National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canada

UNIVERSITY OF ALBERTA

TRANSSECTING DIRECT CONNECTIONS BETWEEN MEDIAL AND SULCAL PREFRONTAL CORTICES ALTERS THE RELATIVE ASSOCIABILITY OF TASTE AND PLACE CUES IN RATS

BY

PETRA MELIKE SCHALOMON

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

DEPARTMENT OF PSYCHOLOGY

Edmonton, Alberta
FALL 1992

National Library of Canada
Canadian Theses Service
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Service des thèses canadiennes

ISBN 0-315-77098-8

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Petra Melike Schalomon

TITLE OF THESIS: Transsecting Direct Connections between
Medial and Sulcal Prefrontal Cortices
Alters the Relative Associability of Taste
and Place Cues in Rats

DEGREE: Master of Science

YEAR THIS DEGREE GRANTED: 1992

10926 - 74 Avenue
Edmonton, Alberta
T6G 0E6

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled TRANSSECTING DIRECT CONNECTIONS BETWEEN MEDIAL AND SULCAL PREFRONTAL CORTICES ALTERS THE RELATIVE ASSOCIABILITY OF TASTE AND PLACE CUES IN RATS submitted by PETRA MELIKE SCHALOMON in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

Dr. Ann M. Robertson

Dr. Douglas L. Wahlsten

Dr. Mathew T. Martin-Iverson

July 8, 1992

I would like to dedicate this thesis to my husband, Mark Schalomon, and to my parents, Dieter and Karin Schalomon of Austria. They are there for me.

I would also like to thank my supervisor, Dr. Ann Robertson, her husband, Andre Laferriere, and the members of my examining committee, Dr. Doug Wahlsten and Dr. Mathew Martin-Iverson for investing their time and providing me with invaluable assistance and guidance.

Abstract

The effects of cutting the corticocortical connections between medial and sulcal prefrontal areas on the conditioning of taste and place cues were examined. In Experiment 1, rats were simultaneously exposed to taste and place cues before injection of 0.15 M LiCl. In controls, a significant conditioned taste aversion (CTA) but no conditioned place aversion (CPA) was observed. In contrast, rats with bilateral knife cuts showed a significant CPA but a weaker CTA. To test whether these results could have been due to the effects of simultaneously exposing the rats to taste and place cues during conditioning, rats were trained independently in either CTA or CPA paradigms in Experiment 2. In the CTA test, rats both in operated and control groups showed a CTA when first tested. Rats with bilateral knife cuts, however, showed a weaker CTA than those in the control group. In the CPA test, rats in the control group did not exhibit a CPA, whereas the knife cut group did. Rats with sham lesions tested in Experiment 2 did not differ from control subjects on either the CPA or the CTA test. Thus, bilateral cuts increased the CPA and decreased the CTA even when tested independently. These results indicate that the relative ease of association of place and taste stimuli may be accounted for in part by the organization of the intrinsic connections of the prefrontal cortex in the rat.

Table of Contents

List of Figures

List of Symbols and Abbreviations

cc: cubic centimentre

CC: corpus callosum

cm: centimetre

CPA: conditioned place aversion

CPP: conditioned place preference

CTA: conditioned taste aversion

CTP: conditioned taste preference

g: gram

ICSS: intracranial self-stimulation

kg: kilogram

LiCl: lithium chloride

M: molar

MC: medial prefrontal cortex

MD: mediodorsal thalamic nucleus

mg: milligram

ml: millilitre

PFC: prefrontal cortex

SC: sulcal prefrontal cortex

US: unconditioned stimulus

Introduction

In early accounts of associative learning, few factors, with the possible exception of an organism's motivational state, were assumed to limit the associability of stimulus and reinforcer (e.g., Mowrer and Lamoreaux, 1946). Pavlov (1927), for example, assumed that whenever a conditioned and an unconditioned stimulus are presented in close temporal contiguity, the conditioned stimulus will ultimately elicit the same reaction originally elicited by the unconditioned stimulus only. This concept - that any cue can be equally effective as any other - has been referred to as equivalent associability (Seligman, 1970). In the past 25 years, however, the concept of equivalent associability has been widely challenged. In a landmark paper published in 1966, Garcia and Koelling showed that in the presence of both an audiovisual and a gustatory stimulus, the avoidance reactions contingent upon electric shock transferred only to the audiovisual stimulus in rats. Avoidance reactions that were contingent upon injections of a toxin (lithium chloride) or X-ray exposure, both of which produce signs of illness, were transferred selectively to the gustatory stimulus. Garcia et al (1968) concluded that the pairing of a cue with a reinforcer was not always sufficient to produce conditioning. They proposed that associative learning occurred only if the cue was "appropriate" for the reinforcer.

In a systematic replication of the original findings of Garcia and Koelling (1966), Miller (1984) found that, whereas rats could learn to avoid the location where footshock was received, they did not learn to avoid a saccharin solution paired with footshock. Miller also failed to find evidence of an association between noise and lithium chloride (LiCl).

This lack of equipotentiality of stimuli in the formation of associations does not mean that certain associations cannot be conditioned. Rather, much research has shown that rats are capable of learning aversions to visual stimuli following LiCl injections and of learning aversions to gustatory stimuli following electric shock (Miller and Domjan, 1981). However, these aversions are generally weaker than those between visual stimuli and shock, or gustatory stimuli and LiCl (e.g. the associations require more trials to acquire; Mackintosh, 1983). Also, associations between visual stimuli and LiCl, for example, require many more pairings or a higher dose of LiCl before reliable conditioned aversive responses can be observed (White and Carr, 1985).

Much attention has been given to the learning mechanisms which underlie the selective associability of distinct reinforcing events to different stimuli. Garcia et al.(1968), for example, suggested that associative learning critically depended on the existence of a common neural substrate for processing both cue and reinforcer. Rescorla and Furrow (1977) proposed that stimuli can be classified along psychologically meaningful dimensions and that there may exist several qualitative relations among stimuli which affect conditioning, one example of which would be a common sensory modality. Rescorla and Furrow suggested that a cue of one specific modality might in fact increase the effectiveness of processing of other stimuli of the same sensory modality and therefore result in faster and stronger conditioning. Lett

(1974), on the other hand, suggested that ease of association depended not on the properties or processing of the stimuli to be associated, but rather upon the amount of interference by other associations that is exerted during conditioning. Nonetheless, both of the above theories of bias in associative learning have in common the notion that any learning will ultimately depend on the sort of central processing of the information that is learned. Indeed, Rozin and Kalat (1971) proposed that selective associability may be directly due to processes in the central nervous system. They pointed out that gustatory receptors involved in taste and visceral receptors involved in nausea are both classified as visceral sensory input to the brain and show a close neurological relationship because inputs from both converge in the medulla and other points along the neuraxis. This implies that the existence of different categories of stimulus processing is a direct consequence of the organization of sensory processing substrates in the central nervous system. Miller and Domjan (1981) later elaborated on this theory and pointed out that different sets of brain nuclei are involved in the processing of the exteroceptive classes of stimuli such as auditory and visual cues as opposed to the interoceptive classes of stimuli such as taste and visceral cues.

In fact, there is much experimental evidence to suggest that specific types of stimulus-reinforcer interactions are critically dependent on dissociable brain areas. One of the key developments in neuroscience over the past 20 years has indeed been the realization that different forms of learning are selectively affected by manipulations of different sets of brain structures (Kolb and Whishaw, 1985). This suggests that learning in the mammalian central nervous system may be organized in a modular fashion rather than dependent on a uniquely localized substrate (Thompson and Donegan, 1986; Olton, 1986). This mechanism of learning could be viewed as reflecting the operation of interactions among separate neural processing systems. A requirement for such interacting systems would be for them to be characterized by different domains of sensory processing, yet at the same time to be connected by a set of neural projections that would support direct functional interactions. These requirements are in fact met in the prefrontal cortex (PFC), which has functionally and anatomically distinct subdivisions that are connected by direct reciprocal projections. Lesions of one of the PFC subdivisions, the insular cortex, interfere with the acquisition of a conditioned taste aversion to lithium chloride (Lasiter and Glanzman, 1982) but are without effect on a conditioned avoidance reaction to spatial cues paired with shock (Dunn and Everitt, 1988). By contrast, there is considerable evidence that lesions of the dorsomedial prefrontal cortex selectively impair spatially mediated learning (e.g., Sutherland, Kolb, and Whishaw, 1982). In addition, Eichenbaum, Clegg, and Feeley (1983) have demonstrated that the constituent parts of the prefrontal cortex (PFC) can be doubly dissociated with respect to their effect on modality-specific learning tasks.

The experiments described in the present paper represent an attempt to examine whether the preferential associability of taste stimuli with nausea over place stimuli with nausea first noted by Garcia and Koelling (1966) is dependent on the organization of PFC subdivisions which are involved in processing these stimuli.

Anatomy and Connections of the PFC

Leonard (1969) suggested that the PFC, across a wide variety of species, includes two segregated processing systems, which can be distinguished by their diverging inputs from the mediodorsal thalamic nucleus (MD). The projections from the MD to the PFC are directed at the rostral quarter of the dorsal bank of the rhinal sulcus (also referred to as dorsal and ventral agranular insular cortex, and orbital area; Kosar et al., 1986b; Krettek and Price, 1977; Beckstead, 1979) as well as the rostral half of the medial hemispheric wall in the frontal lobes (Beckstead, 1979). Krettek and Price (1977) observed that projections from different areas of the MD are topographically organized. Evidence indicates that the central MD segment projects preferentially to agranular areas along the rhinal sulcus, rostral to the middle cerebral artery. Leonard (1969) referred to this area, which is adjacent to the rat gustatory cortex, as part of the sulcal prefrontal cortex or SC. The lateral MD segment, by contrast, projects to the dorsomedial shoulder of the frontal lobes. This area was referred to as medial prefrontal cortex or MC by Leonard (1969).

A major thalamic input to the SC, aside from that from the MD nucleus of the thalamus, stems from part of the ventral posteromedial thalamic nucleus. This nucleus is the thalamic relay for gustatory input from the parabrachial area and it projects to the rat agranular insular cortex, which includes the SC (Kosar, Grill, and Norgren, 1986b). However, there has been some confusion over which parts of the frontal cortex constititute the gustatory cortex in the rat. Early researchers such as

Benjamin and Pfaffman (1955) used the presence of electrical potentials elicited by stimulation of the chorda tympani and glossopharyngeal cranial nerves as an indicator for the location of the gustatory cortex. Using this method, they identified the granular insular cortex as the rat gustatory cortex. Later research indicated that the actual gustatory cortex may be the smaller, but adjacent, agranular insular cortex, the anterior part of which is the SC (Kosar et al., 1986a). This conclusion was based on the presence of specific electrophysiological responses to gustatory stimuli rather than electrical stimulation of cranial nerves.

Leonard (1969) noted that the SC has direct reciprocal connections with basal olfactory areas as well as with the hypothalamus. In fact, one part of the SC (the ventral agranular insular area) is the only neocortical area that has been shown to have reciprocal connections with the primary olfactory cortex (Reep and Winans, 1982b). These observations provide anatomical evidence for the involvement of the SC in the processing of olfactory, gustatory, and visceral sensation.

The MC, on the other hand, does not receive any direct input from olfactory or gustatory structures. It receives projections from polymodal sensory areas, like the temporal and visual cortices (Krettek and Price, 1977). In addition, the MC is known to receive projections from the pretectum and superior colliculi, areas which are known to be important for visual processing (Krettek and Price, 1974). The anatomy of afferent projections to the MC thus clearly suggests that whereas this area is not as intimately connected to gustatory and olfactory processing areas as the SC, it is involved with sensory processes related to exteroceptive stimuli, especially visual information. A similar dissociation applies to the main efferent projection patterns of the MC and the SC.

Efferent projections from the PFC subdivisions have also been described in detail by a number of researchers. The MC has been shown to project to the dorsal and caudal head of the caudate nucleus in the rat (Beckstead, 1979; Krettek and Price, 1977), as well as to the pretectum and deep superior colliculi (Leonard, 1969). The SC, by contrast, projects to the ventrolateral caudate-putamen (Divac and Diemer, 1980) as well as to deep cellular layers of the piriform cortex, and to the lateral entorhinal cortex, hypothalamus, and olfactory tubercle (Beckstead, 1979).

In contrast to the dissociable pattern of connections with various thalamic, subcortical, and cortical areas outside the PFC, the MC and the SC have been shown to be related via corticocortical connections. Beckstead (1979) first described connections between the MC and the ipsilateral as well as the contralateral SC. Ferino, Thierry, Saffroy, and Glowinski (1987) described fibres from the MC to both the contralateral MC and SC which course entirely within the corpus callosum, entering the forceps minor and decussating within the anterodorsal part of the corpus callosum. Furthermore, Reep and Winans (1982a) showed that the projections are topographical, that is, that the dorsal MC is reciprocally connected with the dorsal SC, and the ventral MC with the ventral SC, with some overlap of projections.

Anterior to the forceps minor of the corpus callosum only a few fibres pass between the MC and the SC. In this region, the projecting fibres pass straight from the MC to the SC, medial to layer VI of the orbital cortex, whereas more posteriorly they course through the forceps minor of the corpus callosum, ventral to layer VI of the frontal sensory motor cortex (Reep and Winans, 1982b). Projections between the SC and the contralateral MC, which are less dense than those between ipsilateral MC and SC, run through the corpus callosum as well.

The subdivisions of the PFC therefore appear to differ mainly in the nature of the dominant sensory input to each area. Evidence for the functional roles of the MC and the SC has come mainly from studies examining the behavioural consequences of damage to each of these prefrontal subdivisions. This evidence clearly suggests that the MC and the SC can be functionally dissociated, and that the functional specialization of each constituent area parallels the dominant sensory involvement suggested by the anatomical evidence.

Functions of the MC

Although there are a confusing variety of deficits which have been described as a result of medial prefrontal lesions, many of them may be reduced to a learning deficit specifically related to exteroceptive stimuli and, in particular, visual and tactile stimuli which define spatial locations. Kolb, Pittman, Sutherland, and Whishaw (1982) showed that rats with bilateral lesions of the MC are impaired at performing a radial arm maze or Morris water maze task. Performance on both these tasks is dependent on the rats' ability to find a spatial location based on its location in relation to distal environmental cues. Therefore, MC lesions appear to interfere with the processing of distal stimuli necessary for spatial orientation. This possibility is further supported by the repeated observation of learning deficits in tasks that rely on the use of similar spatial cues. For example, impairments in spatial delayed alternation in a T maze (Wikmark, Divac, and Weiss, 1973), delayed response on a spatial task (Kolb, Nonneman, and Singh, 1974) and spatial reversal learning (Divac, 1971; Kolb et al., 1974; and Nonneman and Kolb, 1974), have all been reported after lesions of the MC.

Furthermore, additional data strongly imply that the above learning deficits occur after MC lesions only when the task demands are explicitly spatial. Eichenbaum, Clegg, and Feeley (1983), for example, found that lesions of the MC, but not SC, impaired performance on delayed response, reversal, and spatial alternation tasks in rats, but did not affect performance on an olfactory discrimination task. In addition, Sakurai and Sugimoto (1985) showed that MC lesions affected the acquisition of a non-spatial go-no go delayed alternation task only marginally and were not associated with the perseverative deficit which characterizes the behaviour of rats with MC lesions in similar tasks using spatially cued responses.

In summary, it can be said that a number of diverse deficits have been described after damage to the MC. However, it may be possible in a number of instances to reduce these deficits to one mechanism involving the disruption of the ability to form associations between specific responses and spatial, or more generally, visual stimuli.

Functions of the SC

Consistent with the anatomical studies showing that the SC has extensive connections with areas of the brain involved in olfactory, gustatory, and visceral sensory processing, it has been demonstrated that removal of the SC, but not the MC, can cause deficits in behaviours dependent on gustatory or olfactory stimulation. Eichenbaum, Clegg, and Feeley (1983), for example, showed that SC lesions, but not MC lesions, result in specific impairments on a go - no go olfactory discrimination task. During this task, water-deprived rats were presented with one of two olfactory stimuli. Rats had to press a bar in response to one odor and refrain from bar pressing in response to the other odor in order to receive a water reward. Although animals with subtotal SC lesions could reach criterion performance, they required significantly more trials than sham operated controls or rats with MC lesions to do so.

Despite earlier and unsuccessful attempts at producing CTA deficits after SC lesions (Divac, Gade and Wikmark, 1975; Kolb, Nonneman, and Abplanalp, 1977), there is solid evidence that lesions directly involving the SC produce a deficit in the acquisition of a CTA without affecting taste thresholds (Lasiter and Glanzman, 1982). Lesions of the gustatory neocortex posterior to the SC, on the other hand, do not produce this CTA deficit (Lasiter and Glanzman, 1982). Lasiter and Glanzman (1985) showed that deficits in the acquisition of a CTA also resulted from lesions of the central and lateral amygdaloid nuclei. However, these deficits were correlated not with the size of the lesions themselves, but with the amount of subsequent transneural degeneration in forebrain areas including the olfactory tubercle and the SC. Based on this evidence they suggested that the SC is not involved in the detection and identification of gustatory stimuli, but rather is involved in the learning of associations involving tastes. As CTA learning depends on the formation of associations between gustatory and aversive stimuli, CTA deficits in the absence of sensory deficits may be due to a failure to form those associations.

The findings reported by Lasiter and Glanzman (1982, 1985) were extended by Dunn and Everitt (1988), who investigated the effects of electrolytic or ibotenic acid lesions of the amygdala and of ibotenic acid lesions of the SC on LiCl-mediated taste aversions and gustatory neophobia. They found that both ibotenic acid lesions of the SC and electrolytic lesions of the amygdala resulted in a decrease in neophobia and a significant decrease in the strength of CTA and faster extinction as compared to controls or subjects with ibotenic acid lesions of the amygdala. These deficits were demonstrably not due to a decrease in taste reactivity. As the excitotoxin ibotenic acid destroys cell bodies in the vicinity of the injection site without damaging fibres of passage, Dunn and Everitt concluded that electrolytic lesions of the amygdala affected CTA performance due to destruction of fibres which course through the dorsolateral amygdala on their way to the SC. Thus, CTA deficits in both subjects with ibotenic acid lesions of the SC <u>and</u> in subjects with electrolytic lesions of the amygdala were presumably due to direct or indirect damage in the SC only. These results therefore make a strong case for an involvement of the SC in the acquisition of aversions to gustatory stimuli paired with LiCl.

A further effect of SC lesions in rats is the disruption of normal food intake characterized by a period of aphagia which may result from orofacial sensorimotor deficits (Kolb and Nonneman, 1975; Glick, 1971). The SC lesions, at the same time, were shown not to affect gustatory sensibility (Kolb and Nonneman, 1975).

These results indicate that, as suggested by its anatomy, damage to the SC selectively interferes with learning processes specifically involving the use of gustatory, olfactory, and visceral information.

Relationship Between PFC Subdivisions

The evidence reviewed above makes a compelling case for the division of the PFC into functionally dissociable, modality-specific areas involved in learning. As argued by Eichenbaum et al. (1983), this organization suggests a possible substrate for the existence of mutually exclusive stimulus-reinforcer associations. However, as noted in the opening section of the present thesis, there is a substantial body of observations suggesting that animals can make associations other than those of taste with illness or visual stimuli with shock. For example, rats can learn to escape shock (as opposed to avoiding a stimulus associated with shock) equally well when cued with a flavour as when cued with a tone (Parker and Smith, 1981) and can learn a CTA to a gustatory cue paired with electric shock (Krane and Wagner, 1975).

Similarly, aversions to the visual aspects of food (Galef and Dalrymple, 1981), the texture of food (Martin and Lawrence, 1979), the container a food was presented in (Revuskv and Parker, 1976), or the environment a food was consumed in (Best, Brown, and Sowell, 1984; Archer, Sjoden, and Nilsson, 1979) have been found in experiments using LiCl as the unconditioned stimulus. These results indicate that it is by no means impossible to condition taste aversions to exteroceptive stimuli or to obtain other aversions using stimulus-reinforcer combinations that do not conform to those used by Garcia and Koelling (1966).

Thus, the observation of bias in conditoning experiments would appear to depend on a process of cue selection or categorization rather than on the existence of mutually exclusive and modality-specific mechanisms. With respect to the possible neural substrates for these effects, this in turn implies that biases in conditioning might be related to interactions between distinct areas involved in the processing of distinct classes of reinforcing events.

Behavioural and anatomical data suggest that the subdivisions of the rat PFC are indeed organized as dissociable extensions of different sensory systems. In the light of this knowledge, the existence of a strong anatomical link between the MC and the SC suggests the possibility of functional interactions between the two areas. There is indeed evidence that the MC and the SC interact functionally. The hypothesis that the MC and the SC interact via a functional link was tested in a series of experiments disconnecting the two areas. When neural connections between the MC and the SC are bilaterally transsected, intracranial self-stimulation (ICSS) of the

MC is significantly, but not permanently, altered (Corbett, Laferriere, and Milner, 1982; Robertson, Laferriere, and Milner, 1986). This implies that activation of the SC via fibres from the MC may be at least partly responsible for self-stimulation of the MC.

Based on these findings, Robertson and Laferriere (1989) suggested a hierarchical organization of the MC and the SC via the fibres connecting the MC and the SC. According to their view, the rewarding effects of MC stimulation are mediated via projections to the SC. In accordance with this hypothesis, these authors found that when rats are exposed to either a novel gustatory or visuotactile stimulus before ICSS of the MC, normal rats develop a conditioned taste preference, but no conditioned place preference. In rats with transsections of MC-SC projections, a reverse pattern of learned associations was found. Robertson and Laferriere suggested that this change in learned associations resulted from a change in the relative associability of the two classes of stimuli used due to transsection of MC-SC fibres. These results therefore suggest that the corticocortical projections linking the MC and the SC may serve to modulate the associative salience of exteroceptive and interoceptive stimuli. As these effects were obtained with the use of an appetitively motivating reinforcer, however, the generality of this hypothesis is questionable.

The present experiments were performed to investigate the function of the MC-SC projection fibres in associative learning when an aversive reinforcer is used. Two experiments were conducted in order to clarify the role of MC-SC projection fibres in the learning of taste and place aversions induced by injections of

LiCl. As reviewed above, there is much evidence to suggest that the aversive consequences of systemic LiCl injections are more readily associated with gustatory (and olfactory) cues than with exteroceptive stimuli (Garcia and Koelling, 1966; Miller, 1984). In both the original demonstration of stimulus-reinforcer specificity (Garcia and Koelling, 1966) and a subsequent replication (Miller and Domjan, 1981), the bias to associate LiCl induced toxicosis with sapid stimuli and footshock with visual or auditory stimuli was demonstrated in a procedure where both types of cues were concurrently present during the pairing phase of the conditioning experiment. In Experiment 1, I therefore investigated the effects of surgical transsection of the projection path between the MC and the SC on the acquisition of taste and place aversions obtained by pairing concurrent spatial and gustatory cues with LiCl injections. This was done in order to obtain a measure of the effects of the surgery on the relative strength of conditioning to each type of stimulus. The hypothesis of a role for the corticocortical prefrontal projections was tested by evaluating the degree and direction of conditioning in operated and control animals.

The results of Experiment 1 were further clarified in Experiment 2, where the effects of disrupting the corticocortical projection fibres between the MC and the SC were studied in separate tests of conditioned aversions to place or taste cues in an attempt to determine whether the effects of the lesions on selective associability seen in Experiment 1 and in other experiments (Garcia and Koelling, 1966; Miller and Domjan, 1981) could be explained as overshadowing effects.

General Method

Methods common to both experiments are described below. Methods specific to an individual experiment are described in the appropriate sections for each experiment.

Subjects and Surgery

Male Sprague-Dawley rats weighing between 270 and 610 grams at the time of surgery were used. All animals were housed in single transparent plastic cages with woodchip bedding at ambient temperatures of 21 degrees C and all were maintained on a 12-hour-light/12-hour-dark cycle. Animals had ad libitum access to food (Purina rat chow) and tap water, except where indicated. Subjects in the surgical treatment group received bilateral knife cut transsections (see Figure 1) aimed at the MC-SC projection fibers (Robertson and Laferriere, 1989). Rats were anesthetized with intraperitoneal injections of 50 mg/cc sodium pentobarbital (Nembutal) at 1 cc/kg body weight and, just before commencing surgery, anesthetized locally with subcutaneous injections of 0.1 cc of Novocaine.

The knife consisted of a piece of 23-gauge stainless steel tubing housing a somewhat longer piece of 30-gauge stainless steel wire that had been coiled previously. The tip of the tubing was finely bevelled and bent such that the wire would extend from it at close to a 45 degree angle (see Figure 2). Depending on the

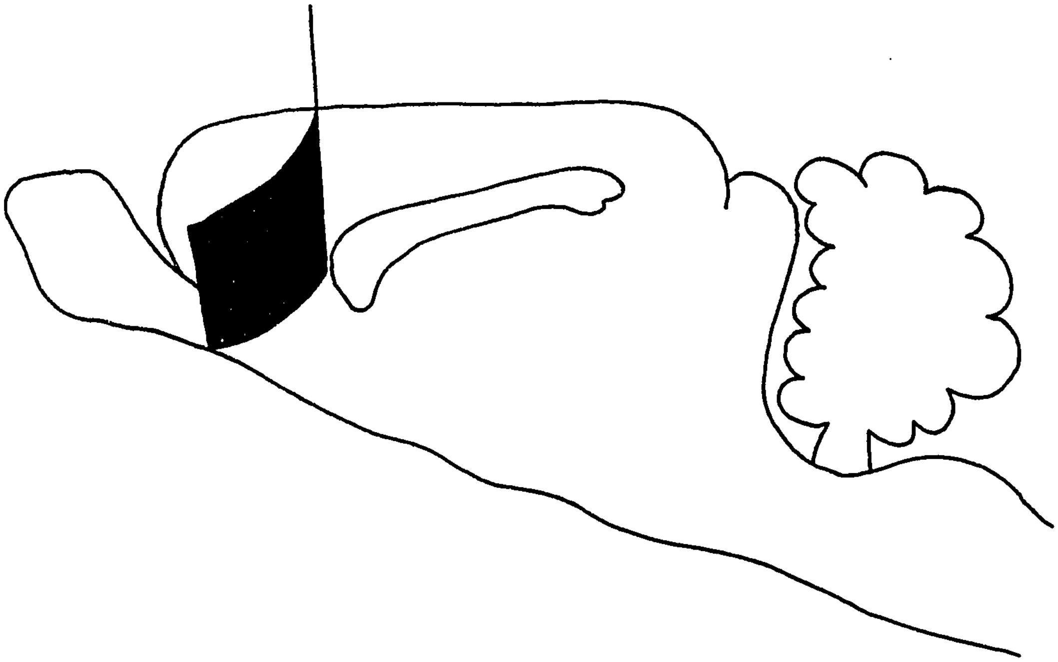


Figure 1. Lateral view of the rat brain showing the approximate rostrocaudal and dorsoventral extent of knife cuts. Actual cuts are not at midline, as shown, but on either side of it.

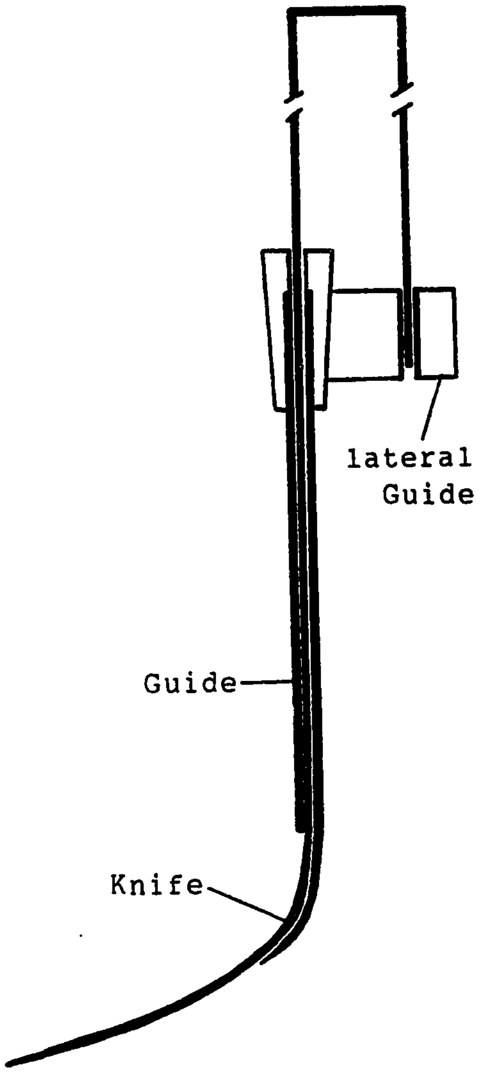


Figure 2. Knife assembly used during surgery. Actual length of the entire assembly was approximately five cm.

setting of the incisor bar between +2.0 mm and +5.0 mm, the coordinates for insertion of the knife guide varied from +1.0 mm to +2.5 mm anterior to bregma. Lateral coordinates were 2.2 mm on either side of bregma in all cases. The knife guide was lowered to -3.5 to -3.7 mm ventral to dura, the knife extended and the whole assembly moved dorsally to 0.1 mm below dura before removal of the assembly.

Subjects were given a minimum of five days to recover from surgery during which period their weight as well as amounts of food and water ingested were monitored daily. Subjects that did not begin to regain weight within two days of surgery were given access to wet mash until pre-surgery weight was reached. This measure was necessary in less than five percent of surgically treated animals.

Taste Conditioning

The general procedure used was similar to that used in previous experiments (eg. Westbrook and Brookes, 1988). Rats were exposed to one novel flavour paired with the unconditioned stimulus (an injection of LiCl[1]), and

---

[1] During the past 20 years, LiCl has frequently been used as an aversive stimulus in conditioning procedures using rats, due to its relative ease of administration and rapid onset of aversive effects (Nachman and Ashe, 1973). Whether LiCl evokes nausea or gastrointestinal distress in rats as well as humans cannot be directly measured. However, Pelchat, Grill, Rozin, and Jacobs (1983) described a constellation of orofacial responses to a sucrose solution paired with LiCl which they interpreted as a sign of distaste due to nausea.

another novel flavour, given on alternate days, paired with the vehicle (an injection of saline).

All rats were water deprived on the day before commencing the experiment. A completely counterbalanced design was used. Each day, equal numbers of animals received each one of the flavours and each flavour was paired with either LiCl or saline in equal numbers of subjects. On pairing days, rats were given 20 minutes' access to a vanilla flavoured solution (4% Scotch Buy Vanilla Extract in distilled water) or an almond flavoured solution (1% Empress Almond Extract in distilled water), both of which had been demonstrated in pilot tests with other animals to be equally likely as tap water to be consumed. Sessions were held in an experimental room outside the animal colony with a background of white noise. Depending on the experiment, subjects were either in their home cages or place conditioning boxes (see below for description) during this time. Ingestion was from a graduated glass drinking bottle using a metal drinking spout with a metal ball bearing to minimize dripping and was measured to the nearest 1 ml. Immediately after the 20 minute drinking period, rats were injected intraperitoneally with either 0.9% saline or 0.15 M LiCl before being returned to the colony room. Additional water was given only to animals that consumed less than 8 ml of fluid during the daily experimental session. These rats were given 10 minutes' access to tap water in their home cages in the colony room at least one hour after the experimental session.

On the test day, rats were given access to both almond and vanilla solutions in their home cages in the experimental room. The position of the two bottles in the home cage was varied randomly to minimize the effects of possible right-left preferences of subjects. The amount of each solution consumed during twenty minutes was measured to the nearest 1 ml. Free access to water was given immediately upon completion of the experiment.

Place Conditioning

The procedure used was similar to that used in previous experiments (eg. White and Carr,1985). Each of two novel environments (conditioning boxes with distinctive visual and tactile cues) was paired with injections of LiCl or saline. This was done in such a way that one environment was paired twice with LiCl and the other environment was paired twice with saline.

The conditioning apparatus (illustrated in Figure 3) consisted of two wooden boxes (30 cm wide by 28 cm deep by 22 cm high) connected by an alleyway (30 cm wide by 19 cm deep by 19 cm high) that could be blocked off on either end by a removeable wood partition. One of the boxes (referred to as white) was painted with transparent polyurethane varnish and had a plexiglass floor with round holes (1 cm diameter) drilled in it. The other box (referred to as black) was also painted with polyurethane varnish, in a pattern of 4 cm wide black and white horizontal stripes. It had a wiremesh floor. The connecting alleyway was made entirely of unpainted wood. All three compartments had a transparent plexiglasss front and were

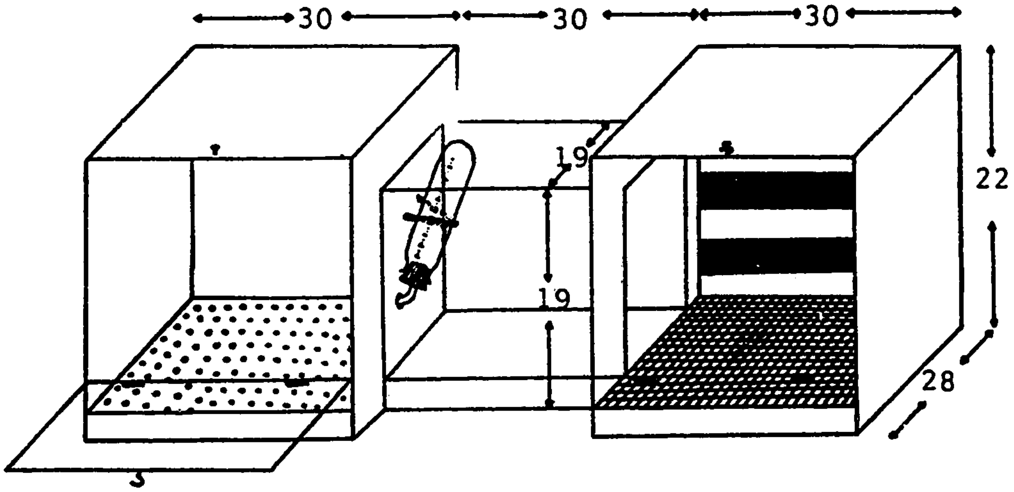


Figure 3. Apparatus used for place conditioning, showing bottle mounted for taste conditioning as used in Experiment 1. Numbers refer to length in cm.

washed with a detergent solution, rinsed with water, and dried after each use in order to minimize odour cues.

Pilot tests in a number of animals not included in this experiment had been conducted using many pairings of the two chambers with saline injections. These tests indicated that, on average, rats had no innate preference for either one of the boxes over the other.

Experimental sessions were held in an experimental room outside the animal colony with a background of white noise. A completely counterbalanced design was used. Half of the subjects in each group were exposed to the white box and the other half to the black box on conditioning days 1 and 3 and vice versa on days 2 and 4 for 20 minutes each session. In half of the subjects the white box was paired with LiCl injections and the black box with saline. In the other half of the subjects the black box was paired with LiCl and the white box with saline. In all cases, the saline or LiCl was administered immediately after the 20 minute exposure to the white or black box.

On day 5, after a total of two exposures to the LiCl-paired box and two to the saline-paired, rats were positioned in the alleyway (to which they were being exposed for the first time) and given free access to the entire conditioning apparatus for 20 minutes. Time spent in the white and black boxes as well as the alleyway was recorded in seconds. The position of head and forelimbs was used to determine the location of the animals.

Data Analysis

Data from both taste and place conditioning tests were analyzed separately using aversion ratio scores (formula: LiCl/(LiCl+saline)). Thus, for data from the CPA test, a ratio score of the time spent in the LiCl paired compartment divided by the total time spent in LiCl plus saline paired compartments, was used. One such ratio was calculated for each of the four consecutive periods comprising one test session, as well as one ratio for the total session. Data from the CTA test were analyzed using a ratio measure computed by dividing the amount of the LiCl paired solution consumed by the total fluid consumption (LiCl paired plus saline paired solutions) during testing. This ratio score is comparable to similar scores used in previous experiments measuring aversions or preferences to specific stimuli (e.g., Dunn and Everitt, 1988). In effect, these ratio scores express the preference or aversion of subjects toward the LiCl paired stimulus, with a value of 0.50 denoting an equal preference for the LiCl paired as for the saline paired stimulus. A value below 0.50 denotes an aversion for the LiCl paired stimulus and a value greater than 0.50 denotes a preference.

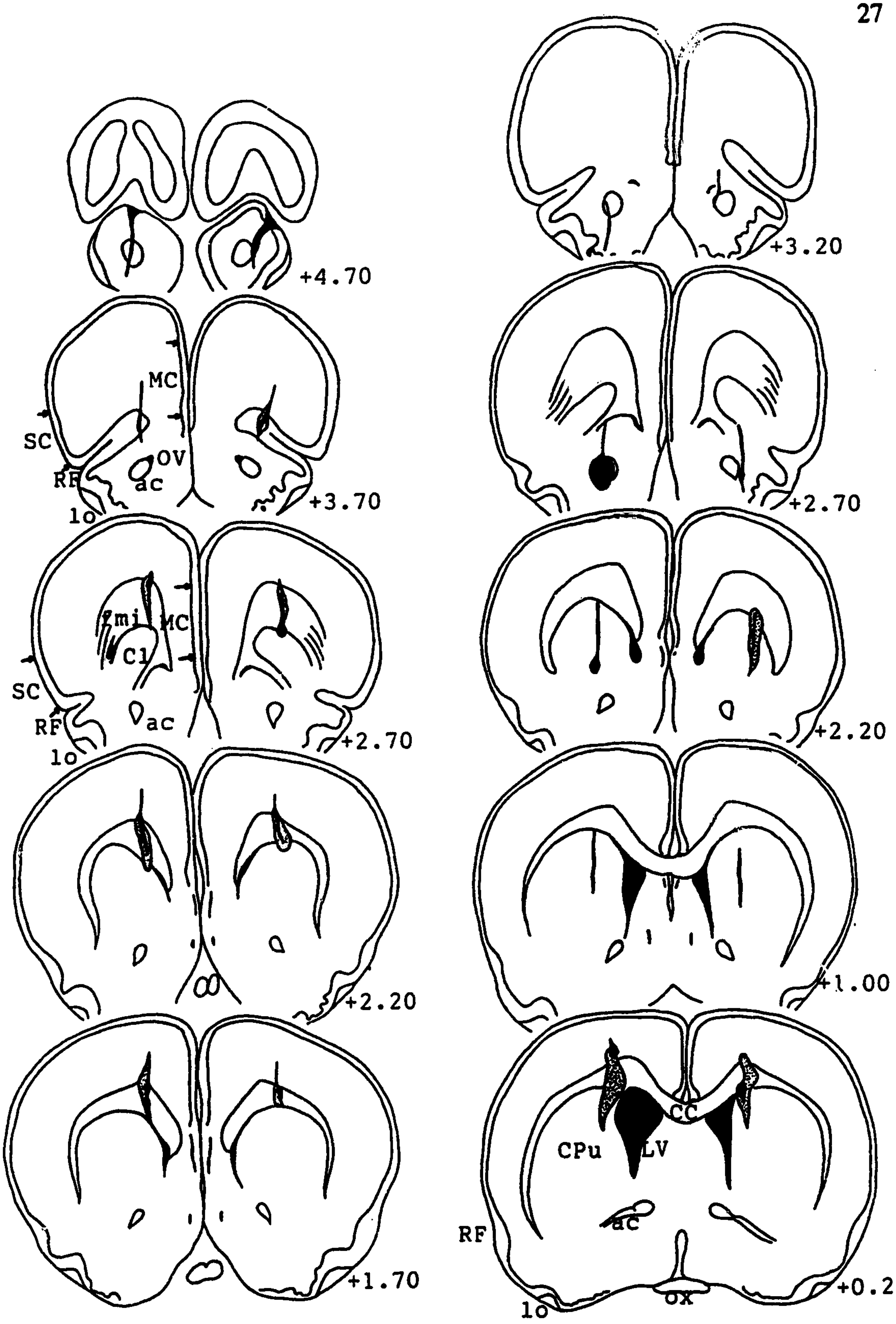
To compare treatment groups, analyses of variance were conducted using the same aversion ratio scores. To determine whether an aversion was present, one-tailed planned Student's t tests were conducted for each group in order to detect whether the ratios were significantly below 0.50.

Histology

Following completion of the experiment, rats were deeply anesthetized with an overdose (1 cc) of sodium pentobarbital (50 mg/cc) and perfused transcardially with 0.9% saline followed by 10% formalin. The brains were removed, stored in 10% formalin for several days and sectioned on a cryostat at 46 micrometers thickness. Every sixth section was mounted on a chromalum coated slide and stained using a modified Kluver-Barrera procedure. Sections were examined under a microscope and several drawings encompassing the full extent of lesions were made with the aid of a projection apparatus.

All ratings of knife cuts were done blindly by an independent observer. Knife cuts were judged successful if they extended minimally from the dorsal boundary of the forceps minor of the corpus callosum to a point level with or ventral to the rhinal sulcus and involved minimal striatal damage. Knife cuts judged unsuccessful were reexamined for possible inclusion in sham groups for Experiment 2. The sham groups included animals in which the corpus callosum had not been lesioned anterior to the level of the anterior commissure and in which minimal striatal damage was observed. Figure 4 a and b shows the extent of damage in representative animals included for analysis.

Figure 4. Tracings of coronal sections from (a) a representative animal in a lesion group, and (b) a representative animal in a sham group. Shaded areas define the extent of actual cuts or bleeding, stippled areas define extensive gliosis. Numbers refer to distance anterior to bregma. Abbreviations used on figure: ac - anterior commissure, CC - corpus callosum, Cl - claustrum, CPu - caudate putamen, fmi - forceps minor corpus callosum, lo - lateral olfactory tract, LV - lateral ventricle, MC - medial prefrontal cortex, OV - olfactory ventricle, ox - optic chiasm, RF - rhinal fissure, SC - sulcal prefrontal cortex.


+4.70
MC
SC
OV
RF
ac
lo
+3.70
fmi
MC
Cl
SC
RF
ac
lo
+2.70
+2.20
+1.70
+3.20
+2.70
+2.20
+1.00
CC
CPu
LV
RF
ac
lo
ox
+0.20

(a) (b)

Experiment 1

The purpose of this experiment was to study the effects of transsecting MC-SC projection fibres on the relative strengths of LiCl-mediated taste and place aversions. To achieve the aversions, subjects were simultaneously exposed to both a taste and an environmental stimulus before toxicosis was induced. The relative strengths of conditioning to each stimulus in two treatment groups, both of which were exposed to the same stimulus compund, were examined.

Method

Forty-eight male Sprague-Dawley rats were used. MC-SC projection fibres were bilaterally transsected in 32 of the subjects. During two conditioning trials subjects were injected with 2.12 ml per 100 g body weight of 0.15 M LiCl. On alternate days, subjects were injected with 2.12 ml per 100 g body weight of 0.9% saline.

For each conditioning trial, subjects were put into the assigned conditioning chamber. A graduated bottle containing the appropriate flavoured solution was mounted outside the conditioning chamber such that the drinking spout extended into the box through the wood partition blocking off the alleyway. Pairings of box (white versus black) with taste (almond versus vanilla) and injection (LiCl versus saline) were fully crossed. After 20 minutes of exposure to the conditioning chamber with free access to the taste solution, subjects were removed from the chamber and immediately injected with either LiCl or saline, as appropriate. The amount of fluid consumed was recorded to the nearest 1 ml and subjects were then returned to the animal colony in their home cages.

On day 5, the test day, the rats were tested in separate sessions for conditioning to each of the two stimuli. Half of the subjects were first tested for taste conditioning and then for place conditioning, whereas the order of testing was reversed in the remainder of animals. Between the two tests, animals were returned to the colony room for a minimum of one hour. Free access to water was given only after both tests had been completed.

Results

Data from all subjects in the lesion group whose surgery had been unsuccessful (see General Methods section) were excluded. Data from subjects in either group that remained immobile for a predetermined portion of the time during place aversion testing were also excluded[2]. Only one subject, in the lesion group, was excluded on this basis. Some animals were also excluded from analysis based on

---

[2] This procedure has been followed in previous research (for example, Miller, McCoy, Kelly, and Bardo, 1986). Miller (1984), in a table of behavioural categories in rats, called this behaviour freezing and defined it as an "absence of all movement, except breathing" (p. 67). For the purpose of the present experiment, animals that remained in one of the three compartments for three of the four consecutive five minute periods comprising a test session and spent a total of less than 20 seconds in the other two compartments were operationally defined as having displayed freezing behaviour.

data collected during taste conditioning: data from subjects consuming less than three ml of fluid during any of the four conditioning sessions were excluded (subjects excluded on this basis included four surgically-treated animals as well as two control subjects). As Domjan and Wilson (1972) have shown, rats that drink less than two ml of a saccharin solution on a pairing trial followed by poisoning acquire a significantly weaker taste aversion than rats consuming larger amounts. This left 18 subjects in the lesion group and 12 subjects in the control group.

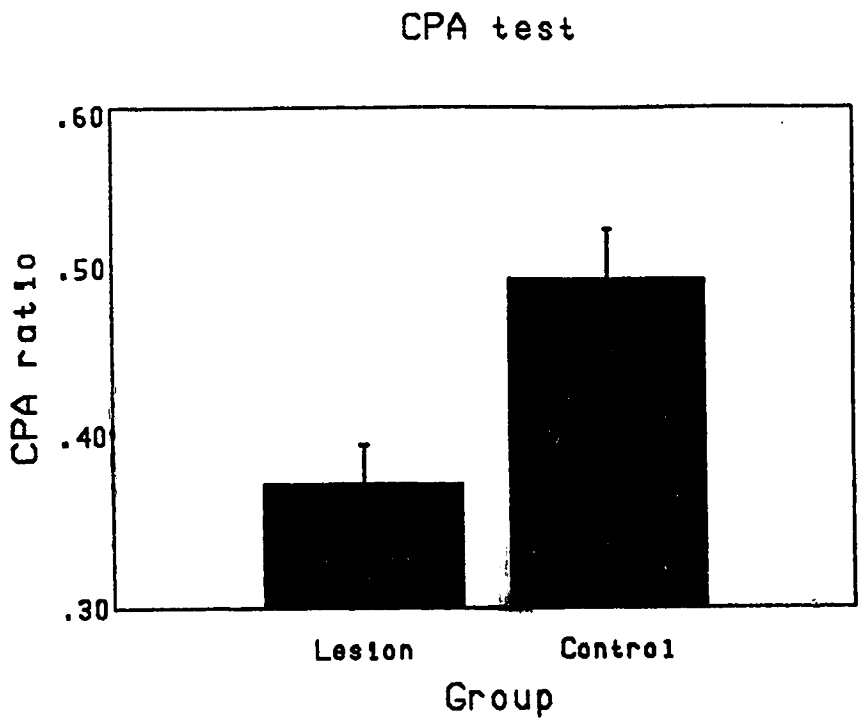
<u>CPA Test</u>

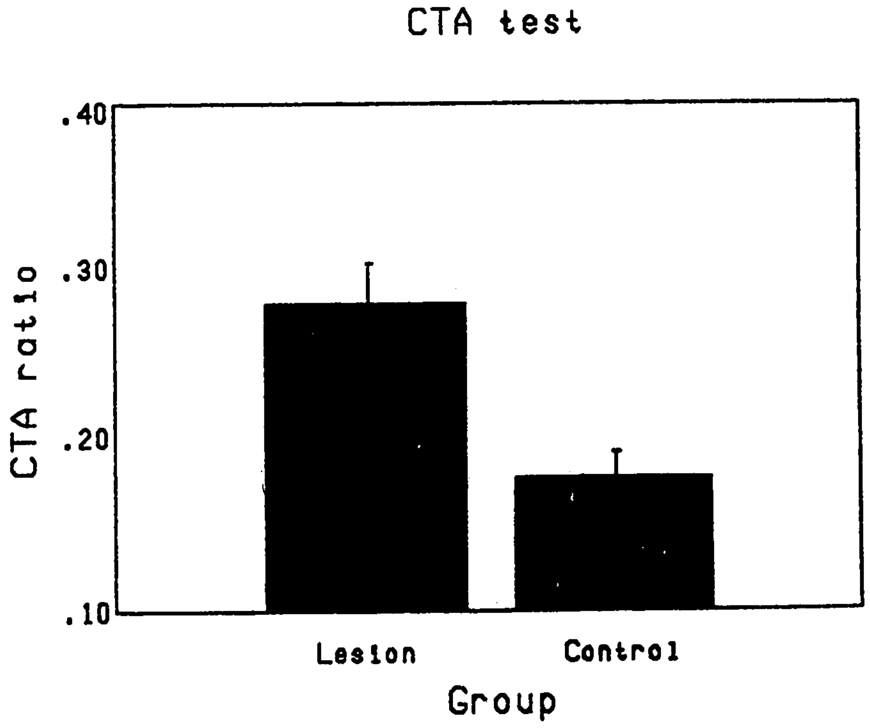
Lesioned animals were found to have a significant CPA ($t=3.46$, 17 df, $p<.005$) in that they spent less time in the LiCl-paired compartment than the saline-paired compartment. Control animals did not have a CPA ($t=.009$, 11 df, $p>.05$). These results are illustrated in Figure 5a.

There was, moreover, a significant difference between mean aversion ratios for the two groups. A 2x4 ANOVA of treatment by period, with period treated as a repeated measure, was calculated. Animals in the lesion group had a significantly lower aversion ratio than those in the control group, ($F(1,112)=7.73$, $p<.01$), with mean aversion ratios of 0.374 and 0.496 respectively. There were no significant time or interaction effects.

Both groups treated the alleyway connecting the two pairing chambers in the same mannner. A oneway ANOVA for the total time spent in this alleyway

Figure 5. Mean aversions of animals in the lesion and control groups used in Experiment 1. Aversions are expressed in ratio form, with ratio values below 0.50 denoting an aversion to the LiCl-paired stimulus. Vertical bars indicate standard errors. (a) Performance on place aversion, and (b) ontaste aversion tests.


CPA test
.60
.50
.40
.30
CPA ratio
Lesion
Control
Group


CTA test
.40
.30
.20
.10
CTA ratio
Lesion
Control
Group

during the test session was run for the two treatment groups and was not significant, F(1,28)=0.55, p>.05.

CTA Test

In the CTA test, both groups consumed equal amounts of fluid on testing day. A oneway ANOVA for the total amount of fluid intake (LiCl plus saline paired solution) on the testing day showed no difference in total intake for the two treatment groups, F(1,28)=2.08, p<.05.

Both surgery and control groups were found to have a CTA, as illustrated in Figure 5b (t=6.71, 17 df, p<.0005 for the lesion group and t=20.15, 11 df, p<.0005 for the control group). That is, rats in both groups consumed significantly less of the solution previously paired with LiCL than of that previously paired with saline injections. However, the CTA was stronger in the control group. Animals in the lesion group had a significantly higher aversion ratio (with a mean of 0.282), than animals in the control group (with a mean of 0.178; F(1,28)=6.42, p<.05).

Discussion

These results demonstrate that the relative magnitudes of conditioned taste and place aversions are changed when the projection fibres between MC and SC are bilaterally transsected.

The results of the CPA test show that control subjects did not develop an aversion to the compartment paired with LiCl injections. Subjects in the lesion group, by contrast, did display a place aversion. The CTA test showed that both groups had developed a significant aversion to the taste paired with LiCl. However, the size of the aversion was lower in lesioned animals than in control animals. Thus it may be that, whereas the taste cue was associated with the aversive effects of LiCl in all subjects, the association was weaker in the surgically treated group. These results suggest that bilaterally transsecting MC-SC projection fibres may have decreased the relative associability of taste cues with the aversive effects of LiCl injections and increased the relative associability of the cues present in the place conditioning chamber with the same aversive effects.

However, several other factors could also account for the relative changes in CTA and CPA following knife cuts. One possibility is that the lesions affected the lesion group's reactions to novel stimuli. As no rats had been exposed to the connecting alleyway before the test day, it was a novel place to all subjects. The fact that rats in both groups spent a comparable amount of time in this compartment indicates that lesioned rats displayed the same response to place novelty as controls. This provides evidence that the effects of of LiCl pairing could not be due to state-dependent learning effects.

Another possibility is that bilaterally transsecting the MC-SC projection path affected gustatory sensitivity. Whereas it is evident that the subjects in the lesion group <u>were</u> still capable of distinguishing between the two tastes (as they still exhibited a significant aversion to the taste paired with LiCl injections), it is possible that the gustatory sensitivity was merely decreased, not abolished, in the lesion group, thus resulting in a weaker taste aversion. However this seems unlikely, because lesioned rats in fact displayed a stronger gustatory neophobia than controls. During the first exposure to both the almond and vanilla solutions, consumption was slightly, but significantly, lower in the lesion group as compared to the control group (this difference disappeared on the second pairing day). This would support the contention that lesioned rats were no less sensitive to gustatory stimuli than control animals.

A factor that could have influenced responding on both CPA and CTA tests is the effectiveness of the unconditioned stimulus, LiCl. If the aversiveness of the LiCl injection was changed in the lesion group, both the CPA and CTA might have changed as well. However, if the LiCl had produced greater aversive effects in the lesion group, then both the CPA and the CTA should have increased in proportion to the aversive effects of LiCl injections. Similarly, if the effectiveness of the LiCl had decreased, the CPA and CTA would have decreased as well. As the strength of the CPA was in fact increased and the strength of the CTA decreased in the lesion group, a change in the effectiveness of the unconditioned stimulus due to surgery can probably not account for the results obtained in this experiment.

One more possible explanation for the observed pattern of aversions exists, however. It is possible that, because the taste and place stimuli were presented simultaneously to each subject, conditioning to the taste stimulus in the control group overshadowed conditioning to the place stimulus and that the effect of the knife cuts was to reduce this overshadowing. The phenomenon of overshadowing has been frequently demonstrated in the past. Foree and LoLordo (1973) showed that when a compound of two stimuli is presented during conditioning, one of the two stimuli may come to control responding significantly better than the other. These authors traingd pigeons to perform an operant response in the presence of a tone - light stimulus compound in order to avoid shock or receive food reward. After the subjects reached criterion performance, the strength of responding to the compound and each of its composite stimuli was measured. The authors found that most responses were made to the stimulus compound regardless of the nature of the reinforcer. When the reinforcer was electric shock, some responding also occurred to the tone and very little to the light. When the reinforcer was food, however, many responses were made to the light and very few to the tone. Foree and LoLordo concluded that overshadowing had in fact occurred due to the simultaneous use of the two stimuli. The direction of overshadowing depended on the nature of the reinforcer used.

If overshadowing had occurred in the present experiment, the place stimulus might not have been conditioned in the control group due to the fact that conditioning to the taste stimulus was simultaneously achieved. It is conceivable that the surgery in the lesion group decreased the amount of overshadowing and therefore increased place conditioning (thowgh this does *not* explain the CTA effect). In fact, as Garcia and Koelling (1966) and Miller and Domjan (1981) used a similar procedure, their results, too, could have been the result of overshadowing of conditioning to one component stimulus of the compound by conditioning to the other.

Experiment 2 was carried out in an attempt to test this explanation.

Experiment 2

If the overshadowing explanation presented above is adequate to account for the changes at least in CPA following knife cuts, then it would be predicted that using independent groups of rats for the CPA and CTA would not produce the same direction of effects. More specifically, one would expect that due to the absence of overshadowing, the control group wowld show an equally strong CPA as a lesioned group. The strength of CTA, on the other hand, would still be weaker in the lesion group. Thus, in Experiment 2, separate groups of rats werg exposed either to taste or to place cues before receiving injections of LiCl.

Another methodological change in the present experiment concerned the groups receiving CTA training only.

As taste aversions in both groups used in Experiment 1 had been significant and strong, the number of conditioning sessions and dose of LiCl used here were reduced in order to differentiate further the treatment groups. Additionally, as the strength of learning can also be measured by resistance to extinction (for example, Seligman and Campbell, 1965), a number of extinction trials were also conducted in this experiment.

Method

CPA Procedure

Thirty-four male Sprague-Dawley rats were used. MC-SC projection fibres were bilaterally transsected, as described in the General Method section, in 24 of the subjects.

Procedures used for place conditioning were the same as described for Experiment 1 except that, as no taste conditioning was conducted, no novel tasting solutions were presented to the rats. All subjects were water deprived in order to ensure a similar physiological state in the rats used in this experiment as in subjects used in Experiment 1. A minimum of one hour after each experimental session, all subjects were given 20 minutes' access to tap water in their home cages.

Tests for place conditioning were conducted as in Experiment 1. Free access to water was given immediately upon completion of the test session.

CTA Procedure

Thirty-four male Sprague-Dawley rats were used. MC-SC projections were bilaterally transsected in 24 of the subjects.

Procedures for taste conditioning were generally as descibed in the General Methods section except that only two conditioning sessions were conducted. During one of these sessions one taste was paired with a LiCl injection, and during the other session the other taste was paired with a saline injection with order of pairings and taste-drug combinations completely counterbalanced. During each session, rats were presented with the appropriate taste solution in their home cages for 20 minutes, after which they were immediately injected with 1.1 cc per 100 g body weight of 0.15M LiCl or 0.9% saline. This constituted a dose reduction of 50% from the dose used in Experiment 1.

A total of nine taste conditioning tests were conducted on nine consecutive days, placement of the two bottles of taste solutions relative to each other being randomized each day.

Results

As in Experiment 1, data from subjects that froze during CPA testing were excluded from analysis. Data from subjects that consumed less than three ml of fluid during at least one CTA conditioning session were also excluded from analysis. Some subjects that had undergone surgery and whose surgery had been unsuccessful were included in a homogenous sham surgery group if they met the appropriate standards set forth in the General Method section. All decisions on inclusion of specific subjects into either lesion or sham surgery groups were made blindly by independent observers. For the CPA procedure, this left 11 subjects in the lesion group, 10 subjects in the control group, and 9 subjects in the sham surgery group. For the CTA procedure, 10 animals each remained in the lesion and control groups and 3 animals in the sham surgery group.

Data from the two tests were analyzed separately, using the same aversion ratio scores as in Experiment 1. Lesion, sham, and control groups were analyzed as three independent treatment groups.

CPA Test

The CPA results are depicted in Figure 6. The lesion group exhibited a CPA ($t=1.86$, 10 df, $p<.05$), whereas the other two groups did not ($t=0.136$, 9 df, $p>.05$ and $t=0.339$, 8 df, $p>.05$ for control and sham groups respectively).

The lesion group differed significantly from the control and sham groups. A 3x4 ANOVA of treatment by period, with period treated as a repeated measure, was calculated, followed by Newman Keul's tests. Only the treatment effect was significant ($F(2,108)=3.75$, $p<.05$). Ratio scores were significantly lower for the lesion group (mean=0.368), than for the control or sham groups (means=0.489 and 0.513 respectively) which were not significantly different from each other. This indicates that the place aversion was indeed stronger in the lesion group as compared to the two other groups.

A oneway ANOVA for the total time spent in the neutral alleyway during the CPA test session was run for the three treatment groups. There were no significant differences among the groups ($F(2,27)=3.14$, $p>.05$), which suggests that

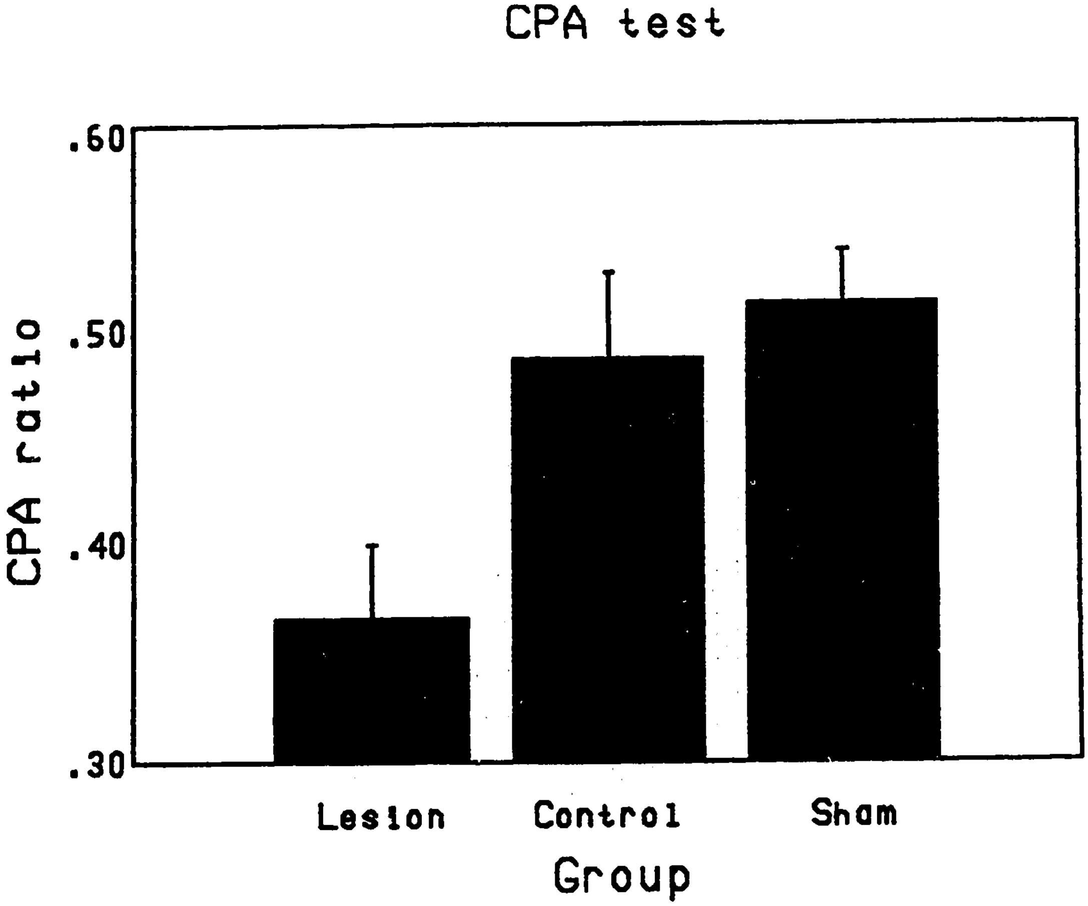


Figure 6. Mean place aversions of animals in the lesion, control, and sham groups used in place conditioning during Experiment 2. Ratio measures significantly below 0.50 denote a place aversion. Vertical bars indicate standard errors.

subjects in all three groups reacted similarly to completely novel, spatial stimuli.

CTA Test

There were no significant differences among the total fluid intakes during the nine CTA test days for the three treatment groups. Total intake measures (LiCl paired plus saline paired solutions) were analyzed in a 3x9 ANOVA for the three treatment groups by the test day, with day treated as a repeated measure. The day main effect was significant^O(F(8,180)=8.62, p<.001) with total consumption rising from a mean of 16.29 ml on day 1 to a mean of 24.03 ml on day 9.

All three treatment groups exhibited a significant CTA on the first test day, as illustrated in Figure 7 (t=8.83, 9 df, p<.0005 for the lesion group, t=2.77, 9 df, p<.01 for the control group, and t=9.04, 2 df, p<.005 for the sham group).

Over the course of the nine CTA tests, the lesion group exhibited a weaker aversion than the other two treatment groups. Data from the nine consecutive CTA tests were analyzed in a 3x9 ANOVA for the three treatment groups by the test day, with day treated as a repeated measure. The treatment effect was significant, (F(2,180)=5.39, p<.01). The mean ratio score over the nine test days was highest in the lesion group (mean=0.349) and equally low for the control and sham groups (means=0.252 and 0.240 respectively). A post hoc Newman-Keul's test showed that the mean for the lesion group was significantly higher than that for the control and

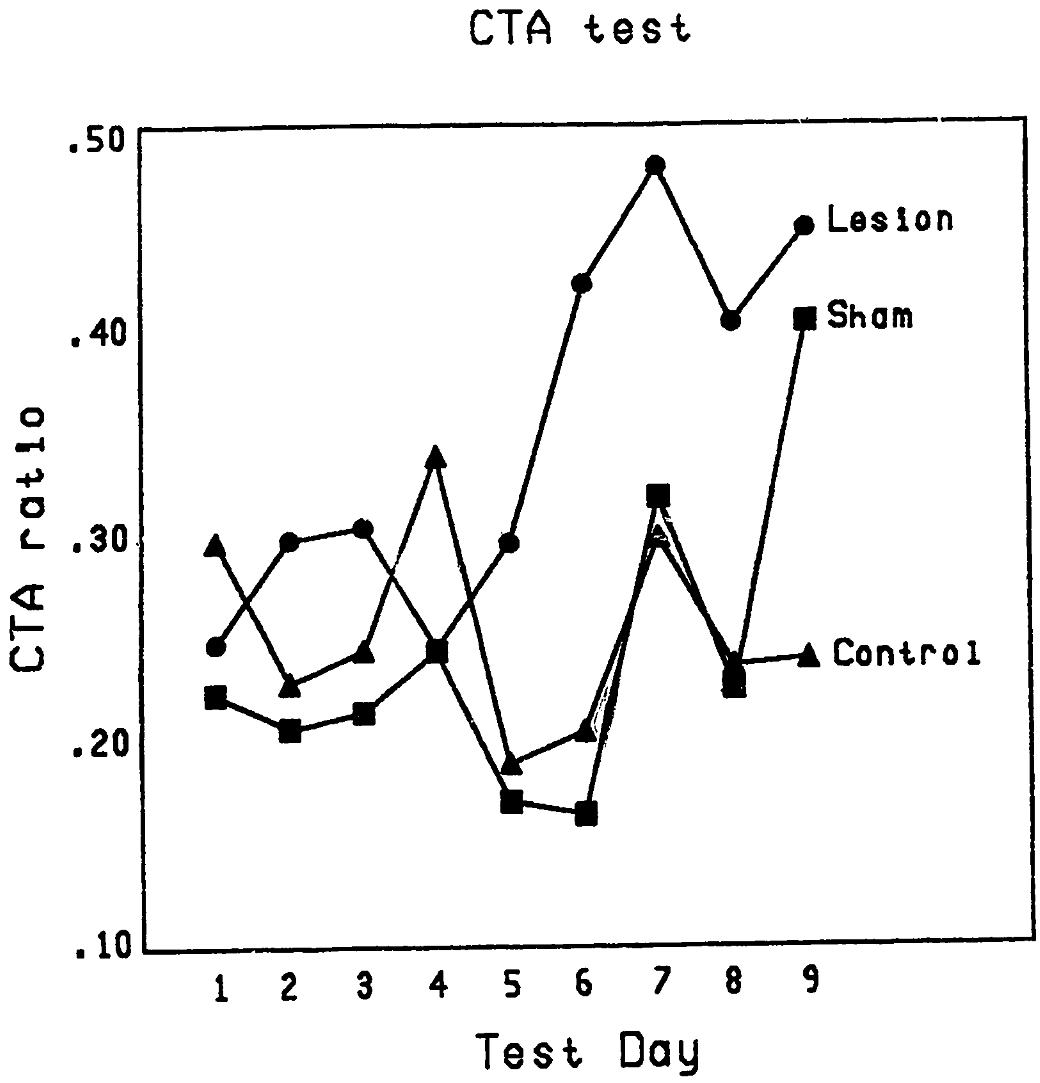


Figure 7. Oean aversions of animals in the lesion, control, and sham groups over the nine days of taste aversion testing in Experiment 2. Ratio measures significantly below 0.50 denote a taste aversion.

sham groups (p<.05) which did not differ from each other. The treatment by day interaction was not significant (F(16,180)=0.75, p>.05) in spite of the fact that all treatment groups initially had a CTA, but the aversion ratio increased significantly over the last three days only in the lesion group (see Figure 7).

Discussion

The results from Experiment 2 clearly parallel those of Experiment 1 in that rats showed an increase in the strength of a CPA and a decrease in the strength of a CTA after bilateral transsections of MC-SC projection fibres.

Due to the fact that the conditioned stimuli were presented separately and to independent groups of subjects in Experiment 2, rather than as a stimulus compound, conditioning to each stimulus could not have been interfered with by the other stimulus. The procedures used in Experiment 2 thus ensured that no overshadowing of conditioning to the cues present in the conditioning chamber by conditioning to the gustatory cues could occur. The fact that changes in conditioning to each stimulus were nonetheless observed must therefore mean that bilaterally transsecting MC-SC projection fibres changed the ease with which taste and place stimuli were associated with the effects of LiCl. That is, as a result of the bilateral knife cuts in the lesion group, the associability of place stimuli with the effects of LiCl injections increased such that a CPA could develop. The associability of taste stimuli with LiCl decreased after this type of surgery. However, a CTA developed in the lesion group, which demonstrates that the associability of tastes with nausea was still significant in these subjects.

The sham surgery group did not differ from the control groups on either the CTA or the CPA test. Thus, knife cuts posterior or ventral to those in the lesion group did not affect the associability of taste and place stimuli. It is unlikely, therefore, that any difference in the relative strengths of CTA or CPA in the lesion group as compared to the other groups were due to general effects of invasive brain surgery. Rather, the change in associability of taste and place stimuli with the aversive effects of LiCl injections may critically depend on the transsection of fibres running in the forceps minor of the CC rostral to the anterior commissure or in areas rostral to the forceps minor of the CC.

General Discussion

In Experiment 1, rats were conditioned using a compound stimulus composed of both a taste and visual and tactile cues followed by an injection of LiCl. As a result of this compound conditioning procedure, control rats displayed a strong CTA, but no CPA. These results closely parallel those first reported by Garcia and Koelling (1966), and by Miller and Domjan (1981) who used similar stimulus-reinforcer combinations. In addition, however, Experiment 1 also showed that rats with bilateral knife cuts transsecting the path of the MC-SC projection fibres exhibited a comparatively weaker CTA and a significant CPA.

As discussed earlier, there are a number of possible explanations for such a pattern of results, most of which can be eliminated based on the performance of the lesioned rats in Experiment 1. The results of Experiment 1 indicate that the bilateral knife cuts in the lesion group did not result in a general learning deficit, in a change in the effectiveness of the reinforcer, LiCl, or in unconditioned effects such as changes in neophobic reactions (see Experiment 1). By virtue of the experimental design, any effects of possible preferences established prior to the experiment were also minimized. It was therefore suggested that the differences in response patterns during the conditioning tests reflected a true difference in conditioning to the two types of cues used. Evidence from Experiment 2, in addition, indicated that the differential conditioning observed in Experiment 1 had not been due to context or overshadowing effects, as a comparable pattern of conditioned effects could be obtained in rats with effective bilateral knife cuts even when each cue type was presented alone, in procedures designed to minimize context effects.

The results therefore suggest that the lesions increased the associability of place cues and concurrently decreased that of taste stimuli. An increase in learning ability following brain damage is not commonly seen. The present results thus constitute a novel effect due to the occurrence of a reliable CPA in rats with bilateral transsections.

This facilitation of CPA learning might be accounted for by the removal of a functional interference of the SC with the MC. That is, assuming that the effects of LiCl preferentially involve SC-related mechanisms, the absence of conditioning to place cues might depend on a functional inhibition of MC-related processes. Thus, the effects of transsecting the MC-SC projection path on conditioning could be described in terms of a hierarchical organization of prefrontal divisions mediated by the direct projections between them. However, in this circumstance, it is difficult to account for the observation that the conditioning of gustatory stimuli actually decreased when MC-SC projection fibres are cut. Whereas it is known that damage to the SC can affect taste conditioning, the SC was not directly lesioned in the animals used in the present study. Therefore, it is unclear what caused the decrease in CTA in Experiments 1 and 2. It is conceivable that the cuts did indirectly lead to some undetected degeneration in the SC or affected other adjacent structures involved in processes related to gustation. On the other hand, it is possible that there is a functional facilitatory effect of the MC on the SC which was removed when MC-SC projection fibres were transsected.

This conclusion is strengthened by the observation that, whereas the changes in susceptibility to conditioning seen in Experiments 1 and 2 occurred in rats with bilateral cuts transsecting the path of MC-SC projections, sham cuts which did not extend through the anterior corpus callosum but inflicted comparable damage in the vicinity of the target area did not effect the same changes. Sham operated animals did not show a CPA or display a weaker CTA in Experiment 2. This would suggest that the critical locus of damage was the area of the anterior corpus callosum already shown to be the main projection path of the fibre projections linking the MC and the

SC (Beckstead, 1979; Reep and Winans, 1982a and 1982b). However, although it can be argued that the cuts in Experiments 1 and 2 interfered with the MC-SC connections, it is not possible to assess whether the behavioural consequences of the lesions were entirely attributable to damage to these fibres.

A comparable conclusion was reached by Robertson and Laferriere (1989), who studied the effects of similar bilateral knife cuts on place and taste conditioning mediated by reinforcing direct brain stimulation of the MC. They found that normal rats rapidly displayed a conditioned taste preference (CTP), but no conditioned place preference (CPP) when rewarded by ICSS of the MC. Rats with bilateral knife cuts had a reverse pattern of preferences, displaying a CPP, but no CTP.

The present results extend these findings to aversively motivated learning: the same interaction between associations involving the stimulus modalities related to the SC and the MC takes place regardless of whether a positive reinforcer, such as ICSS, or an aversive reinforcer, such as LiCl, is used. This would suggest that the modulation of associability ascribed to the MC-SC projection fibres occurs over a wide range of reinforcing events.

Although an extensive body of evidence has implicated the MC and the SC in dissociable forms of learning, based at least in part on the dominant sensory modality of the learning task (see Introduction; Eichenbaum et al, 1983; Lasiter and Glanzman, 1982 and 1985; Kolb et al., 1982), there is little direct evidence for a <u>necessary</u> role of either area in stimulus-reinforcer conditioning of the type represented by the present learning task. For example, although lesions of the insular cortex attenuate the acquisition of a CTA, they do not prevent such learning from taking place (e.g., Lasiter and Glanzman, 1982 and 1985).

This uncertainty over the exact role of the prefrontal area in associative processes makes it difficult to characterize precisely the involvement of the MC-SC projection path in the process of stimulus associability. It is likely that the prefrontal subdivisions exert modulating influences on the necessary substrates for the representations and processing underlying conditioning of the type used in the present experiment. This would be in keeping with current attempts to describe PFC function in terms of a structuring, organizing, or enabling influence on more elemental processes such as stimulus-reinforcer associations (Fuster, 1980; Kolb, 1984). Regardless of the precise nature of the involvement of the PFC in associative learning, the present results strongly suggest the existence of a functional interaction between PFC subdivisions which influences the conditioning of gustatory and visual cues via the corticocortical fibres linking these areas.

Whereas previous research has concentrated on defining the functions of each prefrontal subdivision by characterizing the behavioural deficits associated with lesions to these areas, the results of Experiments 1 and 2 suggest that another aspect of prefrontal function could be uncovered by studying the consequences of disconnecting these same areas. In this context, the observation of altered stimulus associability consequent to the knife cuts performed in Experiments 1 and 2 represents a first step toward describing the role of prefrontal areas in associative learning, and learning, and in particular of a key feature of conditioning phenomena: the existence of stimulus-reinforcer bias. The results of Experiments 1 and 2 demonstrate that the size and direction of such a bias can be altered by the knife cuts performed. That such an effect may be related to the destruction of connecting fibres linking the MC and the SC provides a strong indication that the prefrontal subdivisions may, through their interaction, play an important role in the process of stimulus selection which appears to underlie bias effects in conditioning. The present results therefore extend previous suggestions that the occurrence of stimulus-reinforcer bias reflects the differential activation of distinct sets of neural structures (Rozin and Kalat, 1971; Garcia and Koelling, 1966) by identifying a possible substrate for the variation in associative strength seen with the use of different reinforcing events.

In the present study, only associations between an interoceptive aversive reinforcer (LiCl) and interoceptive or exteroceptive stimuli were examined. Robertson and Laferriere (1989) examined associative learning using a positive reinforcer and obtained results that are fully compatible with those of the present study. However, it remains to be seen whether a similar orthogonal pattern of results could be obtained if an aversive exteroceptive stimulus, such as electric shock, was used. Normal rats readily display a CPA, but not a CTA, with shock as the US (Garcia and Koelling, 1966). Results from research on CTA and CPA learning using shock as the reinforcer could demonstrate more clearly the nature of the interaction between the MC and the SC.

In summary, the results of the present experiment provide a clue to a general feature of the organization of learning: that associative learning involves separate but interacting neural substrates and that prefrontal mechanisms may have a special role in the regulation of these interactions.

References

Archer, T., Sjoden, P.-O., Nilsson, L.-G., & Carter, N. (1979). Role of exteroceptive background context in taste-aversion conditioning and extinction. *Animal Learning and Behavior*, *7*, 17-22.

Beckstead, R. M. (1979). An autoradiographic examination of corticocortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. *Journal of Comparative Neurology*, *184*, 43-62.

Benjamin, R. M., & Pfaffman, C. (1955). Cortical localization of taste in the albino rat. *Journal of Neurophysiology*, *18*, 59-64.

Best, M. R., Brown, E. R., & Sowell, M. K. (1984). Taste-mediated potentiation of noningestional stimuli in rats. *Learning & Motivation*, *15*, 244-258.

Corbett, D., Laferriere, A., & Milner, P. M. (1982). Elimination of medial prefrontal cortex self-stimulation following transsection of afferents to the sulcal cortex in the rat. *Physiology & Behavior*, *29*, 425-431.

Divac, I. (1971). Frontal lobe system and spatial reversal in the rat. *Neuropsychology*, *9*, 175-183.

Divac, I., & Diemer, N. H. (1980). Prefrontal system in the rat visualized by means of labeled deoxyglucose - further evidence for functional heterogeneity of the neostriatum. *Journal of Comparative Neurology*, *190*, 1-13.

Divac, I., Gade, A., & Wikmark, R. E. G. (1975). Taste aversion in rats with lesions in the frontal lobes: No evidence for interoceptive agnosia.

Physiological Psychology, 3, 43-46.

Domjan, M., & Wilson, N. E. (1972). Contribution of ingestive behaviors to taste-aversion learning in the rat. Journal of Comparative and Physiological Psychology, 80, 403-412.

Dunn, L. T., & Everitt, B. J. (1988). Double dissociation of the effects of amygdala and insular neocortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. Behavioural Neuroscience, 102, 3-23.

Eichenbaum, H., Clegg, R. A., & Feeley, A. (1983). Reexamination of functional subdivisions of the rodent prefrontal cortex. Experimental Neurology, 79, 434-451.

Fuster, J. M. (1980). The Prefrontal Cortex. Raven Press, New York.

Ferino, F., Thierry, A. M., Saffroy, M., & Glowinski, J. (1987). Interhemispheric and subcortical collaterals of medial prefrontal cortical neurons in the rat. Brain Research, 417, 257-266.

Foree, D. D., & LoLordo, V. M. (1973). Attention in the pigeon: Differential effects of food-getting versus shock-avoidance procedures. Journal of Comparative Neurology, 85, 551-558.

Galef, B. G., & Dalrymple, A. J. (1981). Toxicosis-based aversions to visual cues in rats: A test of the Testa and Ternes hypothesis. Animal Learning & Behavior, 9, 332-334.

Garcia, J., Ervin, R. R., & Koelling, R. A. (1966). Learning with prolonged delay of reinforcement. Psychonomic Science, 5, 121-122.

Garcia, J., & Koelling, R. A. (1966). Relation of cue to consequence in avoidance learning. Psychonomic Science, 4, 123-124.

Isaac, W. L., Nonneman, A. J., Neisewander, J., Landers, T., & Bardo, M. T. (1989). Prefrontal cortex lesions differentially disrupt cocaine-reinforced conditioned place preference but not conditioned taste aversion. Behavioral Neuroscience, 103, 345-355.

Kolb, B. (1984). Functions of the frontal cortex of the rat: A comparative review. Brain Research Review, 8, 65-98.

Kolb, B., Nonneman, A. J., & Abplanalp, P. (1977). Studies on the neural mechanisms of baitshyness in rats. Bulletin of the Psychonomic Society, 10, 389-392.

Kolb, B., Nonneman, A. J., & Singh, R. K. (1974). Double dissociation of spatial impairments and perseveration following selective prefrontal lesions in rats. Journal of Comparative & Physiological Psychology, 87, 772-780.

Kolb, B., Pittman, K., Sutherland, R. J., & Whishaw, I. Q. (1982). Dissociation of the contributions of the prefrontal cortex and dorsomedial thalamic nucleus to spatially guided behavior in the rat. Behavioural Brain Research, [illegible], 365-37[illegible].

Kolb, B., & Whishaw, I. Q. (1985). Fundamentals of Human Neuropsychology (2nd ed.). New York: W. H. Freeman and Company.

Kosar, E., Grill, H. J., & Norgren, R. (1986a). Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. Brain Research, 379, 329-341.

Kosar, E., Grill, H. J., & Norgren, R. (1986b). Gustatory cortex in the rat. II. Thalamocortical projections. Brain Research, 379, 342-352.

Krane, R. V., & Wagner, A. R. (1975). Taste aversion learning with a delayed shock: Implications for the "Generality of the laws of learning". Journal of Comparative and Physiological Psychology, 88, 882-889.

Krettek, J. E., & Price, J. L. (1974). A direct input from the amygdala to the thalamus and the cerebral cortex. Brain Research, 67, 169-174.

Krettek, J. E., & Price, J. L. (1977). The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. The Journal of Comparative Neurology, 171, 157-192.

Lasiter, P. S., & Glanzman, D. L. (1982). Cortical substrates of taste aversion learning: Dorsal prepiriform (insular) lesions disrupt taste aversion learning. Journal of Comparative and Physiological Psychology, 96, 376-392.

Lasiter, P. S., & Glanzman, D. L. (1985). Cortical substrates of taste aversion learning: Involvement of dorsolateral amygdaloid nuclei and temporal neocortex in taste aversion learning. Behavioral Neuroscience, 99, 257-276.

Lett, B. T. (1974). Visual discrimination learning with a 1-min delay of reward. Learning & Motivation, 5, 174-181.

Leonard, C. M. (1969). The prefrontal cortex of the rat. I. Cortical projection of the mediodorsal nucleus. II. Efferent connections. Brain Research, 12, 321-343.

Martin, L. T., & Lawrence, C. D. (1979). The importance of odor and texture cues in food aversion learning. Behavioral and Neural Biology, 27, 503-515.

Miller, J. S., McCoy, D. F., Kelly, K. S., & Bardo, M. T. (1986). A within-event analysis of taste-potentiated odor and contextual aversions. Animal Learning & Behavior, 14, 15-21.

Miller, V. (1984). Selective assoiciation learning in the rat: Generality of response system. Learning & Motivation, 51, 58-84.

Miller, V., & Domjan, M. (1981). Specificity of cue to consequence in aversion learning in the rat: Control for US-induced differential orientations. Animal Learning & Behavior, 9, 339-345.

Mowrer, O. H., & Lamoreaux, R. R. (1946). Fear as an intervening variable in avoidance conditioning. Journal of Comparative Psychology, 39, 29-50.

Nachman, M., & Ashe, J. H. (1973). Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. Physiology and Behavior, 10, 73-78.

Nonneman, A. J., & Kolb, B. (1979). Functional recovery after serial ablation of prefrontal cortex in the rat. Physiology & Behavior, 22, 895-904.

Olton, D. S. (1986). Interventional approaches to memory: lesions. In J. L. Martinez and R. P. Kesner (Eds.), Learning and Memory. A Biological View (pp.379-397). Orlando: Academic Press.

Parker, H. B., & Smith, R. F. (1981). Flavor- vs. tone-cued shock avoidance. Animal Learning and Behavior, 9, 335-338.

Pavlov, I. P. (1927). Conditioned Reflexes. Oxford University Press.

Pelchat, M. L., Grill, H. J., Rozin, P., & Jacobs, J. (1983). Quality of acquired responses to tastes by Rattus norvegicus depends on type of associated discomfort. Journal of Comparative Psychology, 97, 140-153.

Reep, R. L., & Winans, S. S. (1982a). Afferent connections of dorsal and ventral agranular insular cortex in the hamster Mesocricetus auratus. Neuroscience, 7, 1265-1288.

Reep, R. L., & Winans, S. S. (1982b). Efferent connections of dorsal and ventral agranular insular cortex in the hamster Mesocricetus auratus. Neuroscience. 7, 2609-2635.

Rescorla, R. A., & Furrow, D. R. (1977). Stimulus similarity as a determinant of pavlovian conditioning. Journal of Experimental Psychology: Animal Behavior Processes, 3, 203-215.

Revusky, S., & Parker, L. A. (1976). Aversions to unflavoured water and cup drinking produced by delayed sickness. Journal of Experimental Psychology: Animal Behavior Processes, 2, 342-353.

Robertson, A. (1989). Multiple reward systems and the prefrontal cortex. Neuroscience & Biobehavioral Reviews, 13, 163-170.

Robertson, A., & Laferriere, A. (1989). Disruption of the connections between the mediodorsal and sulcal prefrontal cortices alters the associability of rewarding medial cortical stimulation to place and taste stimuli in rats. Behavioral Neuroscience, 103, 770-778.

Robertson, A., Laferriere, A., & Milner, P. M. (1986). Development of brain stimulation reward in the medial prefrontal cortex: Facilitation by prior electrical stimulation of the sulcal prefrontal cortex. Physiology & Behavior, 28, 869-872.

Rozin, P., & Kalat, J. W. (1971). Specific hungers and poison avoidance as adaptive specializations of learning. Psychological Review, 78, 459-486.

Sakurai, Y., & Sugimoto, S. (1985). Effects of lesions of prefrontal cortex and dorsomedial thalamus on delayed go/no go alternation in rats. Behavioural Brain Research, 17, 213-219.

Seligman, M. E. P. (1970). On the generality of the laws of learning. Psychological Review, 77, 406-418.

Seligman, M. E. P., & Campbell, B. A. (1965). Effect of intensity and duration of punishment on extinction of an avoidance response. Journal of Comparative and Physiological Psychology, 59, 295-297.

Sutherland, R. J., Kolb, B., & Whishaw, I. Q. (1982). Spatial mapping: definitive disruption by hippocampal or medial frontal cortex damage in the rat. Neuroscience Letters, 31, 271-276.

Thompson, R. F., & Donegan, N. H. (1986). The search for the engram. In J. L. Martinez & R. P. Kesner (Eds.), Learning and Memory. A Biological View (pp.3-52). Orlando: Academic Press.

Westbrook, R. F., & Brookes, N. (1988). Potentiation and blocking of conditioned flavour and context aversions. The Quarterly Journal of Experimental Psychology, 40B, 3-30.

White, N. M., & Carr, G. D. (1985). The conditioned place preference is affected by two independent reinforcement processes. Pharmacology, Biochemistry & Behavior, 23, 37-42.

Wikmark, R. E. G., Divac, I., & Weiss, R. (1973). Retention of spatial delayed alternation in rats with lesions in the frontal lobes. Brain, Behavior, Evolution, 8, 329-339.