Role of the insular cortex in morphine-induced conditioned taste avoidance

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ABSTRACT

The present study investigated the role of the insular cortex (IC) in morphine-induced conditioned taste avoidance. The results of Experiment 1 revealed that IC lesions impaired taste neophobia, retarded acquisition of conditioned saccharin avoidance and apparently attenuated the magnitude of that response at asymptote. Using neurologically intact subjects, Experiment 2 established that a safe and familiar saccharin stimulus supports substantially weaker conditioned avoidance at asymptote than does a potentially dangerous and novel saccharin stimulus. This pattern of results does not support the hypothesis that IC lesions disrupt the learning mechanism responsible for morphine-induced conditioned taste avoidance. The data are, however, consistent with the hypothesis that IC lesions impair the perception of the danger and/or novelty of the taste stimulus.

Keywords**:** morphine, avoidance, insular cortex, stimulus preexposure, rat

1. Introduction

In a procedure identical to that used to induce conditioned taste aversions (CTAs), rats will avoid drinking a taste solution that precedes administration of a psychoactive drug (e.g., amphetamine, cocaine, LSD and morphine; Berger, 1972; Booth, Pilcher, D'Mello & Stolerman, 1977; Cappell & LeBlanc, 1971; Domjan & Siegel, 1983; Farber, Gorman & Reid, 1976; Ferrari, O'Connor & Riley, 1991; Goudie, Dickins & Thornton, 1978; Le Magnen, 1969; Parker, 1996; Vogel & Nathan, 1975; also see Gamzu, Vincent & Boff, 1985; Goudie, 1979; Hunt & Amit, 1987; Riley & Tuck, 1985). In the parlance of Pavlovian conditioning, the taste is termed the conditioned stimulus (CS) and the drug is termed the unconditioned stimulus (US). Drug-induced taste avoidance was initially viewed as a CTA (i.e., a consequence of an association between a taste CS and the aversive properties of the drug US). However, research began to emerge that cast doubts on this analysis. In particular, drugs of abuse, unlike the nausea-inducing agent lithium chloride, do not support the acquisition of a conditioned disgust reaction (as indicated by aversive orofacial responses) when the associated taste CS is infused into the mouth (e.g., Parker, 1982, 1988, 1991, 1993, 1996, 2003). It would appear, then, that a drug of abuse US influences intake of a taste CS in manner that is qualitatively different from that of a nausea-inducing US.

The goal of the present research was to determine the role of the gustatory area of the insular cortex (IC) in morphine-induced conditioned taste avoidance. In the first studies to examine this issue, Mackey, Keller and van der Kooy (1986) and Zito, Bechera, Greenwood and van der Kooy (1988), who each viewed the task as a CTA

keyed off the aversive properties of the morphine US, established that lesions of the IC[1] disrupted performance on the two-bottle test trial. However, no data were reported for the conditioning trials in these experiments. So, critical for contemporary analysis of the role of the IC in taste-guided behavior as explained below, it is not known whether the test trial deficit was predicated on a lesion-induced over-consumption of the saccharin CS on the first conditioning trial. Thus, it is difficult to appreciate the nature of the underlying deficit in the IC-lesioned (ICX) subjects in these initial reports. In the next morphine study of this type, Geddes, Han, Baldwin, Norgren and Grigson (2008; Experiment 2), using non-naïve subjects, reported that IC lesions "fully prevent" the suppressive effects of morphine. There are, however, some difficulties with this view. The non-lesioned (SHAM) rats showed the expected pattern of performance: no between-group intake differences on the initial exposure to saccharin and, over trials, saccharin avoidance in the drug-injected group and no saccharin avoidance in the saline-injected control group. Thus, after 6 CS-US pairings the SHAM-Saline group drank ~12 ml of saccharin on trial 7 whereas the SHAM-Morphine rats consumed ~5 ml. On the other hand, the performances of the ICX-Saline and the ICX-Morphine groups were indistinguishable such that on trial 7 each group showed a comparable amount of avoidance by drinking ~6 ml of saccharin. It is not clear how saline (an inert substance administered in the no-US condition) (i) caused the same degree of saccharin avoidance in the ICX rats as morphine did in the SHAM-Morphine and the ICX-Morphine groups and (ii) resulted in different levels of saccharin intake in the ICX-Saline rats relative to the SHAM-Saline subjects. We have no explanation for the pattern of

[1] More specifically, Mackey et al. (1986) and Zito et al. (1988) targeted their lesions at the "visceral" cortex.

results obtained in the ICX rats in this experiment. Presumably, some aspect of their prior experience, and impaired performance, in a procedurally identical task involving cocaine-induced Polycose avoidance (in Experiment 1 of the same study) may have carried-over and influenced their behavior in the later morphine-induced saccharin avoidance experiment. Nonetheless, Geddes et al. concluded that the IC is a critical component of the learning mechanism (reward comparison, in their view) responsible for morphine-induced taste avoidance. In the most recent study in this literature, Roman and Reilly (2009), using experimentally naïve animals, found that ICX rats showed no avoidance of the taste CS following seven saccharin-morphine trials; the saline-injected ICX rats consumed maximal amounts of saccharin (10-12 ml) on each of the 5-min acquisition trials. Roman and Reilly entertained two interpretations of the performance of ICX rats. The first interpretation favored, like Geddes et al., a disruption of the learning mechanism responsible for drug-induced taste avoidance. The second interpretation, derived from work on the effects of IC lesions on taste neophobia and CTA (Lin, Roman, St. Andre & Reilly, 2009a; Roman, Lin & Reilly, 2009), involved an IC lesion-induced deficit in the perception of the danger and/or novelty (henceforth danger/novelty) of the taste stimulus.

The two experiments reported in the present article were intended to evaluate the merits of these two interpretations (or hypotheses) of IC function accounts. To that end, two changes were made to the design of our earlier experiment in order to maximize the opportunity to detect the group differences anticipated by each hypothesis. First, assuming that taste safety/familiarity influences acquisition of morphine-induced saccharin avoidance in the same way as it does CTA (i.e., delays but does not prevent

learning – an effect termed latent inhibition; e.g., Lubow, 1989, 2009), the number of saccharin-morphine conditioning trials was double that employed by Roman and Reilly (2009) in order to provide more opportunity to detect whether ICX rats eventually learn the task to the same performance levels as normal animals. The second design modification concerns the duration of CS access each trial. Our earlier experiment, like that of Geddes et al. (2008), involved 5-min CS access per trial. Recent research (Lin, Roman & Reilly, 2009b) indicates that such a short access time may obscure important group differences, which become evident only when the CS is available for 15 min, because of a tendency towards ceiling effects in the fluid consumption of the water deprived rats. Additionally, to maintain comparability with our earlier experiment, the rats were trained (trials 1-13) using our standard 15-mg/kg dose of morphine. Thereafter, the dose was increased to 30 mg/kg (trials 14-24) to determine whether the obtained lesion effects were dependent upon the magnitude of the US.

2. Results

2.1. Experiment 1

The two hypotheses of the effects of IC lesions on morphine-induced taste avoidance (deficits in the learning mechanism or in the perception of taste danger/novelty) make different predictions regarding the performance of the ICX rats on this task. Irrespective of the nature of the underlying learning mechanism, if, as concluded by Geddes et al. (2008), IC lesions “fully prevent” this form of taste avoidance, then ICX rats should, in the most extreme case, be incapable of avoiding the saccharin CS no matter how many conditioning trials are given. More specifically, the performance of the ICX-morphine

rats should not be significantly different from that of the ICX-saline subjects, and each of these ICX groups would be expected to match the saccharin intake of the non-lesioned (SHAM)-saline subjects (i.e., to show no conditioned taste avoidance). Of course, the actual magnitude of this predicted lesion-induced deficit, whether complete or partial, would be indicated by the difference in performance between the ICX-morphine rats and the SHAM-morphine subjects. It should understood that any form of learning deficit could only be expected to disrupt behavior after, at minimum, the first CS-US pairing has been experienced. That is, a learning deficit account implicitly precludes the occurrence of an IC lesion-induced CS intake deficit on the first conditioning trial. On the other hand, a deficit in the perception of the danger/novelty of taste stimuli predicts that ICX rats would show significantly elevated intake of saccharin on the first conditioning trial (i.e., a deficit of taste neophobia) which, in turn, delays the acquisition of conditioned taste avoidance due to a latent inhibition-like effect. However, given more conditioning trials ICX-morphine rats should, eventually, avoid the saccharin CS to the same performance asymptote as the SHAM-morphine subjects.

2.1.1. Anatomical

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As defined by Kosar, Grill and Norgren (1986; see also Nakashima, Uemura, Yasui, Ozaki, Tabata & Taen, 2000), the gustatory portion of the IC extends along the dorsal aspect of the rhinal fissure ~1.00 mm either side of the middle cerebral artery. Serial schematic reconstructions of the largest (grey) and smallest (black) IC lesions are shown in Fig. 1A. Figs. 1B and 1C show digital photomicrographs of the IC from a SHAM and an ICX rat, respectively. The location of the lesions was determined by the

presence of gliosis and the absence (or shriveling) of cell bodies. Data from 4 ICX rats with lesions that were either unilateral or undersized were excluded from further analyses. The remaining ICX rats had bilateral lesions that damaged the majority of the gustatory IC ranging from 0.00 to 2.20 mm relative to bregma. The lesions spread from the outer cortical layers to the external capsule and, in some cases, caused damage to the claustrum, piriform cortex and secondary somatosensory cortex. However, these encroachments were small and not consistently found across subjects. Thus, in location and size the lesions in this experiment were highly comparable to those used in our other IC research (e.g., Lin et al., 2009a; Roman et al., 2006; 2009; Roman & Reilly, 2007). The final number of subjects in each group was: ICX-Saline, 8; ICX-Morphine, 8; SHAM-Saline, 9; SHAM-Morphine, 10.

2.1.2. Behavioral

Fig. 2 illustrates mean volume of the saccharin CS consumed by SHAM subjects and ICX rats across trials according to the type of US injection (saline or morphine) and morphine dose (15 mg/kg and 30 mg/kg). It is evident from inspection of Fig. 2 (upper panel) that the SHAM subjects displayed a large neophobic response to saccharin on their first exposure, an effect that rapidly habituated in the saline-injected subjects to reach a stable intake level of ~23 ml trial. On the other hand, the morphine-injected SHAM subjects showed evidence of conditioned avoidance after a single CS-morphine pairing and, as a group, reached asymptote levels of avoidance (~5 ml intake) after 2 CS-US pairings. It is also apparent that increasing the morphine dose from 15 mg/kg to 30 mg/kg had little discernible influence on the level of saccharin avoidance in the

SHAM subjects. The performance of the ICX rats (see Fig 2; lower panel) was markedly different from that of the SHAM subjects in a number of ways. First, the ICX rats showed little evidence of neophobia on their first exposure to the potentially dangerous and novel saccharin solution. Furthermore, saccharin intake in the ICX-Saline rats stabilized at a level comparable to that found in the SHAM-Saline subjects. Second, saccharin avoidance in the ICX-Morphine rats was maximal after 3 CS-US pairings. Third, the ICX-Morphine rats never achieved the same degree of saccharin avoidance as the SHAM-Morphine subjects. Finally, as with the SHAM subjects, there was little indication that saccharin intake changed as a consequence of the increase in morphine dose over the last 10 trials of the experiment. As detailed below, these impressions of the behavioral results were substantiated by statistical analyses.

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Using Statistica© software (StatSoft, Tulsa, OK), a three-way analysis of variance (ANOVA) conducted on data from the 15 mg/kg morphine trials found significant main effects of Lesion (SHAM vs. ICX), $F(1,31) = 19.05$, $p < .001$, US (Saline vs. Morphine), $F(1,31) = 79.97$, $p < .001$, and of Trial (Trials 1-14), $F(13,403) = 2.75$, $p < .001$. The same analysis also revealed significant interactions of Lesion x US, $F(1,31) = 13.17$, $p < .01$, US x Trial, $F(13,403) = 12.77$, $p < .001$, Lesion x Trial, $F(13,403) = 2.39$, $p < .01$, and, more importantly, a significant Lesion x US x Trial interaction, $F(13,403) = 3.98$, $p < .001$. Post hoc comparisons (i.e., simple main effects) revealed that ICX rats consumed significantly more saccharin than SHAM subjects on Trial 1, $F(1,403) = 102.24$, $p < .001$, when the solution was potentially dangerous and novel for all animals. Furthermore, while both SHAM-Morphine and ICX-Morphine rats overall drank

significantly less saccharin than their respective saline-injected control animals, the ICX-morphine rats showed significantly less conditioned avoidance than the SHAM-Morphine subjects ($p < .001$). That is, ICX-Morphine rats consumed substantially more saccharin (~10 ml) relative to the SHAM-Morphine subjects. Follow-up analyses of the triple interaction further revealed that although CS intake was significantly suppressed by morphine from Trial 2 through Trial 14 in the SHAM animals ($ps < .05$), a significant suppression was found on Trials 3 - 14 in the ICX-Morphine group ($ps < .05$). Overall, then, IC lesions retarded the acquisition of conditioned taste avoidance as well as the magnitude of that response at asymptote.

To determine whether the increase in morphine dose influenced learning/performance, a four-way ANOVA was conducted on data from the final four trials of each dose (i.e., Trials 11-14 vs. Trials 21-24). This analysis found significant main effects of Lesion, $F(1,31) = 13.06$, $p < .01$, and US $F(1,31) = 85.62$, $p < .001$, and of Trial, $F(10,310) = 3.18$, $p < .001$. There was, however, no significant main effect of Dose ($F < 1$) and no significant Lesion x US x Trial x Dose interaction ($F < 1$). Thus, increasing the morphine dose from 15 mg/kg to 30 mg/kg had no discernible influence on the level of saccharin avoidance in SHAM or ICX rats.

The results of the present experiment are very different from those reported by Geddes at al. (2008) and Roman and Reilly (2009). With regard to the data obtained from normal animals, the SHAM-saline rats displayed a substantial neophobic reaction when they encountered the potentially dangerous and novel 0.15% saccharin solution on trial 1. This innate avoidance response habituated rapidly and asymptote was achieved over the next few trials, as the saccharin became more safe and familiar to the

SHAM-Saline subjects. Conversely, the SHAM-Morphine rats quickly learned to avoid the saccharin CS and, as a group, reached maximal avoidance by the third acquisition trial. Thus, in comparison with the two groups of SHAM subjects at performance asymptote, the magnitude of the conditioned taste avoidance response was approximately twice the size of that reported in neurologically intact subjects by Geddes et al. and Roman and Reilly. Turning now to the ICX rats, three aspects of the performance of these animals are theoretically important. First, relative to the SHAM subjects, each set of ICX rats over-consumed the saccharin solution on trial 1. This finding replicates with 0.15% saccharin an effect that we previously reported with 0.5% saccharin and which we interpret as a lesion-induced deficit in the perception of the danger/novelty of the taste stimulus (Lin et al., 2009a); a deficit in the learning mechanism does not predict any lesion-induced differences on trial 1. Second, compared to the SHAM-Morphine subjects, the ICX-Morphine rats were slow to acquire the conditioned taste avoidance response. These first two deficits are predicted on the basis of a lesion-induced disruption in the perception of the danger/novelty of taste stimuli. The third important aspect of the ICX data is that the ICX-Morphine rats did no achieve the same performance asymptote as the SHAM-Morphine rats. After 13 trials with our standard morphine (15 mg/kg) US and an additional 10 trials with a higher dose of morphine (30 mg/kg), the CS intake of the ICX-Morphine group was consistently 7-10 ml higher than that of the SHAM-Morphine group. The results, then, indicate that in addition to attenuating the neophobic response on trial 1 and delaying acquisition of the conditioned avoidance response, IC lesions are seemingly affecting asymptote levels of performance on the task. This latter deficit is not anticipated by the danger/novelty

hypothesis of IC function and suggests that the lesions might be influencing a second process relevant to morphine-induced conditioned taste avoidance. An immediately obvious candidate is that the lesions may blunt sensitivity to the US property of morphine that is responsible for taste avoidance. However, this account appears untenable because Lin et al. (2009b) found that ICX rats showed normal acquisition of morphine-induced odor avoidance in a procedure that was identical to the taste avoidance task. Thus, the failure of ICX rats to show the same level of taste avoidance as SHAM-Morphine subjects cannot be reduced to a deficit in the perception of, or responsivity to, the US property of the morphine drug state.

2.2. Experiment 2

The finding that the ICX-Morphine rats did not avoid the saccharin CS to the same performance asymptote as the SHAM-Morphine subjects is not predicted by the danger/novelty hypothesis of IC function. This finding encourages the view that the lesion is disrupting a second process that is essential for morphine-induced conditioned taste avoidance learning. However, before considering the merits of a “two deficit” account we need to examine an assumption upon which the first experiment was based. As previously stated, we assumed that taste familiarity (induced in Experiment 1 by the IC lesion) would have the same effect on conditioned taste avoidance as it does on CTA (where it delays but does not prevent acquisition). Experiment 2 examined this assumption by investigating the influence of taste familiarization on the acquisition of morphine-induced taste avoidance in neurologically intact rats. Should the familiar CS group achieve the same level of saccharin avoidance at asymptote as the novel CS

group we would entertain the view that IC lesions also disrupt a second process necessary for morphine-induced taste avoidance. On the other hand, if the familiar CS group shows significantly less morphine-induced saccharin avoidance (i.e., greater saccharin intake) at performance asymptote than the novel CS group we would be inclined to the view that the behavior of the ICX rats in Experiment 1 could be explained in terms of a single deficit in the perception of the danger/novelty of the taste CS. In our examination of the effects of CS preexposure on CTA acquisition in rats with lesions, we find that the more preexposure the better (Roman et al., 2009; Roman & Reilly, 2007). Accordingly, the present design was modeled after the two-phase preexposure procedure of Roman et al. (2009). Thus, in Phase 1 the Familiar group received 25 ml/day of the to-be-CS for 9 days and in Phase 2 saccharin access was capped at 15 ml/day for 5 days. Because of expected differences in the rate of acquisition, rats in the Novel groups received only 6 conditioning trials whereas the rats in the Familiar groups were given a total of 18 CS-US trials.

During Preexposure Phase 1, subjects consumed ~25 ml of the available solution (water for the CS Novel rats, saccharin for the CS Familiar rats) during each 60-min trial except on Trial 1 when the CS Familiar rats encountered saccharin for the first time and drank 23.85 ml. A two-factor ANOVA revealed a significant CS x Trial interaction, $F(8,288) = 4.08$, $p < .001$; neither the main effect of CS nor the main effect of Trial was significant (*ps* > .05). Demonstrating taste neophobia, follow-up analyses (Tukey's test) found that Familiar rats consumed significantly less saccharin on Trial 1 than on Trials 3-9 (*ps* < .05). During Preexposure Phase 2, with little variability, rats consumed the maximal amount (15 ml) of either water or saccharin in each of the 5 trials (*F*s < 1).

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The behavioral data, volume of saccharin consumed, obtained from the conditioning phase of this experiment are displayed in Fig. 3 according to CS (Novel or Familiar) and US (Saline or Morphine) types. A three-way ANOVA conducted on data from the first 6 trials found a significant main effect of CS, $F(1,36) = 51.09$, $p < .001$, US, $F(1,36) = 41.64$, $p < .001$, and Trial (1-6), $F(5,180) = 11.50$, $p < .001$ as well as a CS x US x Trial interaction, $F(5,180) = 5.77$, $p < .001$. Follow-up analyses (simple main effects) revealed that while Novel-Morphine animals consumed significantly less saccharin than the Novel-Saline subjects (*ps* < .05), there was no significant difference in saccharin intake between the two groups in the Familiar CS condition ($p > .05$). That is, as expected, over the initial trials of the experiment, morphine-induced taste avoidance was obtained in the Novel CS condition and, demonstrating latent inhibition, pre-conditioning familiarity with saccharin attenuated this learning effect in the Familiar CS condition.

CS familiarization may delay but it does not prevent taste avoidance learning. As shown in Fig. 3, the Familiar-Morphine rats acquired the avoidance response when additional conditioning trials were given. Conducted on data from the final 4 conditioning trials of each CS type (i.e., Trials 3-6 for Novel CS and Trials 15-18 for Familiar CS), a three-factor ANOVA found a significant main effect of CS, $F(1,36) = 19.75$, $p < .001$, and US, $F(1,36) = 50.10$, $p < .001$; the main effect of Trial was not significant, $F(3,108) = 2.68$, $p > .05$. There was, however, a significant CS x US interaction, $F(1,36) = 13.19$, $p < .001$, but none of the other interactions were significant (*ps* > .05). Post hoc analyses revealed that on the last 4 trials, regardless of the familiarity of the CS,

morphine-treated animals consumed significantly less saccharin than saline-treated rats (*ps* < .05). Furthermore, saccharin preexposure reduced the magnitude of the avoidance effect. That is, Familiar-Morphine subjects consumed significantly more saccharin than did the Novel-Morphine rats (*ps* < .05). Finally, there was no significant difference in saccharin intake between the Novel-Saline and Familiar-Saline rats ($F < 1$). Thus, CS preexposure had no effect on the final levels of saccharin intake in the saline-injected subjects but substantially weakened the magnitude of saccharin avoidance in the morphine-injected rats. Of course, if a 5-min CS duration has been employed in the present experiment, we would, because of a ceiling effect on saccharin intake, have arrived at the erroneous conclusion that CS familiarization prevented the occurrence of morphine-induced conditioned taste avoidance.

3. Discussion

Prior research (e.g., Geddes et al., 2008; Roman & Reilly, 2009) indicated that IC lesions severely disrupt, if not fully prevent, the acquisition of conditioned taste avoidance. However, that earlier research employed CS presentations that were only 5 min in duration. As shown by Lin et al. (2009b) such short CS access may obscure important differences because of a ceiling effect in consumption, if not always in the drug-injected experimental rats then certainly in the saline-injected control subjects. To avoid this problem, the present work used 15-min CS access periods, a duration that also permits more direct comparison with our research on the effects of IC lesions on toxicosis-induced CTA learning. This change in CS duration obtained a very different pattern of results for both normal and ICX rats than those reported by Geddes et al. and

Roman and Reilly. For normal subjects, the occurrence and habituation of the neophobic reaction to the potentially dangerous and novel saccharin solution was observed in saline-injected rats, phenomena that are not evident when 5 min access is employed. Moreover, the habituation of neophobia also shows that the magnitude of the conditioned avoidance response was much larger (about twice the size) than that previously reported. This more complete picture of the performance of normal rats affords a better understanding of the effects of IC lesions on this task. First, ICX rats consumed significantly more saccharin on trial 1 than SHAM subjects, a deficit that we interpret as a disruption in the perception of taste danger/novelty (e.g., Lin et al., 2009a). Second, IC lesions attenuated, but did not prevent, acquisition of morphine-induced taste avoidance. These first two findings parallel the effects of IC lesions on toxicosis-induced CTAs (e.g., Kiefer & Braun, 1977; Roman et al., 2009; Roman & Reilly, 2007). For both taste avoidance and taste aversion learning in ICX rats we consider the latter deficit (retarded acquisition) to be a consequence of the former deficit (failure to perceive taste danger/novelty). So, ICX rats, because they perceive a potentially dangerous and novel taste to be safe/familiar, show retarded acquisition just like neurologically intact subjects do when they are conditioned with a familiar and safe taste CS. The third important finding for ICX rats in Experiment 1 concerns the level of morphine-induced avoidance at performance asymptote. The sustained, higher intake of the CS solution after more than 20 conditioning trials is markedly different from CTA learning in which ICX rats, given a few additional CS-US pairings, show complete avoidance of the taste CS (e.g., Roman et al., 2009). Thus, it would appear that the failure of ICX rats to show the same degree of conditioned taste avoidance as SHAM

subjects at asymptote in the present study cannot be explained as a consequence of a lesion-induced deficit in the perception of taste danger/novelty. Nor, as shown by the normal performance of the ICX rats in the morphine-induced odor avoidance experiment of Lin et al. (2009b), can this deficit in the present study be attributed to a disruption in the detection/processing of the morphine US.

How, then, is the difference in performance asymptote between SHAM and ICX rats on conditioned taste avoidance to be explained? The answer to this question was provided by the results of Experiment 2. We have argued that IC lesions retard acquisition of this avoidance response as a consequence of a primary deficit in the perception of the danger/novelty of the taste CS. We assumed that given enough CS-US pairings the ICX-Morphine rats would eventually show the same degree of taste avoidance at asymptote as SHAM-Morphine subjects, just as occurs with toxicosis-induced taste aversion learning (e.g., Roman et al., 2009). Experiment 2 used a latent inhibition procedure to determine whether neurologically intact rats preexposed to the CS would eventually learn to the same performance asymptote as rats conditioned with a potentially dangerous and novel CS. Three aspects of the results were noteworthy. First, the performance of the Novel groups in Experiment 2 replicated the acquisition curves of the SHAM groups in Experiment 1 - the asymptote levels of consumption in the saline-injected rats like that of the morphine-injected rats were each virtually identical in each experiment. Second, taste familiarization delayed acquisition of the avoidance response. That is, latent inhibition was demonstrated in drug-induced taste-avoidance learning. Third, and most unexpectedly, the results in the Familiar-Morphine group revealed a level of saccharin avoidance learning at asymptote that was

substantially weaker than that found in the Novel-Morphine group. This finding stands in stark contrast to the effect of CS familiarization on toxicosis-induced taste aversion learning.

We believe that the pattern of performance of the ICX rats in Experiment 1 might be entirely explained as a consequence of a lesion-induced disruption in the perception of the danger/novelty of the taste CS. That is, consequent to IC lesions (or, in the case of normal animals, stimulus preexposure), rats that demonstrably treat a taste stimulus as safe/familiar do not show the same performance asymptote as animals conditioned with the same taste stimulus when it is potentially dangerous/novel. By this analysis the asymptote level of saccharin avoidance of the ICX rats in Experiment 1 is not an aspect of performance that needs to be explained by an appeal to a lesion deficit. Indeed, it can be viewed as the expected consequence of conditioning with a familiar taste CS. Thus, IC lesions appear to have the same influence on morphine-induced conditioned taste avoidance as they do on toxicosis-induced conditioned taste aversion learning.

Contrary to our expectations, taste familiarization substantially reduced the asymptote level of morphine-induced conditioned taste avoidance in normal rats. We believe that this pattern of results might be explained by an appeal to other learning phenomena. It is possible, for example, that during the initial conditioning trials the rats familiar with saccharin are not only slow to acquire the saccharin-morphine association but that the morphine enters into association with another stimulus (e.g., injection cues or context) which competes with and limits the amount of saccharin avoidance that morphine can support. That is, CS preexposure may reduce the associability of the saccharin stimulus and thus permit overshadowing by other stimuli during conditioning.

From this perspective, weaker conditioned saccharin avoidance is the consequence of greater competition between the cues present during conditioning for the Familiar-Morphine rats than the Novel-Morphine subjects. To the best of our knowledge, the attenuated level of performance asymptote consequent to CS preexposure as found in Experiment 2 does not occur in CTA learning. Rather, it may occur only with a drug of abuse USs. If this analysis is correct, then a complete account of drug-induced taste avoidance will need to explicitly explain why the drug is less effective with a familiar CS than a novel CS.

The IC does not function in isolation. As the cortical component of the central gustatory system (for reviews see Lundy & Norgren, 2004; Travers, 1993), the IC receives afferent taste information from the gustatory thalamus and the central nucleus of the amygdala. An efferent pathway from the IC projects to the central nucleus of the amygdala (Norgren, 1976; Ottersen, 1982; Pitkanen, Savander & Le Doux, 1997) and each of these areas is connected with the basolateral amygdaloid nucleus and the lateral amygdaloid nucleus (Krettek & Price, 1977a, b; Pitkanen et al., 1997). This latter nucleus also has connections with the gustatory thalamus (Nakashima, Uemura, Yasui, Ozaki, Tabata & Taen, 2000; Turner & Herkenham, 1991). Finally, there are connections between the gustatory thalamus and the central nucleus of the amygdala (Halsell, 1992; Nakashima et al., 2000). These interconnections encourage the view that the behavioral deficits reported here are not so much the product of damage to intrinsic IC neurons but rather are the consequence of a disconnection of the IC from a functional system responsible for taste neophobia. Indeed, we have reported that lesions of basolateral region of the amygdala or medial amygdala (Lin et al., 2009a) or

the gustatory thalamus (Reilly, Bornovalova, Dengler, & Trifunovic, 2003) each result in neophobia deficits. It is, however, unlikely that these areas share the same behavioral function because no behavioral compensation was found consequent to lesions of any one of these structures. That is, if all four structures (IC, basolateral region of the amygdala, medial amygdala, and gustatory thalamus) served the same function then loss of any one would not be expected to reveal a behavioral deficit since the remaining structures should be sufficient for normal expression of taste neophobia. It seems more likely, then, that these structures may each perform a different function that, when integrated in the normal brain, is manifest as a seamless expression of taste neophobia.

This perspective brings into focus a number of important issues concerning the neural basis of taste neophobia. First, what other structures, if any, are involved in this neural system (we are currently addressing this issue by analyzing c-Fos expression consequent to novel or familiar taste exposure). Second, the functional relationships between these structures might benefit from a series of asymmetrical disconnection experiments that contrast the behavioral effects of ipsilateral versus contralateral lesions of any two of the components structures. Three, pharmacological deactivation of each structure might provide valuable insight into the different stages of memory formation (e.g., acquisition, consolidation, retention) that are distributed within the taste neophobia system. Finally, it is expected that advancement in our understanding of the neural substrates of taste neophobia will proceed hand-in-hand with the refinement of a psychological model of taste neophobia, which has been languishing for over thirty year in the two-factor theory of Nachman and Ashe (1974).

To reiterate, we interpret the present results as supportive of the view that IC lesions disrupt the perception of the danger/novelty of the taste CS which, in turn, retards acquisition of morphine-induced conditioned taste avoidance. The finding from Experiment 2 that normal animals familiarized with the taste CS prior to conditioning obtained a substantially weaker avoidance response than rats trained with a potentially dangerous and novel taste CS indicates that the same difference in avoidance learning between the ICX-Morphine rats and the SHAM-Morphine subjects in Experiment 1 is not a consequence of the IC lesion. Thus the conclusions from the present study are consistent with our analysis of the effects of IC lesions on the acquisition of toxicosis-induced CTAs. That is, the retardation of learning found in ICX rats in procedures involving a taste CS is a consequence of a primary deficit in the perception of the danger/novelty of that stimulus.

4. Experimental procedures

4.1. Experiment 1

4.1.1. Subjects

Thirty-nine naïve male Sprague-Dawley rats obtained from Charles Rivers Laboratories (Wilmington, MA) served as subjects in this experiment. They were individually housed in hanging stainless steel cages (Acme Metal Products, Chicago, IL) in a vivarium that was maintained at a temperature of 70˚F with a 12:12 hr light:dark cycle (lights on at 7:30 am). All experimental treatments and procedures occurred during the light phase of the cycle. Food and water were available at all times except as noted below during behavioral testing. The rats weighed ~300 g at the time of surgery and they were

treated in accord with guidelines of the National Institutes of Health and the American Psychological Association. The Institutional Animal Care and Users Committee of the University of Illinois at Chicago approved all treatments and procedures.

4.1.2. Surgery

A total of 20 rats were assigned to receive bilateral IC lesions. These rats were first anesthetized with intraperitoneal (IP) injections of sodium pentobarbital (50 mg/kg) and then fixed into a stereotaxic instrument (ASI, Warren, MI) using non-traumatic earbars. After a midline incision was made, two trephine holes (diameter ~3 mm) were drilled on the skull above the IC. To make excitotoxic lesions, 0.15 M N-methyl-D-aspartate (NMDA; St Louis, MO) was backfilled into a glass micropipette (tip diameter ~70 µm) and infused iontophoretically into the IC with a Midgard precision current source (Stoelting, Wood Dale, IL). There were two infusions per hemisphere at the following coordinates: Site 1, 10-min infusion at AP +1.2, ML ±5.2, DV -5.0 and Site 2, 6-min infusion at AP +1.2, ML ±5.2, DV -4.3. Throughout the surgical procedure, body temperature was monitored with a rectal thermometer and maintained at ~37 ºC with a heating pad (Harvard Apparatus, Holliston, MA). Nineteen animals served as control subjects (Group SHAM): 10 rats received the same surgical procedures as ICX subjects except no NMDA was infused and the other 9 animals received only IP injections of pentobarbital. All rats were returned to their home cages after recovering from anesthesia.

4.1.3. Apparatus

Behavioral testing occurred in the home cage. During the experiment fluids were available from calibrated water bottles with stainless steel sipper tubes. Fluid intake was monitored to the nearest 0.5 ml.

4.1.4. Procedure

The rats were acclimated to a deprivation schedule that permitted 15 min access to water each day. The experiment began when water intake stabilized (~12 days) at which time the rats in each lesion group (SHAM and ICX) were divided into subgroups according to the type of injection they would receive (Saline or Morphine) on conditioning days. Each conditioning trial consisted of 15 min access to 0.15% saccharin followed, 5 min later, by an intraperitoneal (IP) injection of either physiological saline (1ml/kg body weight) or morphine sulfate. On Trials 1-13 the morphine dose was 15-mg/ml/kg body weight. The morphine dose was increased to 30-mg/ml/kg body weight on Trials 14-23; Trial 24 was a saccharin only test trial. A saccharin trial occurred every third day and the rats were otherwise maintained on the water deprivation schedule as described above.

4.1.5. Histology

Once the experimental procedures were completed, ICX rats were injected IP with sodium pentobarbital (~100 mg/kg) and then transcardially perfused with physiological saline and 10% buffered formalin. The brains were extracted and stored in 4% formalin for at least two days and then switched to 20% sucrose for an additional two days.

Thereafter, the brains were frozen, sliced at 50 µm on a cryostat and stained with cresyl violet. Using a light microscope (Zeiss Axioskop 40) photomicrographs were taken with a Q-Imaging camera running Q-Capture software (Quantitative Imaging Corporation, Burnaby, B.C., Canada). Schematic drawings were made on diagrams derived from the Paxinos and Watson (2005) atlas.

4.2. Experiment 2

4.2.1. Subjects and apparatus

The 40 naïve male subjects were obtained and maintained in a manner identical to those described in Experiment 1. The apparatus was that used in Experiment 1.

4.2.2. Procedure

Following stabilization of intake on a deprivation schedule that permitted 15 min access to water each day, the rats were divided into two groups according to the stimulus available during the two phases of preexposure. During the 9 days of Preexposure 1, the rats were limited, as best as possible, to 25 ml of 0.15% saccharin (Group Familiar; n = 20) or 25 ml of water (Group Novel; n = 20) on each 60-min trial. Three days of 15 min water access intervened between Preexposure 1 and Preexposure 2. During the five days of Preexposure 2, the rats were allowed a maximum of 15 min to consume 15 ml of either saccharin (Group Familiar) or water (Group Novel). Prior to the first conditioning trial which occurred on the day after the final Preexposure 2 trial, the rats in each group (Familiar and Novel) were divided according to the US (morphine or saline) they would subsequently receive. During each conditioning trial the rats were allowed

15 min unlimited access to saccharin and, 5 min after the stimulus bottles were removed, injected with either 0.15 mg/ml/kg of morphine (n = 12/subgroup) or an equivalent volume of saline (n = 8/subgroup). A conditioning trial occurred every third day. Because of the expected differences in rates of avoidance learning, rats in the Novel CS condition were given 6 CS-US pairings whereas those in the Familiar CS condition received a total of 18 conditioning trials.

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Figure captions

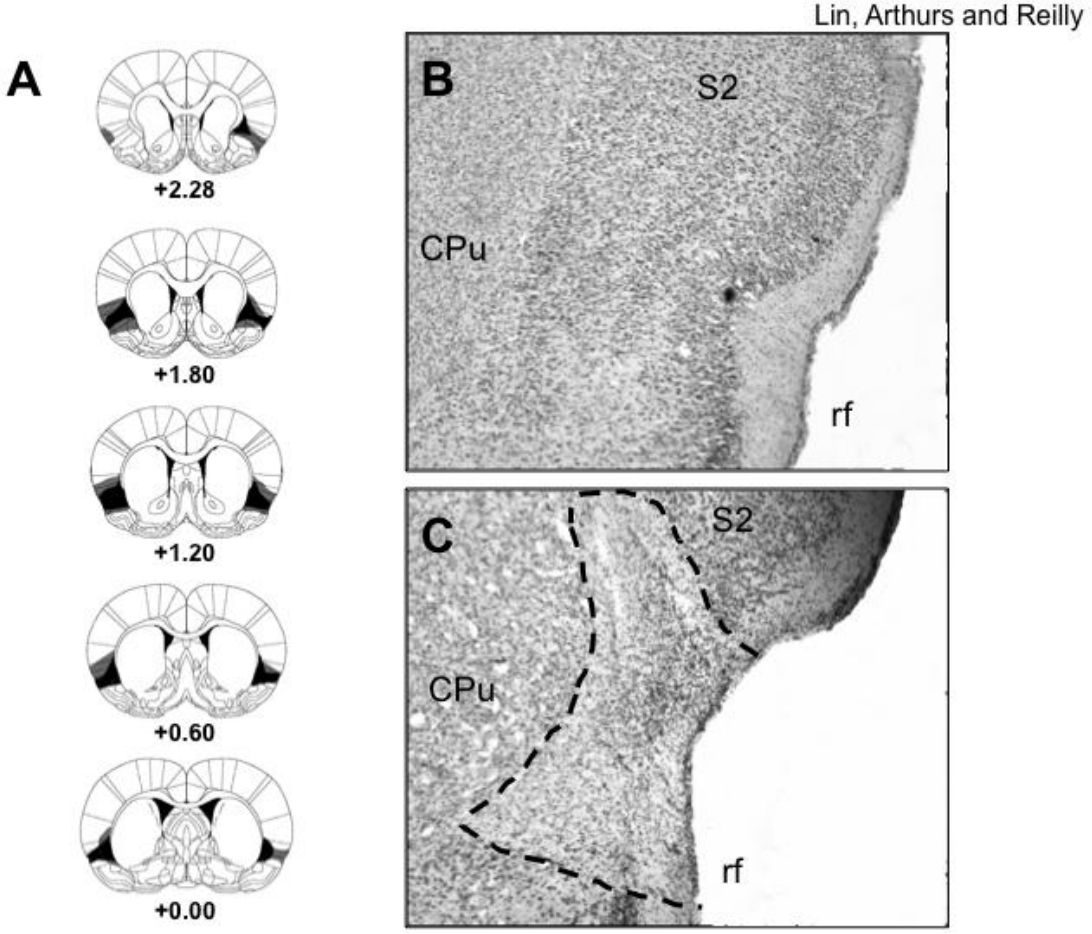
Fig.1 - Histological reconstructions for Experiment 1. Panel A shows serial schematic reconstructions of the smallest (black) and largest (grey) insular cortex (IC) lesions at five coronal levels (+2.28 mm, +1.80 mm, +1.20 mm, +0.60 mm and 0.00 mm relative to bregma). Panels B and C show representative digital photomicrographs of the cresyl-violet stained IC from a neurologically intact (SHAM) subject and from a rat with an IC lesion, respectively. The dashed line indicates the extent of the lesion. CPu = caudate putamen; rf = rhinal fissure; S2 = secondary somatosensory cortex. The diagrams in Panel A were adapted with permission from Paxinos and Watson (2005).

Fig.2 - Experiment 1: Mean (±*SE*) saccharin intake (ml) in normal animals (SHAM; upper panel) and rats with insular cortex lesions (ICX; lower panel) across 24 trials. Each saccharin trial was followed by an intraperitoneal injection of either saline (Saline) or morphine (Morphine), except trial 24, which was a saccharin only test trial. The vertical dashed line indicates the trial when the morphine dose was increased from 15 mg/kg to 30 mg/kg.

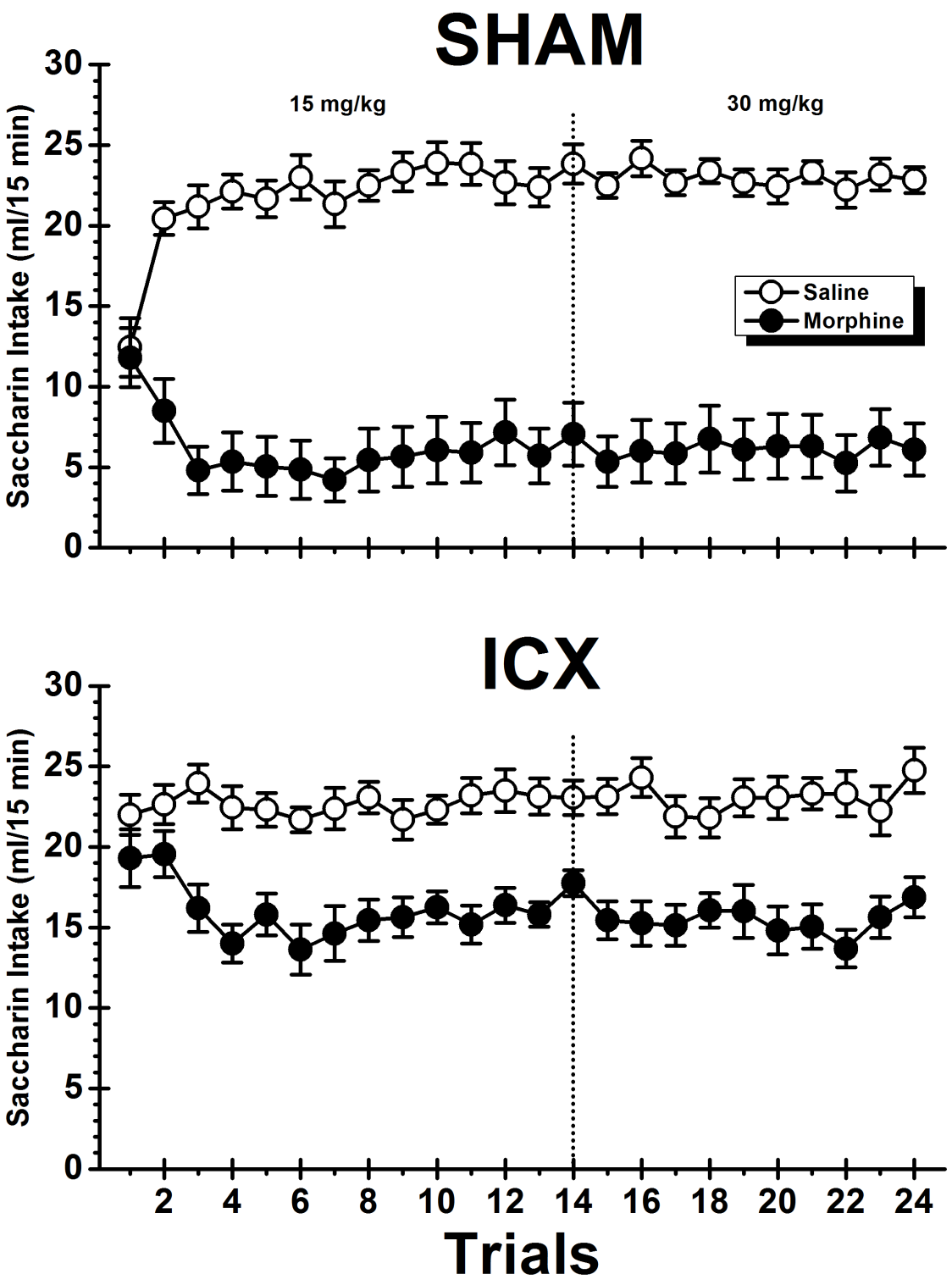
Fig.3 - Experiment 2: Mean (±*SE*) saccharin intake (ml) across 6 acquisition trials in saccharin naïve (Novel; left panel) rats and 18 acquisition trials in subjects that were preexposed to saccharin (Familiar; right panel). The trials were followed by intraperitoneal injections of either saline (Saline) or morphine (Morphine) except the last trial for each group which was a saccharin-only test trial.

Figure 1

Lin, Arthurs and Reilly



Lin, Arthurs & Reilly - Figure 2



Lin, Arthurs & Reilly - Figure 3