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Visceral Cortex Lesions Block Conditioned Taste Aversions Induced by Morphine

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MACKEY, W. B., J. KELLER AND D. VAN DER KOOY. *Visceral cortex lesions block conditioned taste aversions induced by morphine.* PHARMACOL BIOCHEM BEHAV **24**(1) 71–78, 1986.—Rats with bilateral ibotenic acid or sham lesions of the visceral (agranular insular) cortex were tested for a conditioned taste aversion (CTA) to saccharin after five pairings of morphine sulphate injections (15 mg/kg IP) with consumption of a novel solution (0.1% saccharin). Lesioned animals demonstrated no evidence of the morphine-induced CTA that was seen in the sham operated animals. A third group of rats received ibotenic acid lesions but had saccharin consumption paired with saline vehicle injections. This group had the normal preference (seen in naive rats) for saccharin on testing, showing that the visceral cortex lesion had no effect on the ability of the rats to discriminate saccharin from water. In order to test if visceral cortex lesions abolish specifically the CTA induced by morphine, we ran a similar set of CTA experiments using two new novel flavours and either 15 or 75 mg/kg IP lithium chloride (LiCl) as the unconditioned stimuli. Dose dependent CTA's to the LiCl were established in all groups indicating that the visceral cortex plays no role in mediating the aversive effect of LiCl. Using the condition place preference paradigm we investigated the role of the visceral cortex in the expression of morphine's rewarding aspects. Identical place preferences were found in groups of rats with or without visceral cortex lesions suggesting that this cortical region plays no role in either the perception of morphine's rewarding effects or the association of morphine's rewarding properties with sensory stimuli. Visceral cortex lesions also had no effect on the establishment of a conditioned place aversion to a high dose of LiCl (75 mg/kg IP). Thus, visceral cortex appears critical for the establishment of a morphine-induced CTA, but is not crucial for mediating gross taste discrimination, the aversive aspects of LiCl nor the rewarding properties of morphine.

Morphine Lithium chloride Visceral cortex Ibotenic acid Conditioned taste aversion Place conditioning

MORPHINE, along with other psychoactive drugs [13, 14, 34, 35, 36, 37, 48, 51], has been shown to possess both positive [22, 24, 25, 42, 46] and negative reinforcing characteristics [7, 12, 37, 43, 47]. These paradoxical reinforcing effects are not accounted for by variables such as dose, time course of effect, route of administration or previous drug experience [7,47]. Morphine's rewarding aspects may be demonstrated through the intravenous self-administration paradigm [42] or through the conditioned place preference paradigm in which morphine's rewarding properties are paired with visual, olfactory and tactile stimuli from distinctly constructed environments [22, 25, 44]. Morphine's aversive aspects may be demonstrated via the conditioned taste aversion (CTA) paradigm which also makes use of a novel environmental stimulus, but in this case involves only the sense of taste [7, 11, 47]. It appears that the aversive aspects of morphine in rats are preferentially associable with taste stimuli, presumably reflecting the innate "wiring" of the rat.

Given the preferential associability of the aversive aspects of certain drugs to taste in rats, many investigations into neural mechanisms of CTA have looked at the role of the taste cortex and nearby structures [10, 17, 18]. The general finding has been that this region plays a minor role in the establishment of CTA's induced by lithium chloride, copper sulphate and scopolamine methyl nitrate [11], however, contradictory evidence does exist [17,18].

The taste cortex has been defined anatomically as the cortex receiving afferent connections from the thalamic taste area in the ventromedial thalamus. The taste cortex is thus the insular cortex, and debate continues as to whether it is the granular or agranular insular cortex that primarily receives the taste information from the thalamus [29,53]. The agranular insular cortex has been referred to, in broader terminology, as the visceral cortex because of a number of its other connections [45]. First, there is a direct projection from the parabrachial nucleus (the second order relay station in the pons for taste and other visceral information) to the granular and agranular insular cortex [19,38]. Second, the agranular cortex (especially its ventral and posterior divisions) sends direct projections down to the nucleus of the

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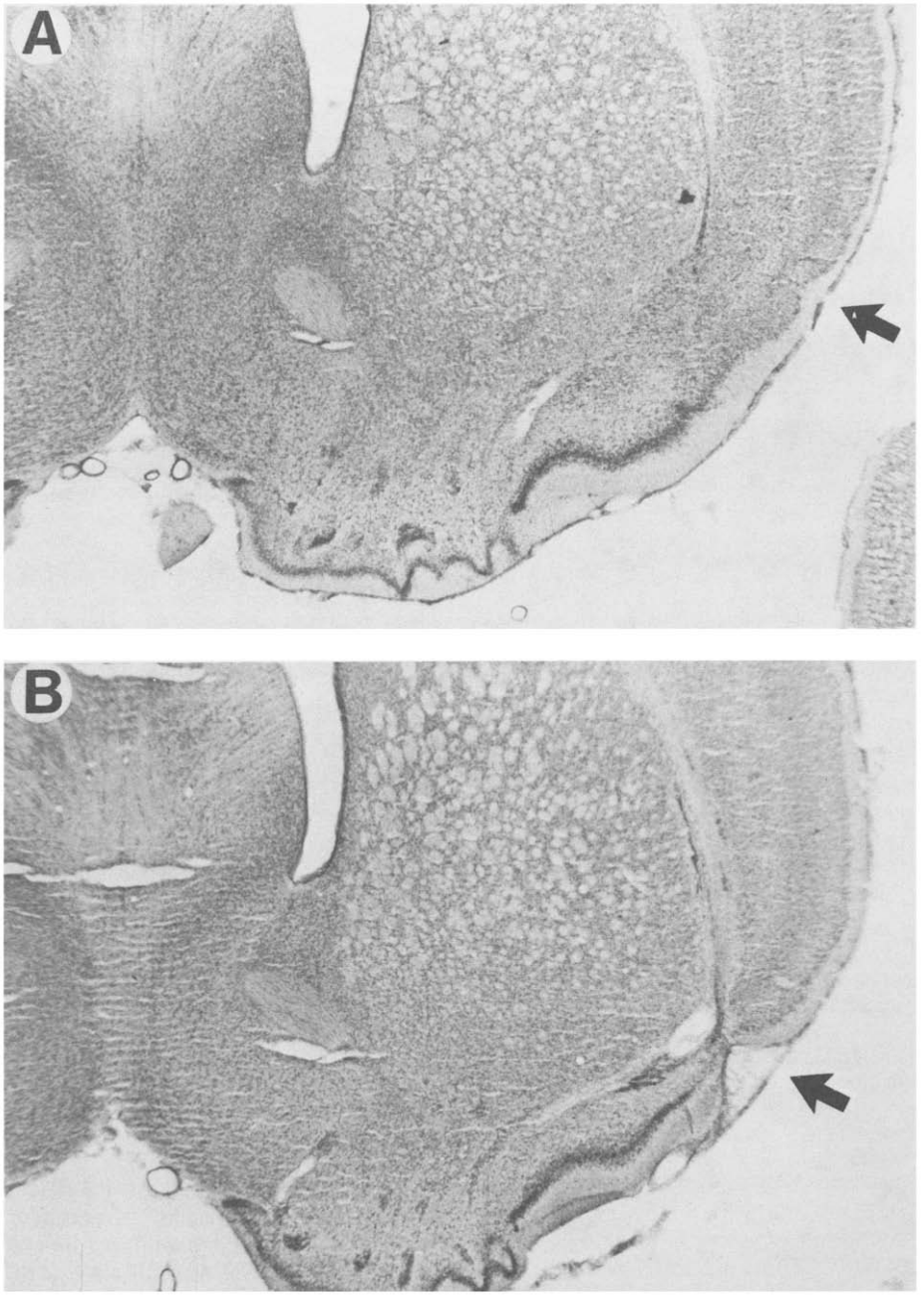

FIG. 1. Representative examples of sham (A) and ibotenic acid (B) lesions of the visceral cortex. Arrows indicate the location of the rhinal sulcus near the site of the agranular insular cortex injection. Needle tracts are not visible with the lengthy survival times post-injection.

solitary tract, the first order relay station in the medulla for taste and other visceral information [45]. Third, the agranular insular or visceral cortex is innervated by dopamine fibers [20,23]. Because dopamine has been implicated in the psychoactive properties of many drugs including the aversive and positive reinforcing aspects of morphine [43,51], we sought to examine the role of the visceral cortex in morphine-induced CTA. Few of the investigations into determining the brain sites involved in morphine's psychoactive properties have considered its aversive properties [37,43]. Determination of the brain structures involved in the expression of these properties is important, however, to shed light on the mechanisms behind the differential associability of the rewarding aspects of psychoactive drugs to certain sense modalities and the aversive aspects of these drugs to other modalities.

In the present experiments we used morphine injections to attempt to form a CTA to saccharin in rats which had received ibotenic acid lesions to their visceral cortex and others which were sham-operated. We then repeated these procedures with two doses of LiCl in order to test the specificity of the visceral cortex lesion to morphine effects. Fi-

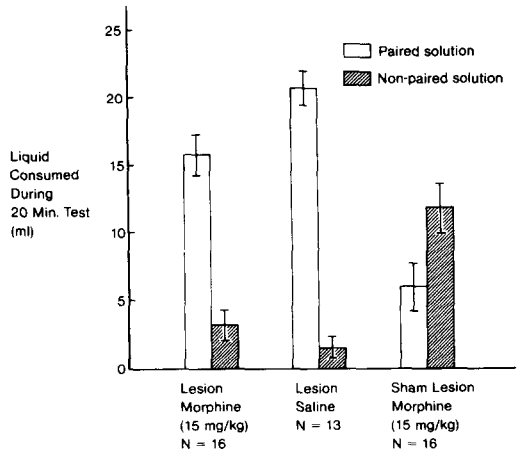

FIG. 2. Conditioned taste aversions elicited by five pairings of morphine (15 mg/kg IP) or physiological saline in rats with either bilateral ibotenic acid or sham lesions of the visceral cortex.

nally, using the place preference paradigm we investigated the effect of the visceral cortex lesions on morphine's rewarding properties.

We now report that the visceral cortex does indeed play a role in the establishment of a morphine-induced CTA. The visceral cortex, however, plays no role in associating the aversive qualities of LiCl with any sensory modality or in associating morphine's motivational properties with sensory stimuli other than taste.

METHOD

Fifty-seven adult male Wistar rats (Charles River) weighing 300–400 g were used. The rats were housed individually in suspended grey wire cages throughout all handling and conditioning procedures in a room kept at 22°C and lit from 0900 to 2100 hr. Purina rat chow was available ad lib throughout all procedures. Water was also continuously available except during CTA training and testing.

Surgery

Under general anesthesia (Nembutal) 48 of the 57 rats were operated upon. Each of the rats was placed in a stereotaxic apparatus, a scalp incision was made and two small holes were drilled in the skull to allow passage of a needle. Each rat was either bilaterally injected with 0.1 μl of a 4% ibotenic acid solution via a 1 μl Hamilton microsyringe over 10 minutes (32 rats) or with physiological saline (16 rats). The needle was left in place for 5 minutes following the infusions. The injection co-ordinates as taken from the atlas of Paxinos and Watson [32] were AP +0.2 mm anterior to bregma, L $\pm$4.8 mm, and DV −5.5 mm below the dura, with the mouth bar set at −3.3 mm below the interaural line.

Two weeks were allowed for recovery from surgery before any experiments were begun, during which each rat was handled at least four times for three minutes at a time. Handling was also performed between experiments since each was separated in time by one month.

Taste Aversion Conditions

Morphine CTA. Two weeks postsurgery all rats were trained to consume water on a limited access regime of 20 minutes a day for five days. On the first experimental day 0.1% saccharin was made available for 20 minutes instead of water and immediately afterward each rat was given an appropriate injection. The rats in group 1 (n=16) had bilateral ibotenic acid lesions of the visceral cortex and were injected intraperitoneally with 15 mg/kg morphine sulphate dissolved in physiological saline. The rats in group 2 (n=13) were also lesioned but were injected with the physiological saline vehicle (three of the 16 original rats from this group died during the experiment). The rats in group 3 (n=16) were the sham-lesioned animals and, like group 1, were injected with 15 mg/kg morphine sulphate. On the second experimental day each rat was merely provided with water for 20 minutes. Treatment on subsequent experimental days continued to alternate between each of these procedures for a total of ten days (i.e., 5 drug pairings). The following day (day 11) a two-bottle choice test was given to each rat by simultaneously presenting both saccharin and water for 20 minutes and the amount consumed of each liquid was recorded for each rat. The two-bottle choice method has been shown to be the most sensitive and reliable of the various CTA testing methods [11]. Glass calibrated drinking tubes held each of the liquids, and the side of the cage where the saccharin tube was placed during conditioning and testing was counterbalanced within each group but remained consistent throughout the experiment for each rat.

LiCl CTA. One month after the morphine CTA experiment, during which time the rats were rehandled, two of the groups of rats were split into two groups of eight (the saline injected group remained intact) and the experiment was repeated except that the morphine injections were replaced by injections of either 15 or 75 mg/kg IP LiCl, and the saccharin solution and water were replaced by solutions of unsweetened grape and cherry Kool-Aid (each 0.3% in tap water). The groups, then, consisted of lesioned rats injected with saline vehicle (n=13, the lesioned, saline injected ras from the first experiment), lesioned and sham-operated animals conditioned with 5 pairings with 15 mg/kg IP of LiCl (n's=8, halves of each of the lesioned, morphine injected, and sham operated, morphine injected groups from the first experiment) and lesioned, and sham operated, animals conditioned with 5 pairings with 75 mg/kg IP LiCl (n's=8, halves of each of the lesioned, morphine injected, and sham operated, morphine injected groups from the first experiment). Unlike saccharin, which is greatly preferred over water by the rats, no preference was expected for one flavour of Kool-Aid over the other. Thus, the flavours were counterbalanced within groups with respect to which flavour was to be paired with the drug injection. Two doses of LiCl were used to increase our chances of finding an effect of the visceral cortex lesion. The 75 mg/kg dose was chosen because it has been shown to be very effective at producing CTA's [34]. The 15 mg/kg dose was chosen since it too produces CTA's [34], but to a lesser degree. It was felt that this lower dose of LiCl would produce an aversion closer in magnitude to the aversion expected of morphine.

Place Conditioning

Place conditioning took place one month after the completion of the second CTA experiment. During this time the rats were rehandled. The procedures used were very similar to those described previously [25]. Briefly, conditioning took place in one of two boxes which differed in colour, texture and smell. One had black walls and a black Plexiglas floor

which was wiped with a 2% vinegar solution just prior to placing each rat inside it. The other box had white walls and a wood chip floor which gave off a slight smell of wood. Each rat received a drug injection one day (15 mg/kg IP morphine or 15 or 75 mg/kg IP LiCl) and a saline injection on the next, and these injections were alternated for a total of eight days (i.e., four drug pairings). When injected with a drug each rat was immediately placed into one box and on alternate days, when injected with saline vehicle, it was placed in the other box. Each pairing lasted 30 minutes. The order of drug and vehicle presentation and the choice of environment paired with drug injection were counterbalanced for the rats in each group.

On the ninth day, each rat was placed into a larger rectangular test box which consisted of environments exactly the same as the two conditioning boxes at each end, separated by a smaller grey area ("neutral zone"). The time that each rat spent on each of the two ends was recorded over a 10 minute period. Evidence exists that this unbiased method of running place preference conditioning is the most reliable [24,44].

Each lesioned and sham operated rat was conditioned with morphine at the dose it had received in the first CTA experiment. The lesioned group that was conditioned with saline in the CTA experiments was now split into two and underwent place conditioning with 15 or 75 mg/kg IP LiCl as the unconditioned stimulus. Two new groups were added in order to test the effects of the two doses of LiCl on place conditioning in naive rats. LiCl has previously been seen to produce place aversions at 60 mg/kg intravenously [25]. During the place conditioning phase of the experiment four rats died from the group of animals which had been lesioned and were receiving morphine injections. Thus, the groups used consisted of lesioned rats conditioned with morphine (n=12, from the lesioned, morphine injected group of the first experiment), sham lesioned rats conditioned with morphine (n=16, the sham lesioned, morphine injected group from the first experiment), lesioned (from the lesioned, saline injected group of the first experiment) and naive groups conditioned with 15 mg/kg IP LiCl (n's=8 and 4, respectively) and lesioned (from the lesioned, saline injected group of the first experiment) and naive groups conditioned with 75 mg/kg IP LiCl (n's=5).

Histology

After the place conditioning procedures all of the rats which were operated upon were deeply anesthetized with Nembutal and perfused through the heart first with saline and then 10% formalin. The brains were cut, mounted on gelatin coated slides, and stained with cresyl violet in order to verify the placement and extent of the lesions. Sections were examined and photographed in brightfield microscopy.

RESULTS

Histology

Representative sections from ibotenic acid and sham lesioned animals are presented in Fig. 1. In all rats the ibotenic acid lesions produced bilateral destruction of the area around the rhinal sulcus, which included most or all of the agranuar insular cortex and large parts of the claustrum. The granular insular cortex was sometimes damaged, but in many rats this region was relatively unaffected by the lesions. The extent of damage to the granular cortex did not seem to correlate with the behavioral results. As assessed in Nissl sections, subcortical structures, with the exception of the claustrum, were not damaged by the lesions.

Morphine CTA

Morphine induced clear CTA's in sham lesioned rats, but not in rats with ibotenic acid lesions of the visceral cortex. The results of the two-bottle choice test after morphine conditioning are presented in Fig. 2. Analysis of variance showed a significant interaction between flavour consumed and lesion in the morphine injected rats, $F(1,30)=20.4$, $p<0.01$. Group 3, which received sham visceral cortex lesions and had morphine injections paired with saccharin consumption, showed the expected aversion to the normally preferred saccharin. On average, only 6.0 ± 1.7 ml of saccharin were consumed during the test (34% of the total fluid intake) versus 11.8 ± 1.8 ml of water. These amounts were significantly different, $t(15)=2.38$, $p<0.05$. In contrast, group 1, which received ibotenic acid lesions of the visceral cortex and had morphine injections paired with saccharin consumption, drank 15.8 ± 1.5 ml of saccharin (83% of the total) versus 3.2 ± 1.1 ml of water. These amounts were also significantly different, $t(15)=6.93$, $p<0.001$. The saccharin preference shown in the ibotenic acid lesioned group was very similar to that seen in group 2, which received ibotenic acid lesions and had saline vehicle instead of morphine injections paired with saccharin consumption. The rats of this group on average drank 20.7 ± 1.3 ml of saccharin (93% of the total) versus 1.5 ± 0.8 ml of water, which again were amounts found to differ significantly, $t(12)=12.9$, $p<0.001$. The normal saccharin preference in group 2 is similar to the normal saccharin preference seen on nonlesioned, saline injected rats, and served as a control showing that ibotenic acid lesions of the visceral cortex do not affect gross taste discrimination nor baseline taste preferences.

LiCl CTA

Ibotenic acid lesions of the visceral cortex had no effect on the acquisition of CTA's induced by LiCl. The results of the two-bottle choice test after LiCl conditioning are presented in Fig. 3. The first group shown (to the far left) consisted of rats which were lesioned and received saline vehicle injections as the unconditioned stimuli. The average consumption from this group was 10.0 ± 1.8 ml of the Kool-Aid flavoured solution paired with the saline injection (49% of the total) versus 11.7 ± 1.4 ml of the other Kool-Aid flavoured solution. No significant differences were seen between these two values, $t(12)=0.22$, $p>0.25$. An overall comparison of the consumption of grape vs. cherry Kool-Aid across all groups showed no preference for one or the other, $t(44)=1.4$, $p>0.25$. Significant dose dependent CTA's were seen in the other four groups all of which were conditioned with LiCl. Analysis of variance showed a significant main effect of flavour paired with drug versus flavour not paired with drug, $F(1,28)=53.34$, $p<0.001$, but no main effect of lesion, $F(1,28)=0.44$, $p>0.5$. At 15 mg/kg, the lesion group drank 7.3 ± 1.4 ml of the flavoured solution paired with the LiCl (28% of the total) versus 18.1 ± 2.4 ml of the other, $t(7)=3.85$, $p<0.01$, and the sham-operated group drank 8.4 ± 2.2 ml of the flavoured solution paired with the LiCl (31% of the total) versus 18.6 ± 1.6 of the other, $t(7)=3.97$, $p<0.01$. At 75 mg/kg, the lesion group drank on average 1.1 ± 0.3 ml of the flavoured solution paired with the LiCl injection (9% of the

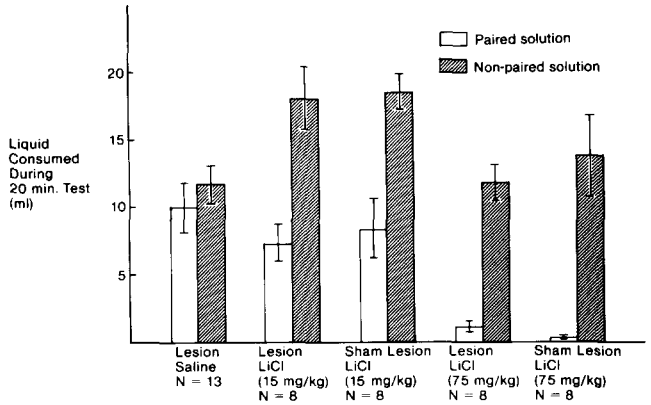

FIG. 3. Conditioned taste aversions elicited by five pairings of LiCl (15 or 75 mg/kg IP) or physiological saline in rats with either bilateral ibotenic acid or sham lesions of the visceral cortex.

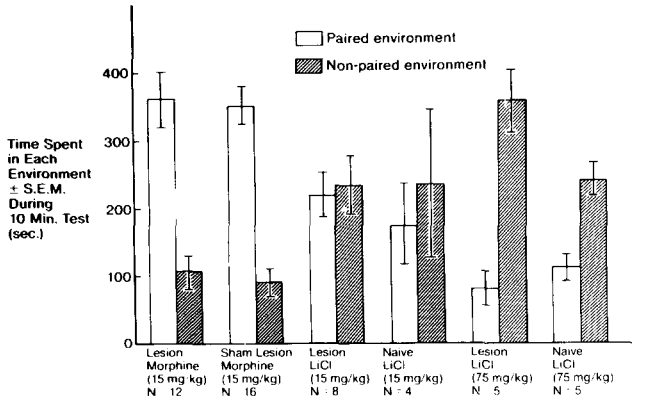

FIG. 4. Place conditioning in groups of lesioned rats and their controls injected with either morphine (15 mg/kg IP) or LiCl (15 or 75 mg/kg IP).

total) versus 11.8±1.4 ml of the other flavoured solution, $t(7)=7.38$, $p<0.001$, and sham-lesioned group drank 0.3±0.1 ml of the flavoured solution paired with the LiCl (2% of the total) versus 13.7±3.5 ml of the other, $t(7)=3.86$, $p<0.01$.

Place Conditioning

Ibotenic acid lesions of the visceral cortex did not modify the place conditioning seen with either mophine or LiCl (Fig. 4). The two groups (a lesion and a sham-lesion group) which received morphine as a conditioning agent showed significant place preferences for the environment in which the rat experienced the effects of the morphine injections. Analysis of variance revealed a significant main effect of morphine versus saline, $F(1,26)=45.56$, $p<0.01$, but no significant main effect of lesion, $F(1,26)=0.67$, $p>0.25$, and no significant interaction between the variables. Comparing times spent in the morphine versus saline paired environments, the rats of the lesion group, $t(11)=5.41$, $p<0.001$, and the sham-lesion group, $t(15)=7.37$, $p<0.001$, spent an average of 256 and 260 seconds more, respectively, in the part of the test box resembling the environment where the morphine was experienced. Twenty-six of the 28 rats in these two groups showed preferences for the environment in which the effects of morphine were experienced.

Only the groups conditioned with 75 mg/kg LiCl showed place aversions, and the lesioned and naive groups did not differ significantly at this dose. Analysis of variance revealed a significant main effect of LiCl paired side versus vehicle paired side, $F(1,18)=7.19$, $p<0.05$, but no significant effect of lesions, $F(1,18)=3.44$, $p>0.05$. At 15 mg/kg the lesion group, $t(7)=0.08$, $p>0.25$, and the naive group, $t(3)=0.16$, $p>0.25$, spent an average of 15 and 59 seconds less time, respectively on the side of the test box where the effects of the LiCl were experienced. At 75 mg/kg, LiCl place aversions were seen in both of the groups which received this dose of drug. The lesion group, $t(4)=5.24$, $p<0.01$, and the naive group, $t(4)=4.20$, $p<0.05$, spent 276 and 129 seconds less time, respectively, on the side of the test box where the effects of the LiCl were experienced.

DISCUSSION

The results of the CTA experiment utilizing morphine as the unconditioned stimulus demonstrated that ibotenic acid lesions of the visceral cortex disrupt the normal aversion developed to novel flavours paired with morphine injections. This is illustrated by the differences in liquid consumption between groups 1 and 3 of the first experiment. The results of Group 2 indicate that the lesion had no effect on the rats' abilities to taste saccharin since it was highly preferred over water when paired with presumably neutral saline injections. This preference is similar to that of normal naive rats (unpublished observations).

A number of studies have investigated the effects of lesions in the area of the visceral cortex on the CTA's induced by a number of agents, but most often LiCl. Even large lesions of the lateral cortex in rat do not abolish the ability to acquire CTA's [4, 5, 15, 16, 18, 21, 53], although in certain cases the CTA's are partially attenuated. Two previous studies employed electrolytic lesions that overlapped significantly the site of our ibotenic acid lesions in the agranular insular cortex. Lasiter and Glanzman [18] showed that dorsal prepiriform cortex lesions, but not lesions more dorsally in the traditional gustatory neocortex, produced a generalized suppression of both water and the flavoured LiCl solution paired on the first day. However, when fluid consumption recovered on the next day, Lasiter and Glanzman [18] reported that clear aversions to LiCl were seen. Yamamoto *et al.* [53] found that electrolytic lesions of an area on the dorsal lip of the rhinal sulcus (ventral and posterior to the traditional gustatory neocortex) diminished a LiCl-induced CTA that was acquired preoperatively.

In order to test the effects of our more specific ibotenic acid lesions on LiCl-induced CTA's we ran two doses of LiCl on the lesioned rats that previously were found to be completely blocked from the acquisition of morphine-induced CTA. Results of the LiCl CTA experiment showed that LiCl produced dose dependent CTA's in both the sham operated and the visceral cortex lesioned rats. Thus, results employing the neurotoxin ibotenic acid, which spares fibers of passage, suggest that the visceral cortex appears to be specific to morphine in terms of its importance to CTA development. Our results also suggest that the slight impairment of LiCl-induced CTA acquisition found in previous studies [18,53] may have been due to the non-specific effects (on fibers of passage) of the electrolytic lesions employed. Visceral cortex, of course, may be involved in the development of CTA's when agents other than morphine or LiCl are used as unconditioned stimuli. The specificity of the visceral

cortex lesion to morphine-induced CTA's further illustrates the fact that different drugs capable of producing CTA's may do so via different mechanisms [8, 37, 48]. Another example of this is the observation that lesions to the area postrema have no effect on amphetamine or morphine-induced CTA's but significantly disrupt scopolamine methyl nitrate-induced CTA's [35,48].

In order to test if the visceral cortex had any effect on the expression of morphine's positive reinforcing properties, place conditioning with morphine was attempted in lesioned rats and sham operated controls. All rats conditioned with morphine showed place preferences to the environment in which they experienced the effects of the morphine. The visceral cortex lesion had no effect on these place preferences, supporting the notion that it is only the aversive properties of morphine or their conditioning to taste stimuli that are affected. These results rule out the possibility that the lesion could have altered the CTA results through an effect on the rewarding characteristics of morphine or through an effect on both the aversive and rewarding aspects of morphine.

The aversive qualities of many drugs are preferentially associated with tastes in rats, rather than with other sensory modalities [11]. It is these other sensory modalities (vision and texture and possibly olfaction) which are exploited in the conditioned place preference paradigm. LiCl, which apparently has only aversive qualities, produced both CTA's and conditioned place aversions. Visceral cortex lesions blocked neither of these aversive effects. Of interest, however, was the finding that only high doses of LiCl produced place aversions in either the sham operated or lesioned rats, whereas both high and low doses of LiCl produce CTA's. This re-emphasizes the difficulty of demonstrating the aversive qualities of psychoactive drugs when employing conditioned stimuli from sensory modalities other than taste.

There are two viable explanations for the blockade of morphine-induced CTA by ibotenic acid lesions of the visceral cortex. First the lesion may have produced a reduction in the perception of morphine's aversive properties. Second, morphine may have prevented the association of the taste cues with morphine's negative aspects or the memory of this association. To differentiate these possible explanations a second measure of morphine's aversive qualities would be required. The recent discovery of a place aversion induced by morphine only at low doses and only via intraperitoneal administration [2], may be such a measure and may allow discrimination between these two possible explanations.

The results of the present experiment are important with respect to the neural mechanisms underlying the differential associability of morphine's positive reinforcing and aversive effects with conditioned stimuli from different sensory modalities. The ability of the visceral cortex lesion to disrupt the CTA's produced by morphine, yet have no effect on morphine's rewarding qualities, demonstrates one anatomical dissociation of these different motivational effects. Other research from our lab has provided evidence which further anatomically separates the rewarding from the aversive aspects of morphine. Morphine's rewarding aspects have been shown to be mediated by central opiate receptors, whereas the aversive aspects appear to originate at peripheral opiate receptors primarily in the gut [2]. Transmission of the aversive information was shown to be via the vagus nerve since subdiaphragmatic vagotomy eliminated morphine-induced CTA's [2].

Combining the present results with prior data, we can speculate on the structures and pathways involved in morphine-induced CTA's. The transmission of taste information through the nervous system seems relatively straightforward. Taste sensations carried by cranial nerves VII, IX, and possibly X, terminate in the rostral nucleus of the solitary tract [1, 27, 30] and from there are relayed to the pontine taste area in the parabrachial nuclei [27,30]. The efferent connections of the parabrachial nuclei in the rat innervate a large number of brain structures. One of these efferents is the classical taste pathway [6, 28, 30, 49], which involves projections from the pontine taste area ascending via the central tegmental bundle to synapse in the ventromedial thalamic nucleus [28,39]. The electrophysiological evidence for the transmission of taste information through the pontine taste area and ventromedial thalamus is well documented [27, 30, 53, 54]. From the ventromedial thalamic nucleus the pathway continues to the taste cortex [31, 52, 53], which overlaps our visceral cortex lesion site, if recent evidence implicating the agranular insular cortex as taste cortex is correct [29]. We assume that the taste information from an ingested solution reaches the visceral cortex via this pathway.

The aversiveness of opiates begins with morphine (or other opiate receptor agonists) binding to receptors located in the gut region [2]. Information from this binding site travels via the vagus nerve [2] to the caudal nucleus of the solitary tract within the medulla. The aversive information from the morphine injection continues (we speculate) from the caudal NTS via a pathway which may include the noradrenaline and dopamine systems at its synaptic points. From the caudal nucleus of the solitary tract the majority of the direct projections to the diencephalon are noradrenergic [40]. Lesions of this and other noradrenergic pathways with 6-OHDA injections in the pons have been reported to attenuate morphine-induced CTA [37]. Previous experiments have also shown that 6-OHDA lesions of the nucleus accumbens attenuate the development of a morphine-induced CTA to the saccharin [43]. One explanation of these results is that the mesolimbic dopamine fibers which synapse in the accumbens are involved in the perception of morphine's aversive qualities [43]. The mesolimbic dopamine system has been implicated in the psychoactive properties of many drugs [50,51].

An alternative explanation for the accumbens 6-OHDA results is that the aversive information about morphine is transmitted to the ventral midbrain, and then on to the visceral cortex via the mesocortical dopaminergic fibers, some of which pass through the medial edge of the nucleus accumbens [3,41]. Disruption of the morphine-induced CTA would then be possible because of the destruction of these fibers of passage by 6-OHDA lesion of the accumbens. CTA experiments utilizing fiber sparing ibotenic acid lesions of the nucleus accumbens would aid in distinguishing between these interpretations. A lack of effect of these cell body specific lesions on morphine-induced CTA would support the suggestion that the mesocortical dopamine pathway carries information about morphine's aversiveness directly from the midbrain to the visceral cortex. Recent anatomical evidence showing that many of the dopamine fibers projecting to the visceral cortex take an alternate lateral route over the optic tract [9] suggests that the transfer of information about morphine (through the nucleus accumbens to the visceral cortex) may be a multisynaptic process.

From the results of the present experiments we speculate that it is at the point of the visceral cortex where the taste

information and the aversive information about morphine are associated. The pathways carrying taste information and aversive opiate information are also anatomically close together at the level of the nucleus of the solitary tract and possibly the parabrachial nuclei, and some association of information may take place at lower levels as well. The association must, of course, be remembered by the rat and utilized on future occasions to reject the conditioned solution upon tasting it. Hence, the visceral cortex may be crucial for storage of the memory that the novel taste is linked to the aversive properties of morphine. Perhaps the associative information about the taste of saccharin and morphine's aversive qualities are relayed to the nucleus of the solitary tract or the parabrachial nuclei for comparison with subsequent tastes via the direct projections from the visceral cortex to the nucleus of the solitary tract and parabrachial nuclei [45].

Clearly, the speculations presented on the structures involved in perception of the aversive aspects of morphine and their association with tastes are incomplete. If our assumption is correct that the visceral cortex lesion only affects the association of the aversive qualities of morphine and taste, then it still remains to describe the rest of structures involved in the acquisition of CTA. For example, extensive work on the neural mechanisms of CTA has implicated the amygdala as important in attaching motivational significance to tastes [11, 26, 33]. A complete description of the structures involved in the perception of the aversive qualities of morphine is also lacking although the receptor population and the initial pathways now seem clear [2]. Given that the visceral cortex lesions specifically disrupt morphine-induced CTA, it is impossible at this time to provide a straightforward general explanation of the neural mechanisms underlying the CTA's induced by all psychoactive drugs.

To summarize, the visceral cortex appears to be critical for the establishment of a morphine-induced CTA. It remains unclear whether this effect is due to a decrease in the perception of the aversive qualities of morphine or whether it is due to the disruption of the association of these qualities with taste. The visceral cortex appears to play no role in the association of any conditioned stimuli with the aversive effects of LiCl nor in the association of sensory stimuli with the positive reinforcing properties of morphine. These results provide an anatomical dissociation of morphine's positive and negative motivational properties, and provide a lead into the problem of the differential associability of these motivational effects with stimuli from different sensory modalities.

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