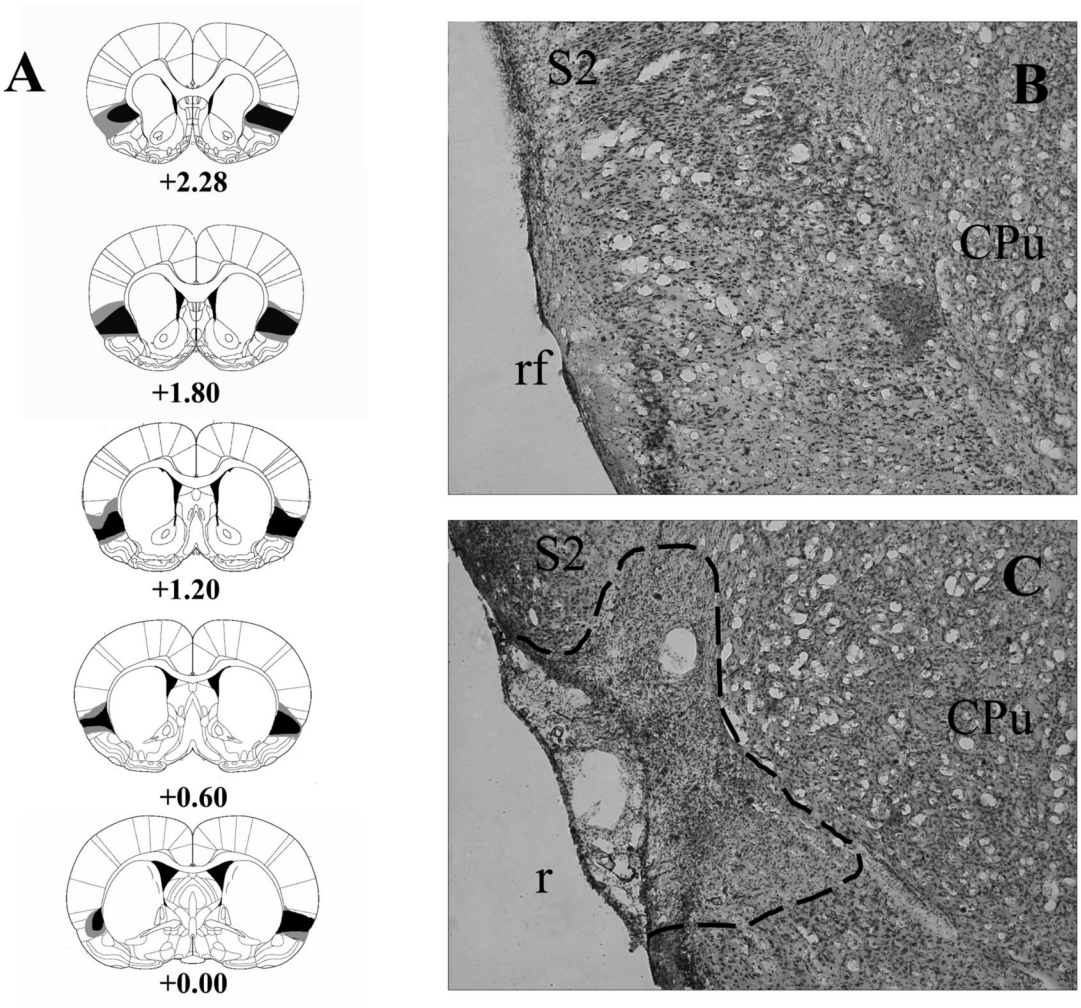

Figure 1.

(A) Schematic reconstructions of the smallest (black) and largest (gray) neurotoxic lesions of the insular cortex (IC; adapted with permission from the Paxinos & Watson, 2005). The numbers (0.00 mm, +0.60 mm, +1.20 mm, +1.80 mm, +2.28 mm) beneath each diagram refer to the anteroposterior coordinates relative to bregma. (B) Digitized photomicrograph of the IC (left hemisphere) taken from a neurological intact subject. (C) Representative neurotoxic lesion of the IC of an experimental rat. The dashed line outlines the extent of the cell loss. CPu, caudate putamen; rf: rhinal fissure; S2, secondary somatosensory cortex.

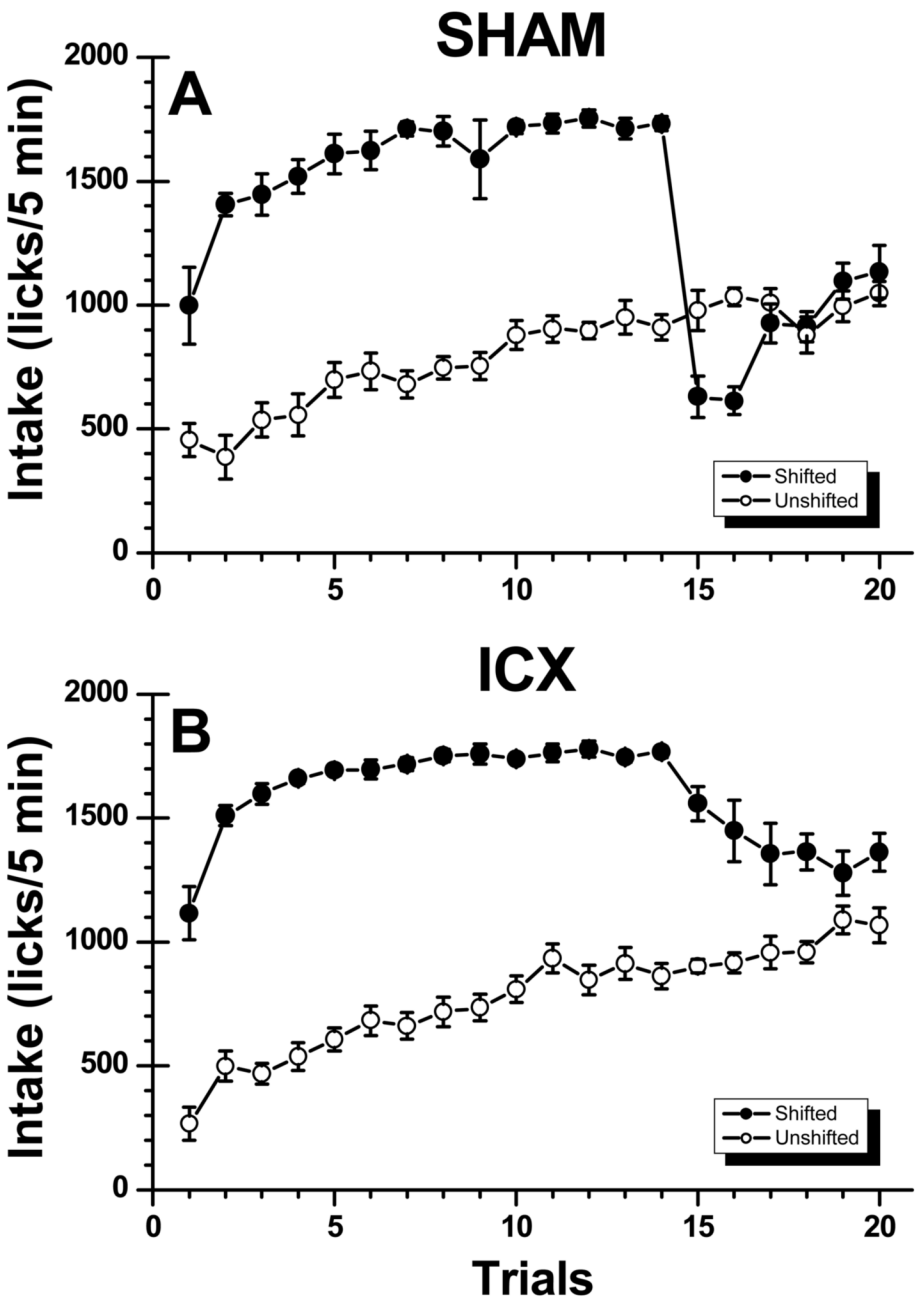

Figure 2.

Mean number of licks (± SE) during the 20, 5-min trials for SHAM subjects (Panel A) and the ICX rats (Panel B). In the Unshifted condition, 0.15% saccharin was presented through the experiment. On the other hand, rats in the Shifted condition were given 1.0 M sucrose during the first 14 trials and 0.15% saccharin in the following 6 trials.