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.

UNIVERSITY OF ALBERTA

SEPTAL LESIONS INHIBIT FEAR REACTIONS IN ANIMAL MODELS OF ANXIOLYTIC DRUG ACTION

BY
CHRISTINE PESOLD

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

(SPRING 1991)

ISBN 0-315-66610-2

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: CHRISTINE PESOLD

TITLE OF THESIS: SEPTAL LESIONS INHIBIT FEAR REACTIONS IN ANIMAL MODELS OF ANXIOLYTIC DRUG ACTION

DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED: SPRING 1991

(Student's Signature)

253 Sedgefield

(Student's Permanent Address)

Pointe-Claire

Québec H9R 1P3

Date: April 22, 1991

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

THE UNDERSIGNED CERTIFY THAT THEY HAVE READ,

AND RECOMMEND TO THE FACULTY OF GRADUATE

STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS

ENTITLED SEPTAL LESIONS INHIBIT FEAR REACTIONS IN ANIMAL MODELS OF ANXIOLYTIC DRUG ACTION

SUBMITTED BY CHRISTINE PESOLD

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

Dallas Treit

Dr. DALLAS TREIT

Charles Beck

Dr. CHARLES BECK

Norman Stacey

Dr. NORMAN STACEY

Date: March 30, 1991

ABSTRACT

The role of the septum in 'anxiety' was studied using two different animal models of anxiolytic drug action: the elevated plus-maze, and the shock-probe burying paradigm. Anxiolytic-like effects were observed in both these paradigms (i.e., an increase in open arm activity, and a decrease in shock-probe burying), after lesions of the entire septum in rats, compared to sham-lesioned controls (Experiment 1). In the second experiment, anti-anxiety effects in these paradigms were found to be anatomically specific since lesions of the posterior septum decreased both indices of 'anxiety', whereas lesions of the anterior septum resulted in levels of 'anxiety' that were comparable to those displayed by sham-lesioned controls. In Experiment 3, it was confirmed that the anti-anxiety effects of lesions to the posterior septum were specific to the cell bodies in that region since the effects of kainic acid lesions, which primarily destroy cell bodies leaving fibers of passage intact, produced results that were generally comparable to those of electrolytic lesioned rats. While alternative interpretations are possible, the major results of the present experiments provide converging evidence that the septum is involved in the modulation of "anxiety"-related behaviors in rats.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to
Dallas Treit for his support throughout all phases
of the research, as well as his guidance and never
ending patience during the writing of this manuscript.

TABLE OF CONTENT

Table of Content (continued)

LIST OF TABLES

LIST OF FIGURES

List of Figures (continued)

INTRODUCTION

The septum is a midline structure present in the forebrain of a variety of animal species. This complex structure, composed of several nuclei, has extensive afferent and efferent connections to many brain structures including the hippocampus, amygdala, hypothalamus, thalamus, raphe nucleus, ventral tegmentum, and habenula. The septum is also traversed by numerous fiber tracts primarily associated with the fornix system and the stria terminalis, which do not synapse in the area (Swanson, & Cowan, 1979).

There are many differing hypotheses regarding the role of the septum in behavior (see Appendix). One hypothesis, which is central to the present thesis, is that the septum, in concert with other limbic structures, plays a role in the modulation of "anxiety"-related behaviors (Gray, 1982). This hypothesis has mainly been derived from the surprising degree of correspondence which is often seen between the effects of clinically effective anti-anxiety drugs (i.e., anxiolytics) and the effects of septal lesions in traditional aversive learning paradigms such as passive avoidance, active avoidance, and classical conditioning of fear (i.e., generally, if following septal lesions, an increase or a facilitation of a particular form of aversive learning is observed, it will also be observed following the administration of anxiolytic agents; if, conversely, a decrease or impairment is obtained, similar results will be observed from an anxiolytic agent).

The first part of this introduction consists of a procedural description of these traditional aversive learning paradigms, followed by a brief summary of the effects of septal lesions and the effects of anxiolytic agents in these paradigms. It will be argued, in the next section of this introduction, that although the correspondence between the effects of septal lesions and anxiolytic drugs in traditional aversive learning paradigms is often impressive, it is far from perfect. Thus, in this section, the problems that arise from studying the effects of septal lesions in these paradigms will be discussed, and arguments in favour of alternative approaches will be provided. The final section will present the rationale and purpose of the present thesis.

Effects on Passive Avoidance

In passive avoidance tasks, an animal must learn to avoid making a response on which punishment is contingent. There are several different passive avoidance tasks. For example, in a runway task, an animal is first rewarded for running down an alley (i.e., with food, water, or the omission of shock). Later, the animal is punished (e.g., with electric foot-shock) for performing the same response. Well-trained animals avoid running down the alley. Although there are exceptions (Fried, 1969), the majority of studies that have examined the effects of septal lesions on passive avoidance in the runway found that septal-lesioned rats were impaired in learning to avoid running down the alley (Beatty, Beatty, O'Brian, Gregoire, & Dahl, 1973; Bengelloun, Burright, & Donovick, 1977). Two other commonly used passive avoidance tasks include "step-down" and "step-through" passive avoidance. In a step-

down task, a rat is placed on a small, elevated platform. When the rat spontaneously steps down onto a larger lower platform, it receives electric foot-shock. In a step-through task, the rat is placed in the large, brightly lit box of a two-box apparatus. When the rat leaves the large brightly lit box to enter a smaller dark box, it receives electric foot-shock. Animals quickly learn to avoid stepping down onto the lower platform (step-down), or going into the smaller box (step-trough). Septal lesions have been found to impair passive avoidance in both step-down (Beatty et al., 1973) and step-through (McDaniel, Donovick, Burtright, & Fanelli, 1980) passive avoidance paradigms, but there are some exceptions (Frank & Beatty, 1974).

Similar to the effects of septal lesions, anti-anxiety drugs such as chlordiazepoxide, diazepam, and oxazepam have generally been shown to impair passive avoidance in step-down (Waddington & Olley, 1977), step-through (Oishi, Iwahara, Yang, & Yogi, 1972), and Y-maze (Kumar, 1971) passive avoidance tasks.

Effects on Escape

In passive avoidance tasks, septal-lesioned animals are often impaired. Whether they are impaired in their anticipation of the shock, or in their perception of the shock is not clear from the passive avoidance data. These possibilities can be partially addressed in studies that examine the effects of septal lesions on escape learning. Escape differs from passive avoidance in that in escape learning, the animal is exposed to an ongoing electric shock from which it can escape by actively making an escape response. For example, in a Y-maze escape task, an animal is placed on

an electrified grid floor. The animal quickly learns to escape the shock by running to the appropriate arm of the Y-maze. Septal-lesioned animals are not reliably impaired in their ability to escape shock (Bunnell & Smith, 1966) suggesting that their perception of, and response to aversive stimuli are not impaired. Escape learning also appears to be unaffected by the administration of anti-anxiety drugs (Waddington et al., 1977). Further evidence that the septal-lesioned animals are not impaired in the perception of painful stimuli comes from studies which found that threshold current intensity necessary to elicit a flinching response was not different in septal-lesioned rats compared to non-lesioned rats (Lints & Harvey, 1969). However, these studies also revealed that jump threshold was lower for septal-lesioned rats, suggesting that while their perception of aversive stimuli such as shock is not impaired, they are more reactive to these stimuli.

Effects on Active Avoidance

Unlike passive avoidance paradigms, in which an animal must refrain from making a specified response in order to avoid punishment, in active avoidance, an animal must perform a specified response in order to avoid punishment. There are many forms of active avoidance, including one-way, two-way, and Sidman bar-press avoidance.

In a one-way active avoidance task, an animal is first placed in a start box. At the onset of a warning signal such as a buzzer or light, the animal must run down an alley to a goal box in order to avoid foot-shock. If the animal does not leave the start

box within a designated amount of time (i.e., the duration of the warning signal), the animal is shocked. Normal animals learn to actively avoid shock by running down the alley to the goal box. The effects of septal lesions on one-way active avoidance are not as consistent as the effects of these lesions on passive avoidance. Some studies have found inpairment of one-way active avoidance following septal lesions (Thomas & Thomas, 1972), while others have found no effect (Thomas & McCleary, 1974). Furthermore, many studies found that septal-lesioned rats show improvements in one-way active avoidance tasks, compared to sham-lesioned control rats (e.g., Zucker, 1965). Similar to the effects of septal lesions, anti-anxiety agents do not appear to have uniform effects on one-way active avoidance. While facilitation of one-way active avoidance has been seen after low doses of anxiolytic drugs (Bignami, De Acetis, & Gatti, 1971), impairments have been observed after high doses. The high-dose effects appear to have been a result of general sedation (Gluckman, 1965).

In a two-way active avoidance task, an animal must also perform a response in order to avoid shock, yet unlike one-way active avoidance, the shock and non-shock areas are continually alternated. For example, in two-way "shuttle" avoidance, an animal is placed in one box of a two-box apparatus connected by an alley. A warning stimulus such as a buzzer or light signals upcoming shock in that box. The animal can avoid the shock by running through the alley to the other box (i.e., a "shuttle-response"). If the animal remains in the first box following the warning signal, it receives foot-shock. The animal can still escape the shock, however, by "shuttling" to the other box, which results in shock termination. The animal remains in this box

until the next warning signal occurs. The animal must then shuttle back to the original box where it had previously been shocked, in order to avoid shock.

Two-way shuttle-avoidance is difficult for rats to acquire (Bolles, 1970), perhaps because the compartment which is presently 'safe' was associated with shock on the previous trial. This association with a punishing stimulus might deter the animals from approaching it (i.e., cause passive avoidance). Hence in shuttle-avoidance there may be a 'conflict' between passive and active avoidance (Gray, 1982). Recall that while septal-lesioned animals are generally not impaired on one-way active avoidance tasks, they generally are impaired in passive avoidance tasks. If septal-lesioned animals maintain their ability to actively avoid shock, but are impaired in their ability to perform in passive avoidance tasks, their ability to avoid shock in a two-way active avoidance task might be expected to be better than normal animals. This expectation is, in fact, supported by the results of a number of studies. The facilitation of shuttle box avoidance in septal-lesioned animals is a well documented phenomenon (Blatt, 1976; Carlson, 1970; Garber & Simmons, 1968; Poplawsky, 1978). Similar improvements in two-way active avoidance have also been observed following administration of anti-anxiety agents (Iwahara, 1971; Robichaud, Sledge, Hefner, & Goldberg, 1973).

In a Sidman bar-press avoidance task, shock is presented at regular intervals. A bar-press response can postpone the shock for a period of time. In this paradigm, there is no warning signal for the presentation of shock, other than the passage of time. An animal must bar-press within regular response/shock intervals in order to

avoid getting shocked. Studies revealed that septal-lesioned animals perform better at this task, as measured by a reduction in shocks taken, as well as by a reduction in responses emitted (greater efficiency), than normal animals (Morgan & Mitchell, 1969; Sagvolden, 1976; Sodetz, 1970, & 1972). Similar improvement in performance of Sidman bar-press avoidance tasks was observed following the administration of anti-anxiety agents at low doses (Davidson, 1970). While impairments were found at high doses (Sansone, Renzi, & Amposta, 1972), these seem to be a result of the sedative effect of these drugs at high doses.

Effects on Conditioned Suppression

Classical conditioning of 'fear' is another form of aversive learning that has been employed in the study of 'anxiety'. These paradigms examine the effects of "conditioned fear" on ongoing behavior. However, very few of these tasks have been used to study the effects of both septal lesions and anxiolytic drugs. The most commonly used of these tasks is called "conditioned suppression" or "conditioned emotional responding" (Estes & Skinner, 1941).

In conditioned suppression, a neutral stimulus such as a light (conditioned stimulus), that has been previously paired with an aversive stimulus such as foot-shock (unconditioned stimulus), is superimposed on an ongoing behavior such as bar-pressing for food, and the resulting suppression in this latter behavior is the index of 'fear' or 'anxiety'. Even though presentation of shock is not affected by the rats' bar-pressing response (response-independent), animals normally suppress their bar-

pressing behavior following the presentation of a signal for shock (Estes & Skinner, 1941; Lauener, 1963). Early studies that examined the effects of septal lesions on conditioned suppression yielded inconsistent results. While some researchers observed a deficit of response suppression in septal-lesioned animals (Harvey, & Lints, 1965), others did not (Brady, & Nauta, 1955). Later studies compared rats' bar-pressing response rates on two different signals for shock. One signal represented response-independent shock, while the other represented shock whose probability was dependent on the rats' bar-pressing response (response-contingent shock). In normal animals, suppression of bar-pressing behavior was found to be greater when the signal represented response-contingent shock than when it represented response-independent shock (Huppert, & Iversen, 1975). Studies which compared the effects of septal lesions on response suppression toward these two signals for shock found that septal lesions alleviated response suppression, but did so equally whether the signal warned of response-contingent or response-independent shock (Dickinson, 1975; Feldon, Rawlins, & Gray, 1982). Anti-anxiety agents also antagonized the response suppression produced by both types of signals. However, this effect was greater for the signal warning response-contingent than response-independent shock (Rawlins, Feldon, & Gray, 1980; Huppert, & Iversen, 1975).

Summary of Septal Lesion Effects in Traditional Aversive Learning Paradigms

While the effects of septal lesions on passive avoidance have been generally consistent, the effects of septal lesions on active avoidance have been more varied.

For example, performance on one-way active avoidance tasks has been found to be impaired, improved, or unchanged following septal lesions in rats. In both two-way active avoidance and Sidman bar-press avoidance tasks, septal lesions in rats have often been shown to facilitate avoidance. While the effects of septal lesions on conditioned suppression have been rather equivocal, they suggest that septal lesions may impair conditioning to signals that warn of punishment.

Overall, it appears that in tasks in which shock-avoidance requires a decrease in responding, septal-lesioned rats perform poorly, whereas in tasks which require an increase in responding, septal-lesioned rats perform better than controls. These data are in accordance with Gray's (1982) Behavioral Inhibition System (BIS) model of anxiety. This hypothesis states that inputs such as novelty and signals of punishment produce, among other things, behavioral inhibition. In tasks where behavioral inhibition competes with avoidance responding, one would expect normal rats to have poor performance, and conversely to perform well in tasks in which avoidance requires inhibiting a response. Furthermore, if we abolish the BIS, which according to Gray is dependent on the functioning of the septo-hippocampal system, we would expect the opposite results (i.e., impaired performance when avoidance requires inhibiting a response, and improved performance when avoidance requires the performance of a response).

Nevertheless, while there is considerable evidence that septal lesions modulate "anxiety"-related behaviors in traditional aversive learning paradigms, the data are not

entirely consistent, and their interpretation is not particularly straightforward (cf., Thomas, 1988). The main problem stems from the large number of inconsistencies in septal-lesion effects in these paradigms. This lack of agreement across studies could be due to a combination of factors such as variations in lesion parameters (e.g., size, extent and type), and/or variations in behavioral parameters (e.g., shock-intensity, task complexity).

Due to the complex nature and interconnections of the septum, differential septal damage could cause fairly different behavioral effects. For example, many studies have noted different behavioral results following medial versus lateral septal damage. Lateral septal lesions appear to produce increased exploratory behavior (Myhrer, 1989), "emotionality" (Poplawsky & Johnson, 1973), and startle amplitude (Lee, Lin, & Yin, 1988), as well as decreased 'anxiety' in the "social interaction test" (Clarke & File, 1982), and decreased rearing (Lee, et al., 1988). Medial septal lesions have been shown to produce decreases in exploratory behavior and activity (Myhrer, 1989), and normal "emotionality" (Clody & Carlton, 1969). Other studies have noted differences when comparing anterior with posterior septal lesions. For example, anterior septal lesions were found to enhance defensive behaviors in rats, whereas posterior septal lesions were found to have no effect (Blanchard, Blanchard, Lee, & Nakamura, 1979).

The other factor that might contribute to inconsistent results is the heterogeneity of the behavioral paradigms used to study the role of the septum in anxiety (e.g., step-down passive avoidance, step-through passive avoidance, one-way

active avoidance, 2-way active avoidance, Sidman barpress avoidance, conditioned suppression). Moreover, many of these traditional aversive learning paradigms involve food or fluid as appetitive reinforcers. Since septal lesions produce changes in appetitive motivation indicated by increases in food (Donovick, Burright, & Gittelson, 1969) and water intake (Lubar, Schaefer, & Wells, 1969), behavioral paradigms which involve such reinforcers might not be appropriate for the study of septal lesions. Finally, it should be noted that these behavioral paradigms were not specifically designed to study animal 'anxiety'. Instead their main purpose was to study the 'general laws of learning'.

In light of these problems, it is not surprising that support of the hypothesis that the septum is involved in the modulation of anxiety has not been entirely consistent. Nor, perhaps, is it surprising that the interpretation of the exact role of septal nuclei in anxiety has not been uniform (Gray, 1982; Thomas, 1988).

In the next two sections, arguments in favour of alternative approaches to studying the role of the septum in anxiety will be presented. These arguments will center on pharmacological and behavioral considerations.

Pharmacological Considerations

Effects of anxiolytic drugs that are similar to the effects of septal lesions in these aversive learning paradigms lend support to the notion that septal lesions are involved in the modulation of anxiety. However, the behavioral effects of anxiolytic drugs in these paradigms are often inconsistent, and often lack drug-class specificity

(i.e., non-anxiolytics have effects that are similar to anxiolytic agents). For example, non-anxiolytic agents such as amphetamine and morphine have also been found to have effects like those of septal lesions and anxiolytic drugs on conditioned suppression (Capell, Ginsberg, & Webster, 1972; Hill, Pescor, Belleville, & Wikler, 1957), and active avoidance (McMillan & Leander, 1976; Morrison & Stephenson, 1973). The lack of drug-class specificity of behavioral effects in these paradigms raises the question of whether the effects of anxiolytic drugs in these paradigms actually reflect a specific reduction in anxiety. Thus, behavioral paradigms that have satisfied pharmacological criteria for a test for anxiolytic drug action may be valuable for studying the role of the septum in 'anxiety'. The major criterion for a model of anxiolytic drug action is that it can distinguish standard anxiolytic from non-anxiolytic agents (Treit, 1985).

Behavioral Considerations

Another approach to studying the role of the septum in anxiety may be to utilize behavioral paradigms that measure animals' untrained reactions to novel or painful stimuli. For example, there is a considerable amount of evidence suggesting that high fearfulness is accompanied by high defecation scores (Broadhurst, 1975; Gray, 1971, & 1979). Interestingly, there are many reports that septal lesions reduce defecation in novel situations such as in open field tests (e.g., Jonason & Enloe, 1971; Nielson, McIver, & Boswell, 1965). Further evidence that septal lesions reduce untrained 'fear' reactions comes from findings that septal lesions reduced latency to

eat in a novel environment (Ross, Grossman, & Grossman, 1975), and reduced emergence latency to go from a familiar to an unfamiliar place (Thomas, Moore, Harvey, & Hunt, 1959). The suppressive effects of novelty on eating, drinking, and emerging in novel environments are also reduced following the administration of anti-anxiety agents (Soubrie, Kulkarmi, Simon, Boissier, 1975; Soubrie, de Angelis, Simon, & Boissier, 1976; and Simon & Soubrie, 1979, respectively).

Two recently developed tests that employ rats' untrained 'fear' responses, as well as satisfy pharmacological criteria for a test of anxiolytic drugs, are the elevated plus-maze test (Pellow, 1986), and the shock-probe burying test (Treit, Pinel, & Fibiger, 1981). Moreover, these tests are not confounded with appetitive motivation since neither of these tests involve food or fluid reward.

In the elevated plus-maze, two adjacent arms are open, and two adjacent arms are enclosed with walls. On first exposure, rats normally avoid the open arms, restricting most of their activity to the closed arms. This avoidance of the open arms is likely to reflect 'fear' since rats display significantly more 'anxiety'-related behaviors (freezing, immobility, defecation) in the open arms, and have significantly higher plasma corticosterone levels when they are confined to the open arms (Pellow, Chopin, File, & Briley, 1985). Open-arm activity in the plus-maze is defined as the amount of time that the rat spends in the open arms relative to total time in any arm (i.e., % open/total), as well as the amount of crosses into the open arms relative to the total number of crosses (i.e., % open /total). General activity is measured as the

total number of crosses into any arm. The relatively low level of open arm activity (10-20%), is a measure of 'anxiety' in this paradigm.

Pharmacological studies revealed that a selective increase in open-arm exploration was observed following the administration of clinically effective anxiolytic agents such as diazepam, chlordiazepoxide, phenobarbitone, and tracazolate (Pellow, et al., 1985). Conversely, agents that supposedly increase anxiety (i.e., anxiogenics) such as yohimbine, amphetamine, caffeine, and pentylenetetrazole (PTZ), suppress rats' open arm activity below baseline control levels (Pellow, & File, 1986). Furthermore, non-anxiolytic agents including antidepressants (i.e., imipramine and mianserin) and major tranquilizers (i.e., haloperidol) have no specific effects on open arm activity (Pellow, et al., 1985).

Similar effects have been found in the shock-probe burying paradigm. Rats shocked from an electrified probe mounted on a wall of a test chamber will characteristically spray bedding material from the floor of the chamber toward the probe, with rapid alternating movements of the forepaws. This "burying" behavior has been observed toward a variety of aversive or 'threatening' stimuli, such as airblasts, physical blows, light flashes (Terlecki, Pinel, & Treit, 1979), noxious smells (Pinel, Gorzalka, & Ladak, 1981), and fluids previously paired with toxicosis (Wilkie, MacLennan, & Pinel, 1979). Rats appear to rapidly associate the shock (or other aversive stimulus) with a spatially contiguous cue (e.g., the probe), and following first exposure to the shock, proceed to bury the source of the aversive stimulus with whatever material is present, e.g., bedding, sand, or wooden blocks (Pinel & Treit,

1979). The burying response appears to be an 'untrained' fear reaction in rats, since depriving rats of any type of bedding material from birth does not prevent burying response on first exposure to this test (Pinel, Symons, Christensen, & Tees, 1989). There is also some evidence that burying behavior may be a response that rodents display toward stimuli that are aversive or threatening to them in their natural environment. For example, "territorial threat" from conspecifics has been reported to induce rats to bury the entrance hole of their underground burrows (Calhoun, 1962). In addition, ground squirrels will reportedly defend themselves from predatory snakes by spraying material directly at the snakes (Owings & Coss, 1978).

In the shock-probe burying test for anxiolytic drug action, rats are shocked from an electrified probe, and the amount of bedding material that they spray toward the probe (i.e., burying) is a major index of 'anxiety' (Treit, Pinel, & Fibiger, 1981). A variety of clinically effective anxiolytic agents such as diazepam, chlordiazepoxide, and pentobarbital, have been shown to suppress rats' burying response in a dose-dependent fashion (Treit et al., 1981). This reduction in burying behavior appears to be specific to anxiolytic agents. Although there are exceptions (Craft, Howard, & Pollard, 1988), most studies have shown that non-anxiolytic agents such as imipramine, morphine, d-amphetamine, pentylenetetrazole, and picrotoxin have no significant effect on burying behavior (Beardslee, Papadakis, Fontana, & Commissaris, 1990; Treit, 1990; Treit et al., 1981). Furthermore, blocking the site at which benzodiazepine-type anxiolytics exert their effects [GABA/benzodiazepine receptor complex (Haefely, Pieri, Polc, & Schaffner, 1981)], blocks the effects of

these anxiolytics on burying behavior (Treit, 1987; Treit, Pinel, & Fibiger, 1982). Moreover, 'anxiogenic' agents such as yohimbine and beta-CCE have been shown to facilitate the amount that rats bury the shock probe (Tsuda, Yoshishige, & Tanaka, 1988; Tsuda, Nishimura, & Tanaka, 1989).

These behavioral and pharmacological characteristics suggest that the elevated plus-maze test and the shock-probe burying test may be useful for the study of septal lesions on 'anxiety'. Their combined use may be particularly advantageous: while the plus-maze paradigm measures a passive, untrained 'fear' reaction to novel elevated platforms (i.e., open-arm avoidance), the shock-probe burying paradigm measures an active, untrained 'fear' reaction toward a painful stimulus (i.e., shock-probe burying). Moreover, since an anti-anxiety effect in the plus-maze test is measured as an increase in a specific activity (open arm activity), whereas in the shock-probe test it is measured as a suppression of a specific activity (burying), results from these two tests may provide valuable converging evidence for the role of the septum in anxiety. If the septum is involved in the modulation of anxiety, it would be expected that septal lesions in rats will produce a specific pattern of 'anti-anxiety' effects in the shock-probe burying and in the elevated plus-maze tests. Specifically, relative to controls, septal-lesioned rats should exhibit higher percentages of open arm activity in the elevated plus-maze, but suppressed burying behavior in the shock-probe burying paradigm.

Thus, the purpose of the present thesis was to study the effects of septal lesions in rats, on two pharmacologically validated tests of anxiolytic drug action: the elevated plus-maze test and the shock-probe burying paradigm.

Experiment 1

In the first experiment, the effects of electrolytic lesions of the entire septum on rats' reactivity in two pharmacologically justified tests of anxiety (the elevated plus-maze test and the shock-probe burying paradigm), were examined. While there is evidence that electrolytic lesions of the septum reduced fear reactions in the shock-probe burying paradigm [i.e., septal lesions eliminated burying toward the shock-probe (Gray, Terlecki, Treit, & Pinel, 1981)], the effects of septal lesions in the elevated plus-maze have not been documented. The first purpose of Experiment 1 was to replicate the earlier findings of reduced fear reactions in the shock-probe burying paradigm following complete septal lesions (Gray et al., 1981), as well as to extend these findings by using different shock parameters (i.e., parameters that have been found to be sensitive to the anxiolytic effects of drugs on defensive burying and shock-probe avoidance). The second purpose of this experiment was to examine the effects of septal lesions on open-arm activity in the elevated plus-maze.

METHOD

Subjects

The subjects were 50 naive, 265-465 g male albino Sprague-Dawley rats from Charles River, Canada. They were handled and tail marked one day prior to surgery. Following surgery, rats were individually housed in polycarbonate cages and maintained on a twelve hour light/dark cycle (lights on at 0700), food and water available ad lib. Behavioral testing occurred between the hours of 1000 and 1700.

Apparatus

Plus-Maze. A separate testing room contained the elevated plus-maze apparatus. The wooden plus-shaped maze, elevated to a height of 50 cm, consisted of two adjacent 50 X 10 cm open arms, and two adjacent 50 X 10 X 50 cm enclosed arms, each with an open roof. The testing room was quiet, and dimly lit at the time of testing. Behavioral data were collected by an observer who was unaware of the surgical history of the rat, and who sat quietly one meter from the maze.

Shock-Probe Burying. The shock-probe burying apparatus was kept in a quiet testing room at the time of testing. The floor of this 40 X 30 X 40 cm Plexiglas chamber was covered evenly with 5 cm of bedding material (odour-absorbent kitty

litter). On one of the walls of the Plexiglas chamber, 2 cm above the bedding material, was a small hole through which the shock-probe could be inserted. The 6.5 X 0.5 X 6.5 cm probe was wrapped with two copper wires through which the electric current was administered. Shock intensity was adjusted with a variable resistor in series with a 1000 V shock source, and was set at 2 mA. The behavior of each rat was recorded on video tape via closed circuit television, and later measured by an observer who was unaware of the surgical history of the rats.

Surgery and Histology

Following sodium pentobarbital anesthesia (Nembutal, 60 mg/kg i.p.), the animals were placed in a Kopf stereotaxic instrument. Bilateral septal lesions (n=30) were produced by passing a 1.2 mA, 12 second anodal current though a stainless steel electrode insulated with varathane to within 0.5 mm of its tip. Using flat skull coordinates, the electrode was lowered, angled 15 degrees medially in order to avoid perforating the sagittal sinus, such that its tip was positioned in the septal area (1.2 mm anterior and 2.0 mm lateral to bregma, 5.5 mm ventral to dura). Pilot studies revealed a 0.5 mm anterior/posterior discrepancy between the atlas coordinates and the instrument coordinates for the septal area in these rats, therefore a +0.5 mm adjustment in stereotaxic coordinates were made. Sham-lesioned controls (n=20) were treated in the same way except that no current was passed through the electrode. At the conclusion of the behavioral testing, rats were overdosed with

chloral hydrate and perfused intracardially with physiological saline followed by 10% formalin. Brains were extracted and placed in 10% formalin for a minimum of three days, after which they were frozen and sliced using a cryostat. In order to determine exact location and extent of the lesions, every fourth 32 um section through the lesion area was mounted on a slide, stained with thionine, and examined microscopically. Lesions were rated by at least two observers, and data for the animals with misplaced lesions were discarded. The location and extent of these lesions were transcribed onto the appropriate Paxinos and Watson atlas plates.

Procedure

Plus-Maze. Following three days of post-surgical recovery, ten septal-lesioned rats and ten sham-lesioned rats were handled every day for four consecutive days. On the fifth day (8 days post-surgery), each rat was placed individually in the center of the plus-maze. An observer scored total time spent in the open arms, the total number of crossings into the open arms, and the total number of crossings into the closed arms for five minutes. A crossing was defined as all four paws in the arm. For the purpose of analysis, open arm activity was measured as both the amount of time that the rat spent in the open arms relative to the total time of the test (% open/total), as well as the number of crosses into the open arms relative to the total number of crosses (% open/total). The maze was cleaned after each rat was tested.

Shock-Probe Burying. Following eight days of post-surgical recovery, the other ten rats from each of the two lesion conditions were tested in the shock-probe apparatus. On each of the four consecutive days prior to the shock-probe test, the rats were individually habituated to the Plexiglas test chamber for 15 minutes. On the fifth day, rats were placed in the chamber containing the constantly electrified probe, which had been inserted 6 cm into the chamber just prior to the test. When the rat touched the constantly electrified probe with its forepaws or snout, it received a brief, electric shock. Following the first shock, the duration of time each rat spent spraying bedding material toward the probe (i.e., burying behavior) was measured for fifteen minutes, as well as the total number of shocks each rat received from the probe. The rat's behavioral reaction to each shock was measured on the following four point scale: 1) flinch involving only head or forepaw, 2) whole body flinch and ambulation to far end of chamber, 3) hopping away and running, 4) jumping away and running. A mean shock reactivity score was derived for each rat by summing their reactivity scores to each shock, and dividing this by the number of shocks obtained. The total duration of all the rats' activity (e.g., locomotion, sniffing, rearing, digging, grooming) was also measured to assess the effects of the septal lesions on general activity.

Septal hyperreactivity. One day prior to the shock-probe and plus-maze tests, all rats were evaluated for septal hyperreactivity by scoring the rats' resistance to capture according to the following five point scale: 0) no reaction, 1) shied from hand, 2) avoided hand by running and/or struggling when capture, 3) jump to avoid capture, 4) bit and/or tried to bite when captured.

RESULTS AND DISCUSSION

Histology

The maximal extent of the lesions, as well as the extent of a typical lesion, are illustrated in Figure 1. While damage to the sham-lesioned rats was restricted to very minor track-lesion damage of the corpus callosum and of the dorsal surface of the neocortex, damage to the septal-lesioned rats included most of the lateral and medial septal nuclei. These lesions extended as far rostral as the most anterior portion of the genu of the corpus callosum, and as far caudal as the anterior fornix. Extra-septal damage occasionally included very slight damage to the corpus callosum, cingulate cortex, medial striatum, vertical limb of the diagonal band, and anterior fornix. Data for misplaced lesions were discarded leaving 20 rats in the sham-lesioned group, and 20 rats in the septal-lesioned group.

Plus-Maze

The mean percent (open/total) of time spent in the open arms as well as the mean percent (open/total) of crossings into the open arms, for sham- and septal-lesioned rats, are shown in Figure 2. It is apparent that septal-lesioned rats spent more time in, and crossed more often into the open arms than sham-lesioned controls. These differences in percent open-arm activities were found to be significant with independent measures t-tests [percent open-arm time: $t(18) = 4.73$, $p < 0.001$; percent open-arm crosses: $t(18) = 3.14$, $p < 0.006$]. These results show that like the

effects of anxiolytic agents, complete lesions of the septum in rats produce a reliable increase in open arm activity. The total number of arm entries for the septal-lesioned rats was significantly lower than that for the sham-lesioned rats [$t(18) = 2.16$, $p<0.05$; see Table 1]. These differences in overall activity do not, however, appear to account for the differences in open arm activity, since the absolute duration of time spent in open arms was higher for the septal-lesioned rats than the sham-lesioned controls [$t(18)=4.723$, $p<0.001$].

Shock-Probe Burying

The mean duration of time that the septal- and sham-lesioned rats spent burying the probe is shown in Figure 3. Consistent with the results of previous experiments (Gray et al., 81), sham-lesioned rats spent a considerable amount of time spraying bedding material toward the probe (i.e., burying), whereas not a single septal-lesioned rat displayed this behavior. Independent measures t-tests confirmed that the difference in burying duration between the septal- and sham-lesioned rats was significant, $t(18)= 2.56$, $p<0.02$. The behavioral effects of septal lesions appear to be specific to burying behavior since there were no significant differences between septal- and sham-lesioned rats in either the number of shocks obtained [$t(18)=0.64$, $p>0.5$; see Table 2], or in reactivity to those shocks [$t(18)= 1.72$, $p>0.1$; see Table 2]. Furthermore, this suppression of burying activity in the septal-lesioned rats cannot be attributed to an overall decrease or change in general activity during the test, since there were no significant differences between septal- and sham-lesioned rats on

this measure [$t(18) = 0.48$, $p > 0.5$; see Table 2]. These results indicate that, like anti-anxiety agents, complete lesions of the septum appear to produce a reasonably specific suppression of defensive burying.

Handling Reactivity

The mean resistance to capture of septal-lesioned and sham-lesioned rats was not significantly different on the day before the plus-maze [$t(18) = 1.00$, $p > 0.5$; see Table 1] and shock-probe test [$t(18) = 0.268$, $p > 0.5$; see Table 2]. These results suggest that differences in behavior displayed by septal- and sham-lesioned animals in the latter tests were not due to "septal hyperreactivity".

Figure 1

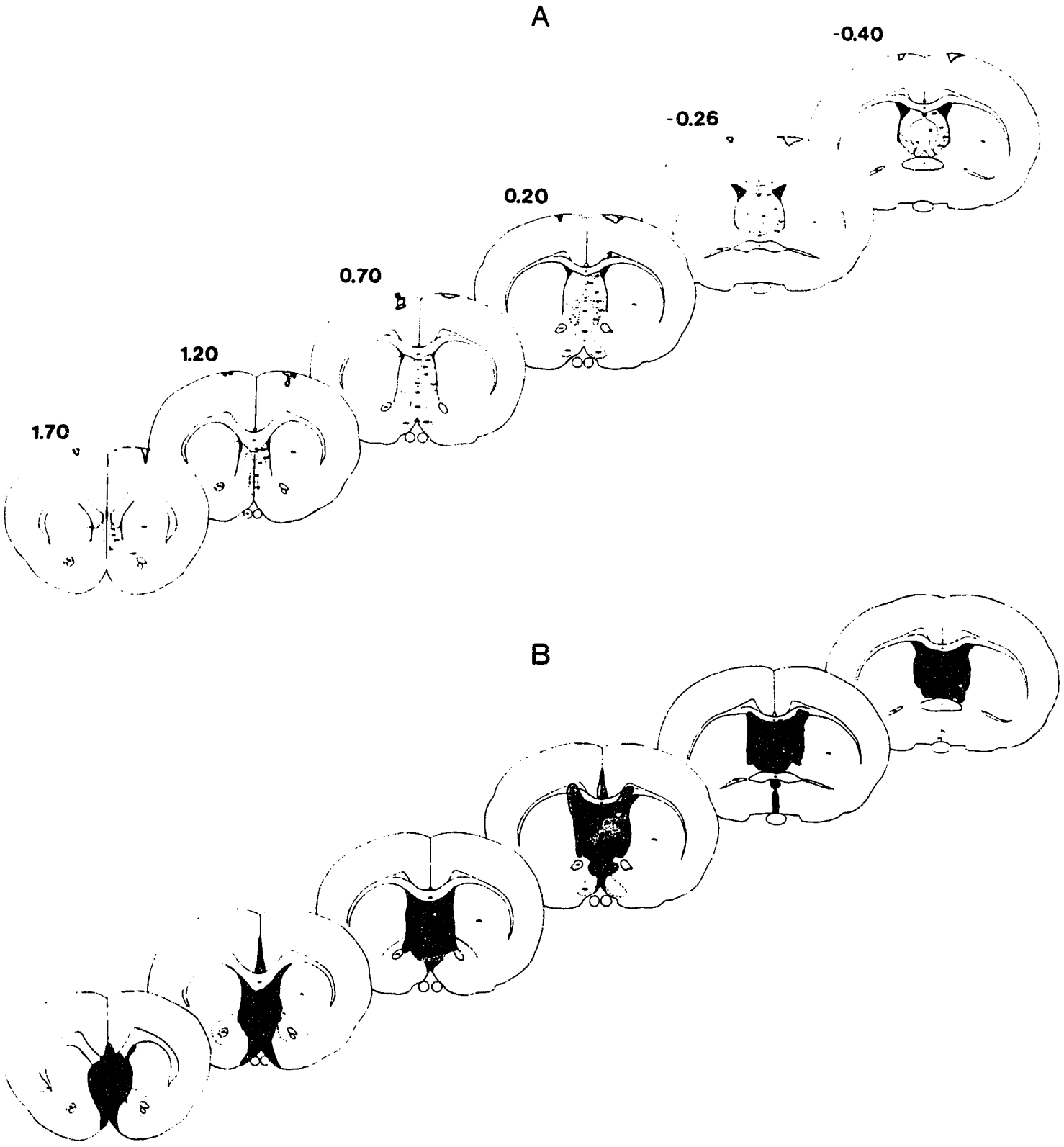

Figure 2

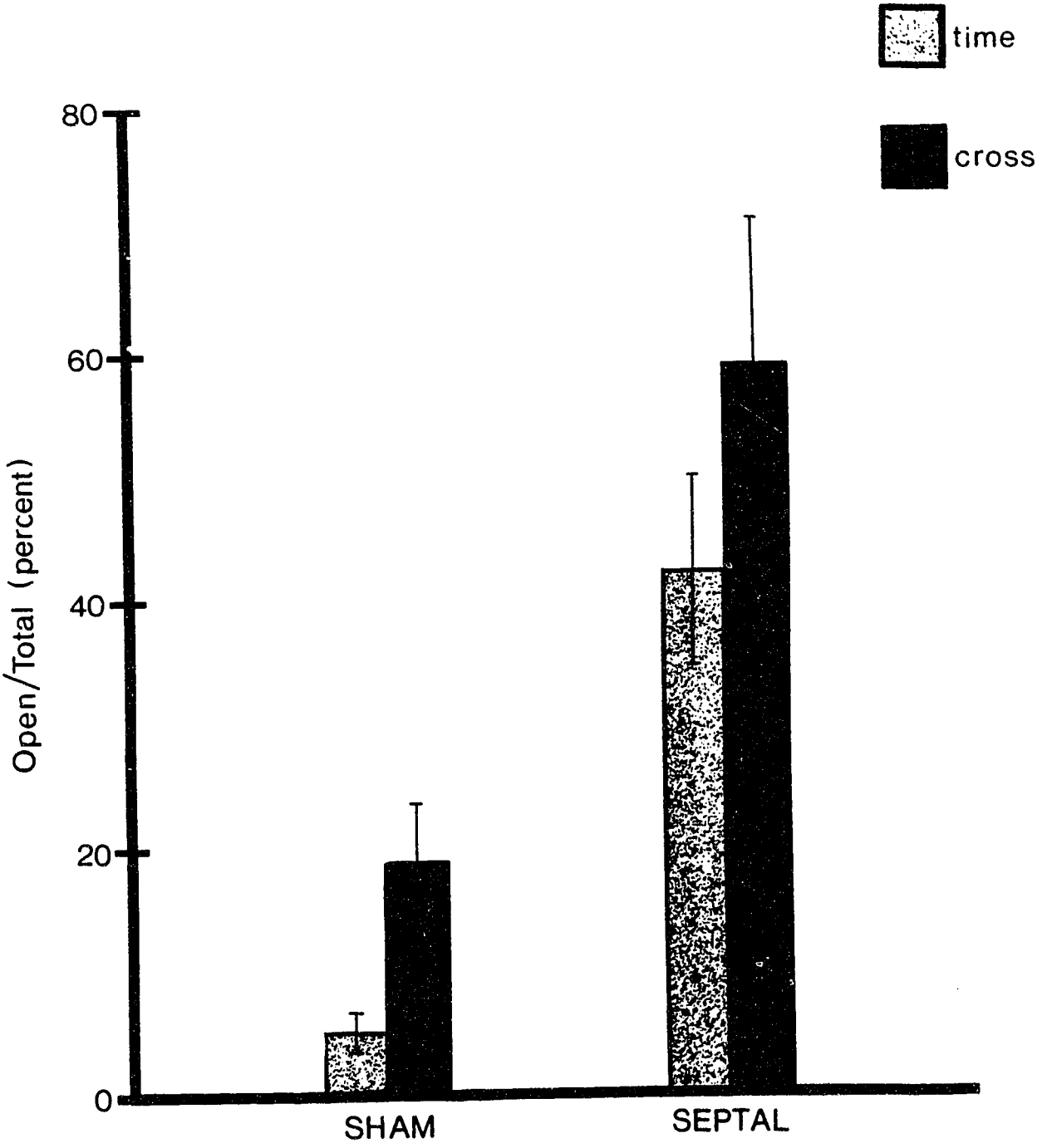

TABLE 1

	SHAM (n=10)	SEPTAL (n=10)
Total Number of Arm Entries	10.80 (1.04)	5.90 (2.02)
Handling Reactivity	0.00 (0.00)	0.20 (0.20)

Figure 3

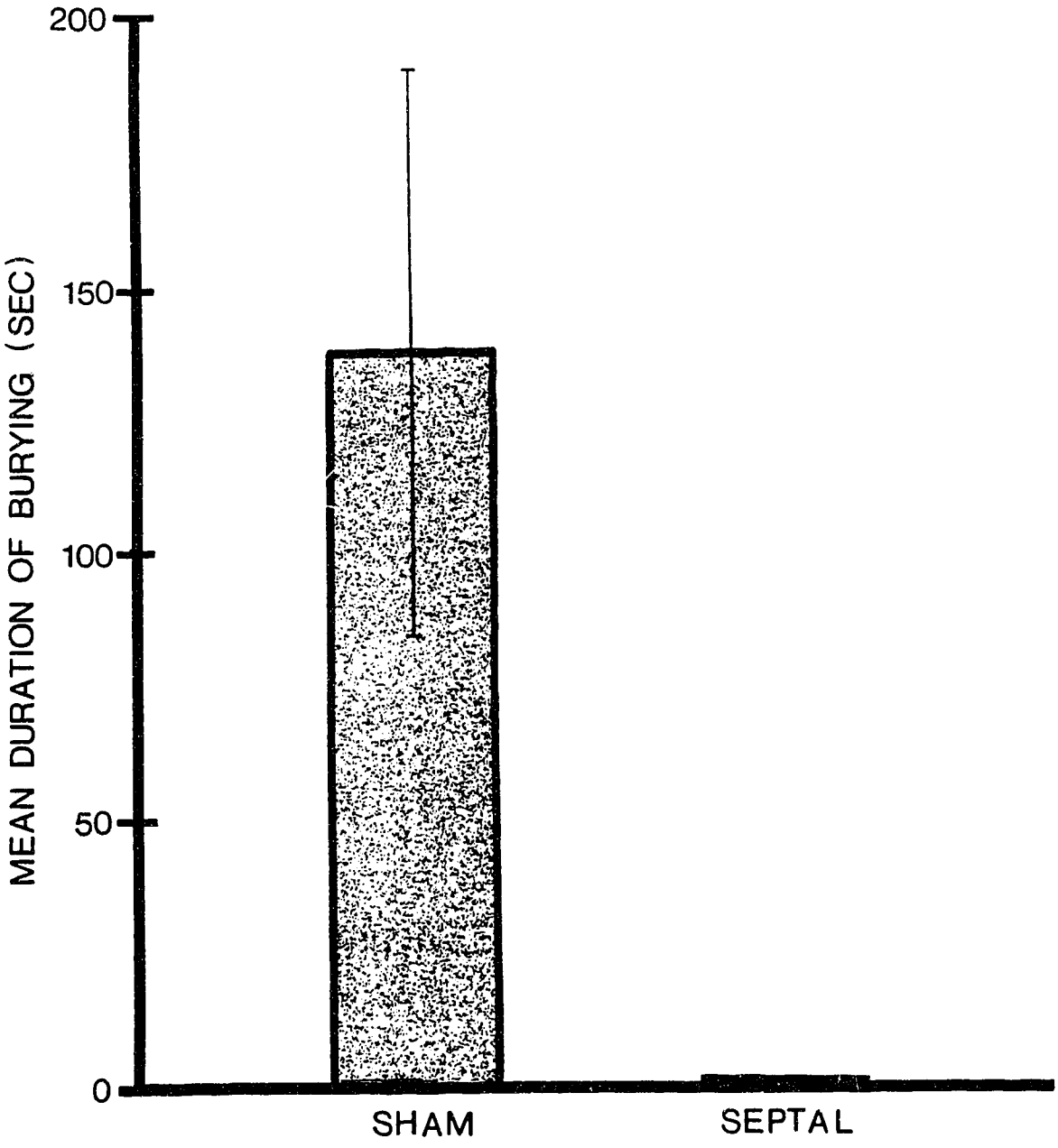

TABLE 2

	SHAM (n=10)	SEPTAL (n=10)
General Activity (sec)	807.3 (36.7)	826.8 (16.3)
Number of Shocks	1.6 (0.22)	1.4 (0.21)
Shock Reactivity	1.1 (0.24)	1.7 (0.29)
Handling Reactivity	0.30 (0.2[illegible])	0.40 (0.31)

Experiment 2

Results from the first experiment revealed that complete septal lesions reduced fear reactions in two tests of anxiolytic drug action (the elevated plus-maze test, and the shock-probe burying paradigm). Previous studies have suggested that different septal nuclei might play different roles in the modulation of emotion-related behaviors. For example, Gray and his colleagues (1981) compared the effects of anterior with posterior septal lesions on the shock-probe burying paradigm and found the suppression of burying behavior to be specific to the posterior portion of the septum. Thus, the first purpose of Experiment 2 was to replicate the previous findings of reduced fear reactions in the shock-probe burying paradigm following posterior septal lesions but not anterior septal lesions. The second purpose of Experiment 2 was to compare the effects of anterior and posterior septal lesions in the elevated plus-maze test.

METHOD

Subjects

The subjects were 40 naive, male albino Sprague-Dawley rats weighing 185-225 g at the time of surgery. Housing and feeding conditions were the same as those

used in Experiment 1, as were shock-probe and plus-maze apparatus.

Surgery and Histology

Following nembutal anesthesia, the rats were placed in a Kopf stereotaxic instrument. With the electrode positioned according to flat skull coordinates, 15 rats received bilateral lesions (1.0 mA for 10 sec) to the anterior portions of the septum (2.1 mm anterior and 2.0 mm lateral to bregma, 6.7 mm ventral to dura, with the electrode angled 18 degrees medially), and 15 rats received bilateral lesions (1.0 mA for 10 sec) to the posterior portions of the septum (0.7 mm anterior and 2.0 mm lateral to bregma, 5.5 mm ventral to dura, with the electrode angled 15 degrees laterally). Pilot studies revealed a 0.5 mm dicrepancy between the atlas and instrument coordinates for the posterior septal area, and a 0.9 mm discrepancy for the anterior septal area. Therefore, +0.5 mm and +0.9 mm adjustments were made for posterior and anterior septal lesions, respectively. Half of the ten sham-lesioned rats were treated the same way as the anterior septal-lesioned rats and half as the posterior septal-lesioned rats, except that no current was passed through the electrode. Since there were no behavioral differences between these two sham-lesioned groups, their data were combined for purpose of analysis. Histological procedures and assessments were the same as those described in Experiment 1.

Procedure

The procedures were similar to those used in Experiment 1 except that each rat was tested in both the plus-maze and the shock-probe apparatus.

Following eight days of post-surgical recovery, the rats from each of the three lesioned groups (anterior, posterior, and sham) were tested on the elevated plus-maze. Immediately following the plus-maze test, the rats were habituated 15 minutes to the shock-probe apparatus for the first of four consecutive habituation days. On the fifth day (12 days post-surgery), the probe was inserted into the Plexiglas chamber, and all rats from each group were tested in the shock-probe test. This order of testing was based on pilot studies which revealed that behavior in the shock-probe test was not affected by prior exposure to the 5-min plus-maze test; however behavior on the plus-maze test was disrupted as a consequence of prior exposure to the shock-probe test. Otherwise, handling and testing procedures were the same as those described in Experiment 1. Resistance to capture was also assessed on the day prior to each behavioral test.

RESULTS AND DISCUSSION

Histology

The maximal extent of the lesions as well as the typical lesions for both the anterior and the posterior septal-lesioned rats, are illustrated in Figure 4. Damage to sham-lesioned rats was minimal, similar to that of sham-lesioned rats in Experiment 1. Damage to anterior septal-lesioned rats was restricted mainly to the medial and lateral septal nuclei, beginning as far rostral as the most anterior portion of the genu of the corpus callosum, and extending as far caudally as 1.0 mm anterior to the fornix. Damage to this lesion group occasionally included slight damage to the corpus callosum, cingulate cortex, and the vertical limb of the diagonal band. Damage to the posterior septal-lesioned rats was also primarily restricted to the medial and lateral septal nuclei, extending as far rostral as 1.0 mm anterior to the fornix, and as far caudally as the anterior fornix. Extra-septal damage in this lesion group occasionally included slight damage to the corpus callosum, medial striatum, vertical limb of the diagonal band, and anterior fornix. Data for misplaced, unilateral, or complete septal lesions were discarded leaving 10 rats in the sham-lesioned group, 12 rats in the anterior septal-lesioned group, and 11 rats in the posterior septal-lesioned group.

Plus-Maze

The mean percent (open/total) open arm activity (time spent and crosses into

open arms) for the posterior and anterior septal-lesioned rats as well as the sham-lesioned controls, are presented in Figure 5. Anterior septal lesions had little effect on rats' open-arm activity as compared to sham-lesioned controls. Posterior septal lesions, similar to complete septal lesions in Experiment 1, produced a substantial increase in open arm activity. One-way analysis of variance revealed a significant effect of lesion type on both percentage of time spent in open arms, and percentage of crossings into the open arms [$F(2,30) = 12.58$, $p<0.001$, and $F(2,30)=9.96$, $p<0.001$, respectively]. Subsequent pairwise comparisons (independent t-tests, alpha=0.05) confirmed that the anterior septal- and the sham-lesioned rats did not differ significantly from one another on either measure, but that they both differed significantly from posterior septal-lesioned rats on both measures. In contrast to the results of Experiment 1, the three groups did not differ significantly in their overall level of activity as measured by total number of entries [$F(2,30)=2.966$, $p>0.05$; see Table 3]. In agreement with the findings of Experiment 1, these results show that septal lesions, and more specifically, posterior septal lesions, produce anti-anxiety effects in the elevated plus-maze.

Shock-Probe Burying

Consistent with the plus-maze results, anterior septal lesions had little effect on rats probe burying behavior compared to sham-lesioned controls, whereas lesions of the posterior septum completely suppressed this behavior (see Figure 6). An analysis of variance performed on the duration of burying data revealed a significant

effect of lesion type [$F(2,30)=5.77$, $p<0.01$]. Pairwise comparisons (t-tests, alpha=0.05) confirmed that while there was no significant difference in burying duration between the anterior septal-lesioned rats and the sham-lesioned rats, posterior septal-lesioned rats buried significantly less than either of the two latter groups. These differential results are specific to burying behavior since there were no significant differences between the three groups on other measures such as number of shocks obtained [$F(2,30)=0.69$, $p>0.5$; see Table 4], shock reactivity [$F(2,30)=0.82$, $p>0.5$; see Table 4], or changes in general activity [$F(2,30)=0.53$, $p>0.5$; see Table 4]. These results, which replicate the findings of Gray et al., (1981), show that posterior but not anterior septal lesions produce specific and complete suppression of probe burying behavior. These results and those of the plus-maze test support the hypothesis that posterior portions of the septum modulate "anxiety"-related behaviors in these two paradigms.

Handling Reactivity

There were no significant differences in resistance to capture scores between the three groups of rats on the day prior to the plus-maze test [$F(2,30)=2.18$, $p>0.1$; see Table 3] or on the day prior to the shock-probe test [$F(2,30)=0.01$, $p>0.5$; see Table 4]. These results suggest that any differences in behavior observed between the three groups of rats on either of the two tests were not a consequence of septal-hyperreactivity.

Figure 4

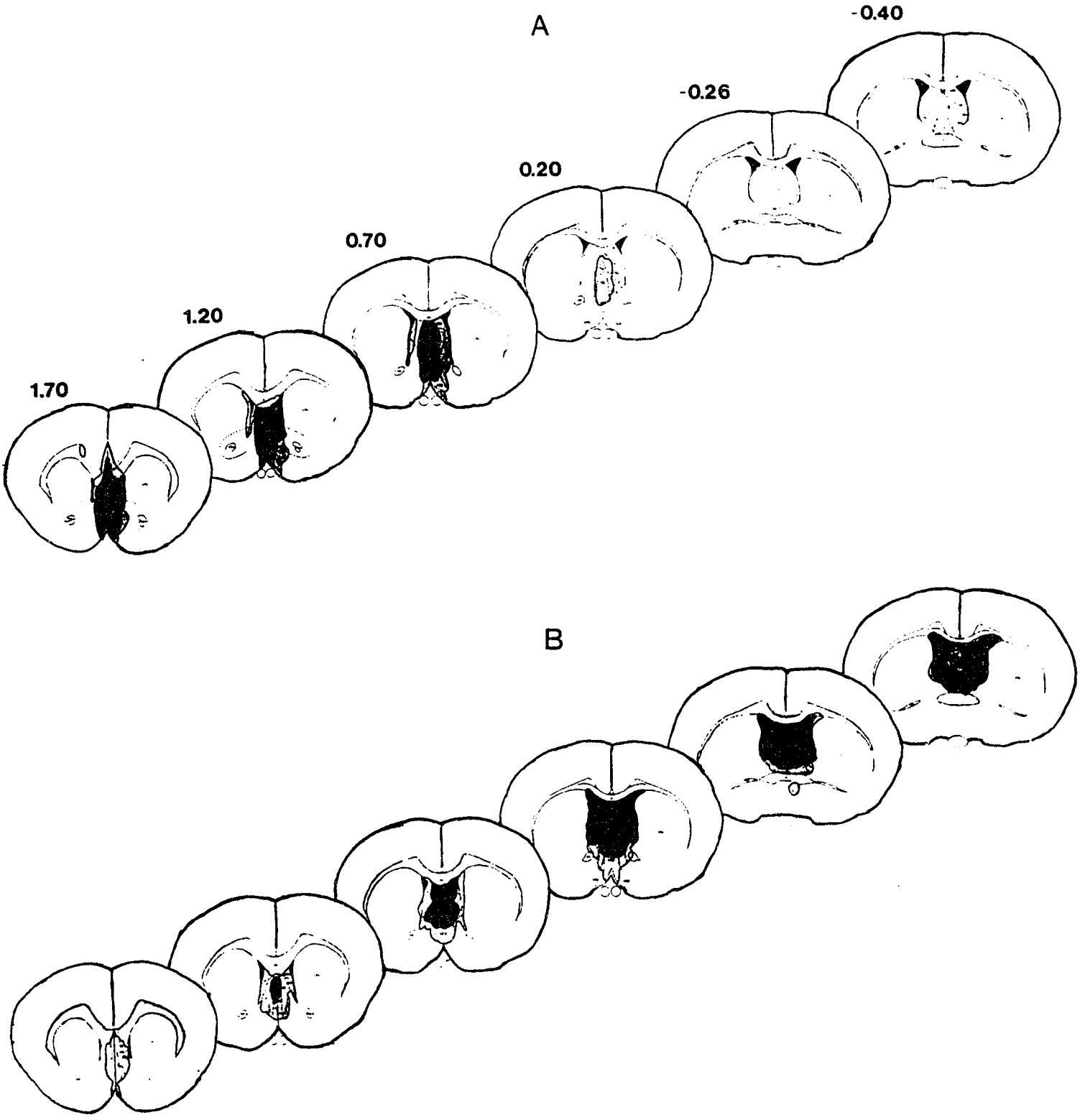

Figure 5

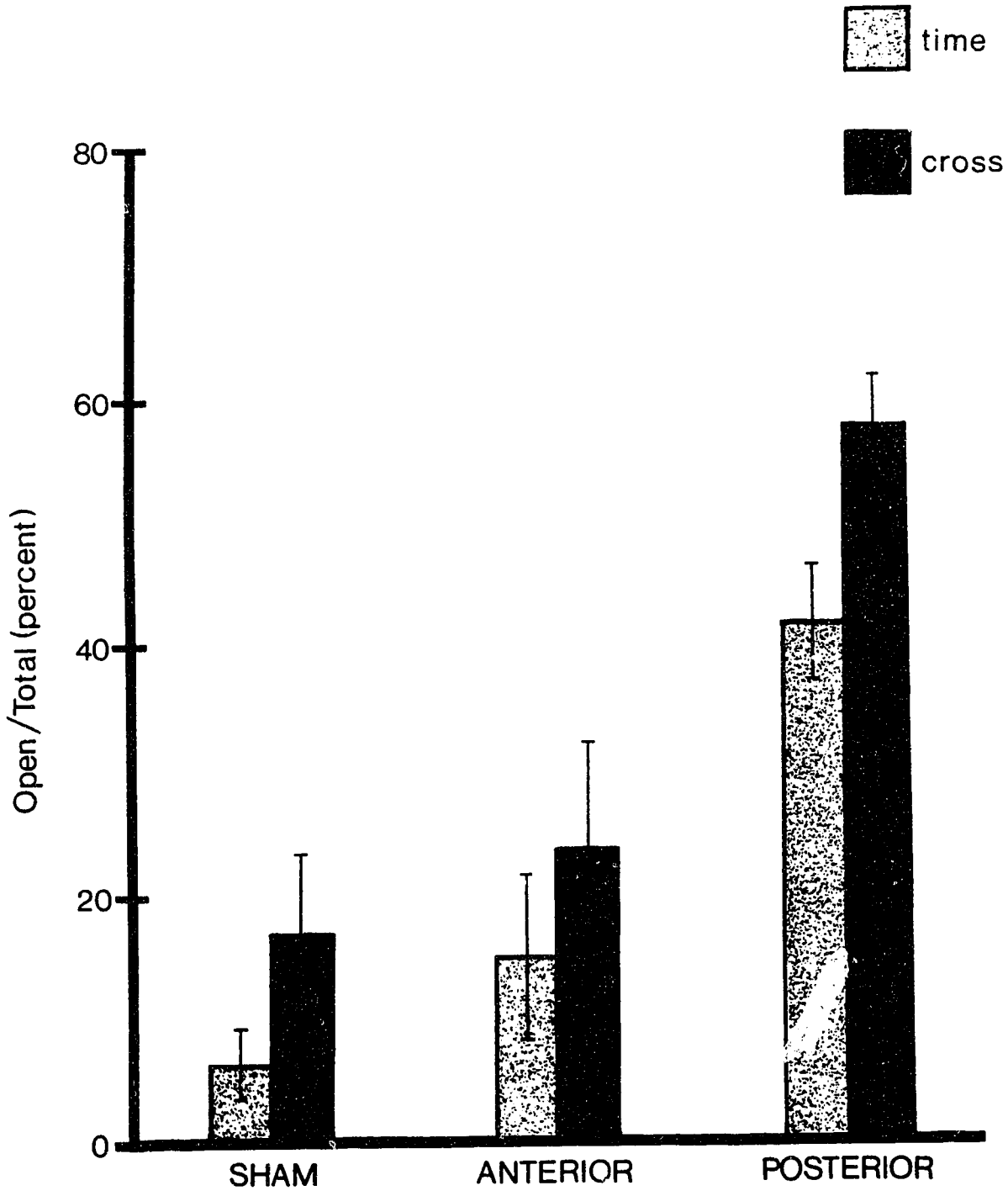
time
cross
80
60
40
20
0
Open/Total (percent)
SHAM
ANTERIOR
POSTERIOR

TABLE 3

	SHAM (n=10)	ANTERIOR SEPTAL LESION (n=12)	POSTERIOR SEPTAL LESION (n=11)
Total Number of Arm Entries	6.10 (1.31)	11.08 (2.21)	11.27 (1.18)
Handling Reactivity	0.7 (0.26)	0.0 (0.0)	0.18 (0.18)

Figure 6

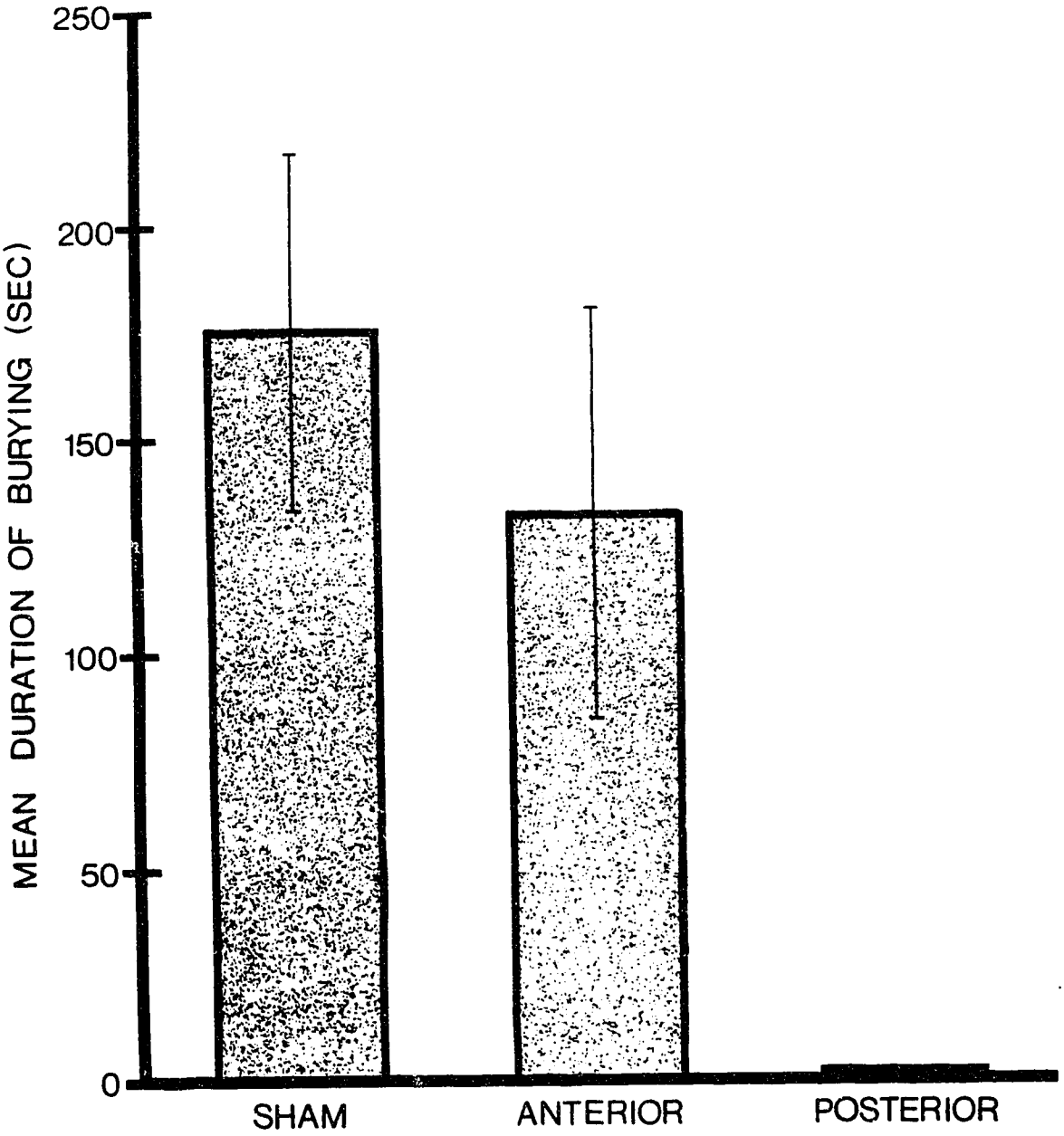
250
200
150
100
50
0
MEAN DURATION OF BURYING (SEC)
SHAM
ANTERIOR
POSTERIOR

TABLE 4

	SHAM (n=10)	ANTERIOR SEPTAL LESION (n=12)	POSTERIOR SEPTAL LESION (n=11)
General Activity (sec)	815.2 (40.8)	854.8 (16.4)	817.5 (35.0)
Number of Shocks	1.8 (0.25)	2.2 (0.29)	1.7 (0.30)
Shock Reactivity	2.1 (0.22)	1.7 (0.28)	2.2 (0.30)
Handling Reactivity	0.0 (0.0)	0.08 (0.08)	0.0 (0.0)

Experiment 3

Results from Experiment 2 suggest that the anti-fear effects observed following septal lesions are specific to the posterior septum since electrolytic lesions of the posterior, but not anterior septum, suppressed fear reactions in both the elevated plus-maze and the shock-probe burying paradigm. However, due to the septum's extensive reciprocal connections with other areas of the limbic system, it is unclear whether the anti-fear effects of electrolytic lesions can be directly attributed to the destruction of septal nuclei themselves, or fibers of passage. The use of an excitatory amino acid such as kainic acid, which primarily destroys cell bodies leaving fibers of passage intact, may help determine whether anti-fear effects of posterior septal lesions are due to loss of cell bodies in the posterior septum, or simply a result of destroying fibers which course through the area. Thus, the purpose of Experiment 3 was to clarify the role of the septum in fear, by comparing the effects of kainic acid and electrolytic lesions of the posterior septum, on rats' reactivity in both the elevated plus-maze and the shock-probe burying paradigm. If fear reactions are specifically modulated by the posterior septum, then kainic acid lesions should produce anti-fear effects that are comparable to those of electrolytic lesions.

METHOD

Subjects

The subjects were 52 naive, male albino Sprague-Dawley rats weighing 240-400 g at the time of surgery. Housing and feeding conditions were the same as those in the first experiment, as were shock-probe and plus-maze apparatus.

Surgery and Histology

Following nembutal anesthesia the rats were placed in a Kopf stereotaxic instrument. Using flat skull coordinates, bilateral electrolytic lesions (n = 13) were produced by passing a 1.0 mA anodal current for 10 seconds through an electrode positioned in the septal area (0.7 mm anterior and 2.0 mm lateral to bregma, 5.5 mm ventral to dura, with the electrode angled 15 degrees medially). Kainic acid lesions (n=25) were produced by bilaterally infusing 0.75 ul of kainic acid (2.2 ug/ul, dissolved in phosphate buffered saline, pH=7.4) at a rate of 0.1 ul/min through a stainless steel cannula positioned in the septal area (0.7 mm anterior and 2.0 mm lateral to bregma, 5.3 mm ventral to dura, with the cannula angled 13 degrees medially). The cannula was connected via polyethylene tubing to a 200 ul Hamilton microsyringe. The syringe was left in place 7 minutes following the infusion of the acid in order to allow it to diffuse away from the cannula tip. Post-surgical seizure

activity was carefully monitored in the kainic acid-lesioned rats, and a 5-10 mg/kg dose of diazepam was administered (i.p.) if and when the seizuring reached a moderately severe level (i.e., full body rigidity and tremoring). Half of the fourteen sham-lesioned rats were treated the same as the electrolytic-lesioned rats except that no current was passed, and the other half were treated the same as the kainic acid-lesioned rats except that no kainic acid was infused, and no diazepam was administered.

Histological procedures and assessments were also the same as in Experiment 1. Kainic acid lesions were quantified in terms of a loss of magnocellular cells, and a respective increase in gliosis.

Procedure

The procedures were virtually the same as in Experiment 2, except for the extended post-surgical recovery time allotted to all groups. This extended post-surgical recovery time was determined by pilot studies which showed that a reasonable amount of cell death (and gliosis) was apparent by 15 days following the excitatory amino acid infusion.

Following fifteen days of post-surgical recovery, the rats from each of the three lesion groups (electrolytic, kainic acid, and sham) were tested on the elevated plus-maze, then individually habituated for 15 minutes on each of four consecutive days in the shock-probe apparatus, and tested with the shock-probe in place on day

5 (i.e., 19 days post-surgery). In addition to the measures taken in Experiments 1 and 2, the total time that the rats spent in the closed arm for the five minute test was also assessed. In this experiment, open arm time was defined as the amount of time that the rat spent in the open arms relative to the total amount of time in any arm (% open/total). Otherwise, handling, habituating and testing procedures were the same as those described in Experiment 1. Resistance to capture was assessed on the day of each behavioral test.

RESULTS AND DISCUSSION

Histologies

Data for misplaced lesions, as well as data for kainic acid lesions which included total tissue loss, were discarded leaving a total of 31 lesioned animals: 13 sham, 7 electrolytic, and 11 kainic acid. Both typical and overall extent of the lesions are displayed in Figure 7. Similarly to Experiment 1 and 2, damage to sham-lesioned controls was restricted to very slight neocortical and corpus callosum damage, as well as slight ventricular enlargement. Despite the slightly larger track lesions produced by lowering the cannula, there were no behavioral differences between the two sham-lesioned groups and therefore their data were combined for the purpose of analysis. Damage to the electrolytic-lesioned animals encompassed most of the medial and lateral septal nuclei, beginning approximately 1.0 mm anterior to the fornix and

extending as far caudally as the anterior fornix. Extra-septal damage occasionally included slight damage to the corpus callosum, medial striatum, vertical limb of the diagonal band, and anterior fornix. While the kainic acid-lesioned animals had slightly more corpus callosum damage than the electrolytic-lesioned rats, the rostro-caudal and medio-lateral extents of lesions were the same.

Plus-Maze

The mean percentage (open/total) of time spent in and number of crosses into the open arms for sham-, electrolytic-, and kainic acid-lesioned rats, is shown in Figure 8. A one-way analysis of variance revealed a significant effect of lesion type on both percentage of time spent in open arms [$F(2,28)=34.267$, $p<0.001$], as well as percentage of crossings into the open arms [$F(2,28)=24.536$, $p<0.001$]. In agreement with results obtained in Experiment 1 and 2, electrolytic-lesioned rats displayed significantly elevated open arm activities as compared to sham-lesioned controls (independent t-tests, alpha=0.05). The effects of kainic acid lesions were found to be comparable to those of electrolytic lesions, since they also differed significantly from sham-lesioned controls on both measures of open arm activity while not differing significantly from electrolytic-lesioned rats on either measure (independent t-tests, alpha=0.05). While there was a trend for kainic acid-lesioned rats to display even greater open arm activity than electrolytic-lesioned rats, this difference was not significant. Both groups of lesioned rats showed a level of open arm activity that exceeded chance level (t-tests, alpha=0.05), suggesting a

"preference" for the open arms.

While the lesioned groups displayed higher percentages of open arm activity than the sham-lesioned controls, there were no significant differences among any of the groups in terms of total number of arm entries [$F(4,44) = 0.427$, $p > 0.5$; see Table 5]. Given that kainic acid primarily destroys cell bodies, leaving fibers of passage intact, the finding that kainic acid lesions of the septum produced at least as powerful 'anxiolytic' effects as electrolytic lesions, provides increasing support for the hypothesis that regions of the posterior septum play a role in modulating "anxiety"-related behaviors.

Shock-Probe Burying

In accordance with predictions, not a single rat in either of the two lesion groups displayed burying behavior. Unfortunately, normal baseline levels of burying were not obtained for the sham-lesioned controls (i.e., only 3 rats buried) yielding non-significant differences in duration of burying between any of these groups of rats [$F(2,28) = 1.572$, $p > 0.2$]. While it would be tempting to speculate that the suppression of burying in the lesioned groups was reflective of an anxiolytic effect, it could have also been a consequence of another factor, which suppressed burying behavior in the sham-lesioned controls.

A one-way analysis of variance revealed a significant effect of lesion type [$F(2,28) = 4.515$, $p < 0.05$], on number of shocks obtained (Figure 9). In agreement with the results obtained in Experiment 2, pairwise comparisons (independent t-test,

alpha = 0.05) confirmed that there was no significant difference in the number of shocks taken by electrolytic- and sham-lesioned rats (see Table 6). However, pairwise comparisons also revealed that kainic acid-lesioned rats obtained significantly more shocks than sham-lesioned controls (see Table 6). This increased number of shocks taken by the kainic acid-lesioned rats cannot be explained by a reduced sensitivity toward the shock-probe since there were no significant differences between any of the groups on reactivity to shock scores [$F(2,28) = 0.271$, $p > 0.1$; see Table 6]. While a lesion effect was also revealed for general activity scores [$F(2,28) = 6.101$, $p < 0.01$], the increase in number of probe contacts does not appear to be attributable to an overall non-specific increase in activity, since general activity scores for the kainic acid-lesioned rats were significantly lower than those of sham-lesioned controls [$t(22) = 4.537$, $p < 0.0005$], while not differing significantly from electrolytic-lesioned rats (see Table 6). Interestingly, similar increases in number of shocks taken have also been observed in this paradigm following the administration of anxiolytic compounds (Treit, 1990).

While these results are certainly interesting, it would be premature to draw any strong conclusions at this time since baseline level of burying response normally observed by control animals in this paradigm were not obtained.

Handling Reactivity

A lesion effect was found for the mean resistance to capture scores on both the day of the plus-maze test [$F(2,28) = 11.339$, $p < 0.001$; see Table 5] and the day of

the shock-probe test [$F(2,28)=7.914$, $p<0.005$; see Table 6]. Pairwise comparisons (independent t-tests, alpha=0.05) revealed that on both these days, sham- and electrolytic-lesioned rats were significantly less reactive than kainic acid-lesioned rats, while not significantly different from each other on this measure. While these results indicate that kainic acid-lesioned rats were hyperreactive to handling, they were not more reactive to shock, and their general activity in the two tests of anxiety was either lower than, or not different from, sham-lesioned controls.

Figure 7

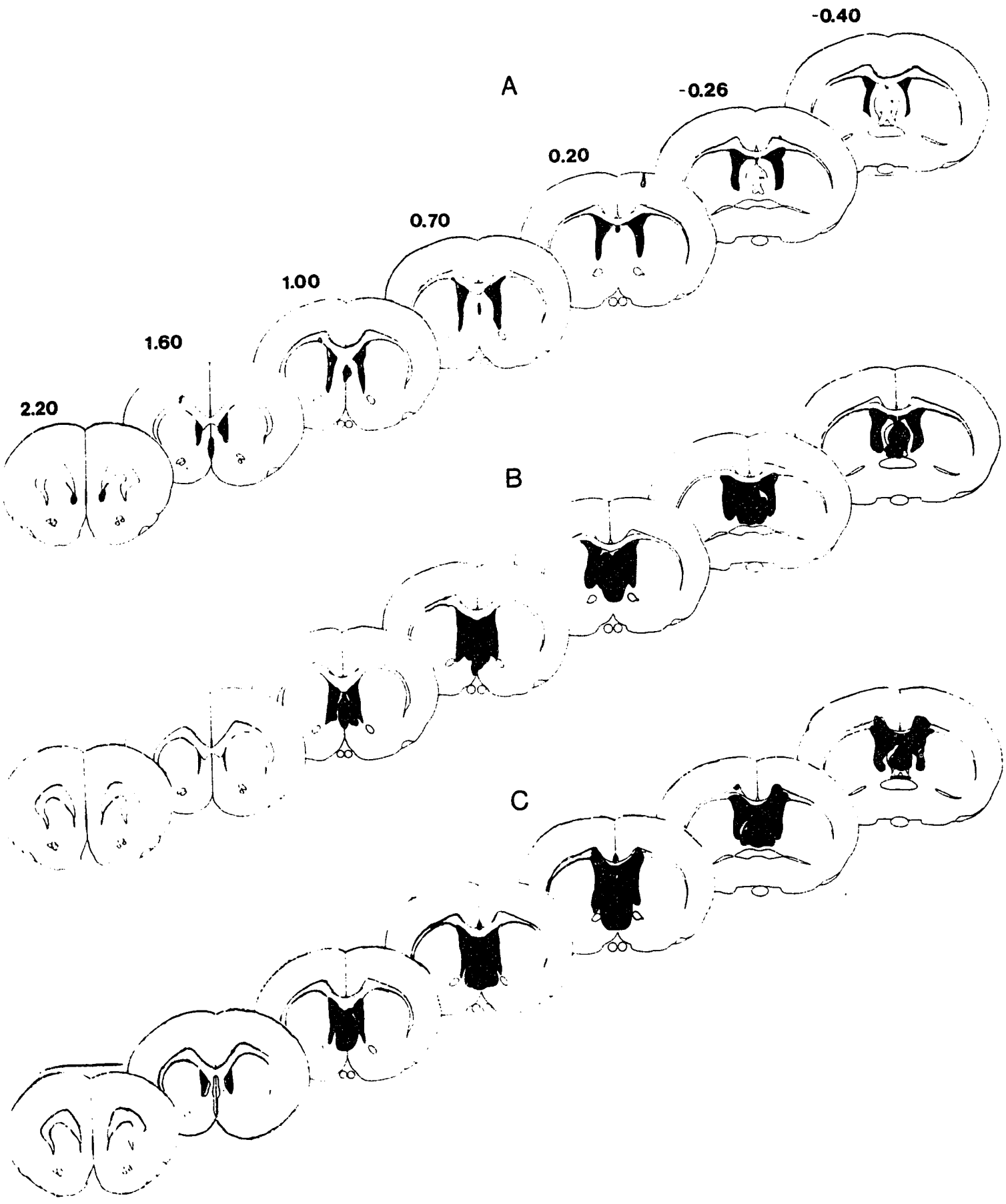

Figure 8

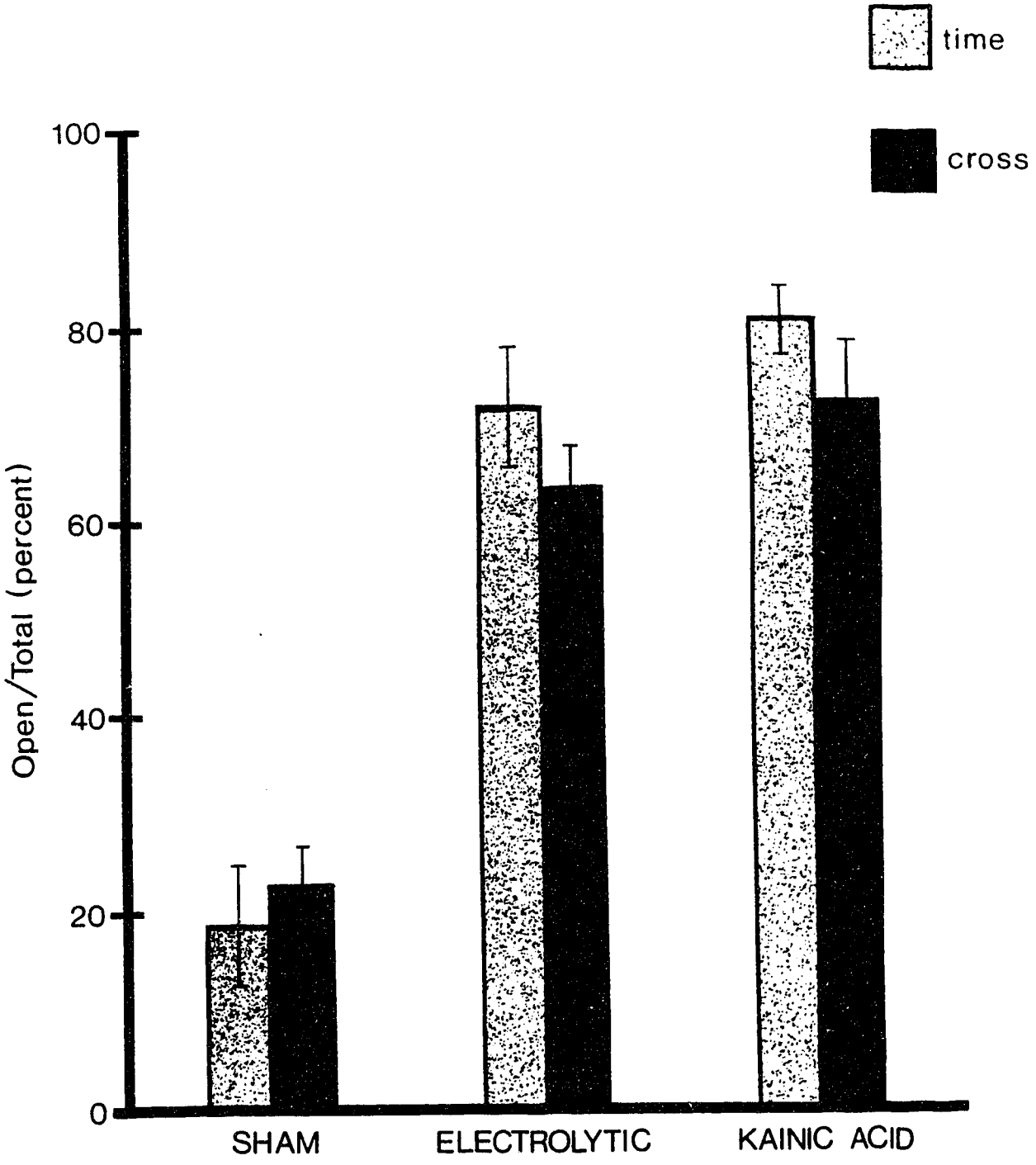
time
cross
100
80
60
40
20
0
Open/Total (percent)
SHAM
ELECTROLYTIC
KAINIC ACID

TABLE 5

	SHAM (n=13)	ELECTROLYTIC SEPTAL LESION (N=7)	KAINIC ACID SEPTAL LESION (N=11)
Total Number of Arm Entries	10.31 (1.08)	12.43 (1.78)	12.73 (2.08)
Handling Reactivity	0.89 (0.20)	0.86 (0.24)	2.18 (0.25)

Figure 9

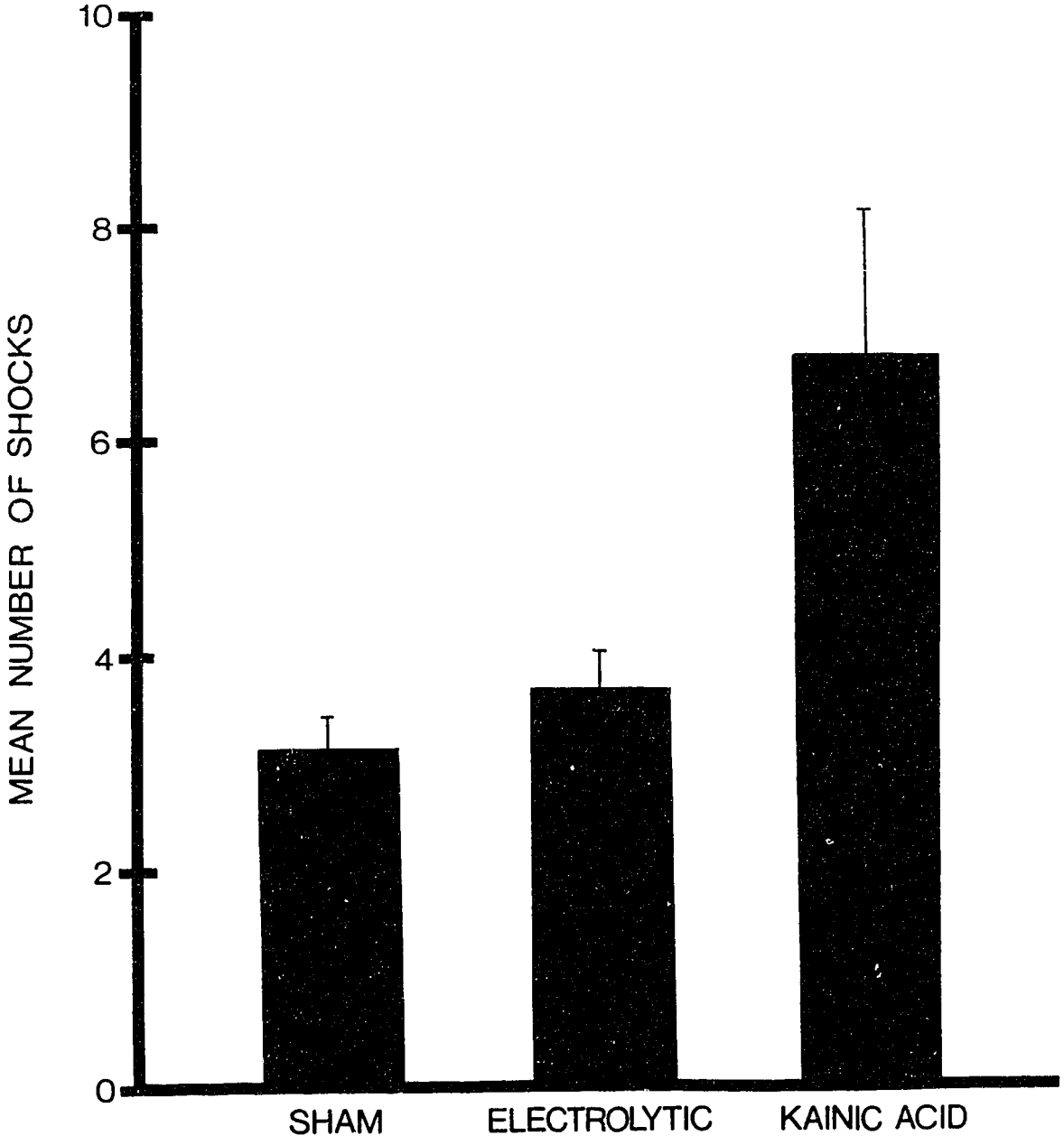
10
8
6
4
2
0
MEAN NUMBER OF SHOCKS
SHAM
ELECTROLYTIC
KAINIC ACID

TABLE 6

	SHAM (n=13)	ELECTROLYTIC SEPTAL LESION (N=7)	KAINIC ACID SEPTAL LESION (N=11)
General Activity (sec)	829.69 (19.75)	716.14 (89.04)	565.82 (58.97)
Number of Shocks	3.08 (0.33)	3.57 (0.37)	6.73 (1.39)
Shock Reactivity	1.58 (0.15)	1.47 (0.12)	1.43 (0.14)
Handling Reactivity	0.96 (0.14)	1.07 (0.23)	2.18 (0.30)

GENERAL DISCUSSION

The results of these experiments support the hypothesis that the septum is involved in the modulation of "anxiety"-related behaviors in the rat. In Experiment 1, complete septal lesions suppressed fear reactions in two different animal models of anxiolytic drug action: the plus-maze test and the shock-probe burying paradigm. In the plus-maze test, septal lesions suppressed rats' tendency to avoid the open arms, and in the shock-probe burying paradigm, septal lesions completely suppressed rats' burying behavior toward a continuously electrified probe. In Experiment 2, it was demonstrated that these anti-fear effects could be localized to a fairly restricted region of the septum, since anterior septal lesions did not produce any significant changes in either the plus-maze or the shock-probe test, whereas posterior septal lesions suppressed both open-arm avoidance and shock-probe burying. The results of Experiment 3 suggested that these anti-fear effects of posterior septal lesions were specific to that region, and not the result of disrupting fibers which pass through the area, since the effects of kainic acid lesions were comparable to many of the effects of electrolytic lesions. Both electrolytic and kainic acid lesions of the posterior septum produced comparable suppression of rats' tendency to avoid the open arms in the elevated plus-maze. While the rats' tendency to bury the shock-probe was suppressed for both electrolytic and kainic acid-lesioned rats in Experiment 3, normal baseline levels of burying were not obtained from the control rats in this experiment, rendering any conclusion regarding the effects of these lesions on burying behavior

merely speculative.

These anti-fear effects following septal lesions are at least as large as those observed following the administration of anxiolytic agents in these two paradigms. While there were some exceptions, lesion effects were behaviorally restricted to open-arm avoidance and probe-burying. The results of these experiments provide converging evidence that the septum is involved in the modulation of "anxiety"-related behaviors in the rat.

While there is substantial evidence consistent with this view (Gray, 1982), most of the behavioral paradigms used to study the role of the septum in anxiety are not pharmacologically validated e.g., the effects of anxiolytic agents do not show drug-class specificity, they have not been shown to be blocked by antagonists of the GABA/benzodiazepine receptor complex, and these tests are not always sensitive to anxiogenic agents. Furthermore, these behavioral paradigms often employ the use of food and fluid as rewards. Septal lesions, like anxiolytic agents, have repeatedly been shown to have effects on consummatory motivation. While this strengthens the apparent similarities between the effects of septal lesions and those of anxiolytic agents, it may nonetheless be more prudent to employ tests that are not confounded by such appetitive motivators, especially in the study of the role of the septum in anxiety. Unlike traditional aversive learning paradigms, the elevated plus-maze test and the shock-probe burying test have been shown to satisfy most pharmacological criteria of anxiolytic drug action, and are not confounded by the use of food or fluid as appetitive motivators.

The combined use of the plus-maze and shock-probe paradigms in the analysis of septal lesion effects is important for two reasons. First, the elevated plus-maze and the shock-probe burying paradigm employ two different types of fearful stimuli (novel elevated arms in the plus-maze and painful aversive stimuli in the shock-probe burying paradigm). Second, 'fear'-reduction is primarily measured by a facilitation of a specific activity in one paradigm (plus-maze) and an inhibition of a specific activity in the other (shock-probe). Therefore, effects observed are not specific to one type of 'fearful' stimulus (e.g., shock), or one behavioral index of 'anxiety' (e.g., response suppression). Thus, reduced fear reactions in these two tests following septal lesions provide strong converging evidence that the septum plays an important role in the control of anxiety.

Nevertheless, septal lesions have been shown to produce a wide variety of behavioral effects, some of which could have contributed to the present results. Furthermore, many of the behavioral effects of septal lesions have also been found following the destruction of anatomically related areas, leading to the notion that these effects may not necessarily be specific to the septum, but a result of destroying its connections or inputs to related structures (e.g., the amygdala, hippocampus, and hypothalamus). The following section will critically evaluate a number of behavioral and anatomical alternative explanations for the results of the present experiments.

Alternative Interpretations: Behavioral

General Activity. Septal lesions have been reported to have various effects on general activity. Rats with septal lesions have been reported to be hypoactive in wheels (Douglas, & Raphelson, 1966), tilt boxes (Trafton, 1967), and home cages (Thomas, et al., 1959), but hyperactive in situations which encourage exploratory behavior such as novel mazes (Nielson, et al., 1965).

In the elevated plus-maze, lesions of the entire septum, and lesions of the posterior septum but not the anterior septum, produced significant increases in open-arm activity that appeared to be unrelated to any changes in general activity in this test. That is not to say, however, that there were no changes in general activity following septal lesions. General activity in the elevated plus-maze, as measured by total number of arm crossings, was significantly lower for complete septal lesions as compared to controls in Experiment 1, and slightly higher for posterior septal-lesioned rats as compared to controls in Experiment 2. These differences in open arm activity may be related to the inconsistent and complex effects that septal lesions have on ambulation in the open field. It is important to note, however, that despite the fact that general activity is significantly lowered in the first experiment, slightly elevated in the second experiment, and unchanged as compared to controls in the third experiment, a significant increase in open-arm activity was uniformly observed in all three experiments. It therefore seems unlikely that changes in general activity can account for the increase in open arm activity observed in the elevated plus-maze

after septal lesions.

The complete suppression of burying behavior after electrolytic lesions of the septum observed in Experiments 1, 2, and 3 was not associated with any significant changes in general activity in this test. However, the complete suppression of burying behavior seen in kainic acid-lesioned rats in Experiment 3 was associated with a change in general activity. While general activity of these rats was not significantly different from that of electrolytic-lesioned rats, it was significantly lower than that of sham-lesioned controls. These rats tended to engage in significantly more immobile activities such as resting or sleeping. In any case, no conclusions can be made regarding the suppression of burying behavior observed in lesioned animals in Experiment 3, since normal baseline levels of behavior were not observed in sham-lesioned control rats.

Hyperreactivity. Hyperreactivity to handling is a well documented behavioral effect of septal lesions (Fried, 1973). Resistance to capture was measured to assure that behavioral changes in these paradigms were not correlated with increases in this measure. While the resistance to capture score of electrolytic septal-lesioned rats was higher than sham-lesioned controls post-operatively (data not presented), all rats were handled for four days prior to testing, lowering the reactivity of these rats to the same level as controls by testing day. It is therefore unlikely that hyperreactivity had any effect on the behavior of electrolytic-lesioned rats in these paradigms.

Interestingly, resistance to capture scores of kainic acid septal-lesioned rats

never decreased to the level displayed by electrolytic or control rats at the time of testing, remaining significantly higher following several days of habituation. However, there is no reason to speculate that the increased resistance to capture of these rats could have produced any of the behavioral changes observed in these paradigms, since reactivity to shocks was not different for these rats, and general activity was either not different than, or lower than sham-lesioned controls.

Memory Deficit. Septal lesions produce well documented memory impairments in rats. For instance, septal lesions have been found to impair performance in the radial arm maze task (Miyamoto, Kato, Narumi, & Nagaoka, 1987), Morris water maze task (Kelsey & Landry, 1988), item recognition task (Kesner, Crutcher, & Beers, 1988), fixed-goal task, variable-goal task (Poucet & Herrmann, 1990), and delayed matching task (Dunnett, 1985). Could memory deficits have contributed to the present results?

It seems unlikely that a memory deficit could account for the increased open arm activity on the elevated plus-maze, since the maze does not appear to involve a clear memory requirement. If these rats were impaired in remembering which arm they had previously visited, we would expect to see a random number of entries into any arm. The observed 'preference' for the open arms by septal-lesioned rats in Experiment 3 argues against this hypothesis.

The shock-probe burying paradigm might involve a memory component in that the rats had to remember that they had received a shock, as well as from where they

had received it. If septal-lesioned rats' memory was impaired, we might expect to see an increase in the number of shocks taken by these rats. The findings that these rats passively avoided the shock-probe argues against a memory deficit in the electrolytic septal-lesioned animals, although the increased number of shocks that kainic acid-lesioned took from the probe does not argue against a memory deficit.

Behavioral Disinhibition. Septal-lesioned rats are characterized by their propensity to over-respond in situations which require response inhibition. Impairment of passive avoidance is an example of septal-lesioned rats' behavioral disinhibition. Are the present results simply due to a general 'disinhibition'?

The percentages of open-arm activity displayed by electrolytic-lesioned rats in Experiment 1, 2, and 3, and by kainic acid-lesioned rats in Experiment 3, were significantly higher than those of sham-lesioned controls. This increase in open arm activity may be attributable to a general disruption of passive avoidance, or 'disinhibition'. However, the suppression of the burying response in the shock-probe burying paradigm by septal-lesioned rats does not appear to support a response disinhibition hypothesis, which might predict more burying. Furthermore, with the exception of kainic acid-lesioned rats, rats in Experiments 1, 2 and 3 were clearly able to inhibit shock-probe contacts. If septal lesions simply disinhibited responding, these rats would presumably take more shocks than sham-lesioned controls.

While general 'disinhibition' is not clearly apparent in electrolytic septal-lesioned rats in the shock-probe burying paradigm, the behavioral effects of kainic

acid are not as clear in this test. Kainic acid-lesioned rats behaved quite differently in the shock-probe paradigm than the electrolytic-lesioned rats. While electrolytic-lesioned rats did not take significantly more shocks than sham-lesioned controls, kainic acid-lesioned rats did. Anxiolytic agents are also reported to increase, in a dose-dependent fashion, the number of shocks taken from the probe in this test (Treit, 1990). This increased number of contact-induced shocks received by kainic acid-lesioned rats is probably not attributable to any differences in reactivity toward shocks, since electrolytic- and sham-lesioned rats were not different on this measure. Moreover, the increased number of shocks taken cannot be attributable to a non-specific increase in general activity, since the general activity of kainic acid-lesioned rats was not significantly different than that of electrolytic-lesioned rats, and significantly lower than that of sham-lesioned controls.

The higher number of shocks taken by kainic acid- as compared to electrolytic-lesioned rats can be interpreted in other ways, however. It can be speculated that a more specific septal lesion, such as that produced by kainic acid, which presumably does not destroy fibers of passage, results in a more 'fearless' rat, one that resembles more closely a rat under the influence of anxiolytic agents in this paradigm. It could also be speculated that the kainic acid-lesioned rats' failure to passively avoid the probe may be attributable to damage of extra-septal areas such as the hippocampus (Ben-Ari, Tremblay, Ottersen, & Naquet, 1979). While diazepam was administered during the surgery for kainic acid lesions (in order to minimize extra-septal damage), it may have been insufficient to prevent extra-septal damage

completely. Since the septum has many efferent connections to the hippocampus, and since lesions of the hippocampus have also been found to produce passive avoidance deficits (Boast, Zornetzer, & Hamrick, 1975; Thompson, 1978), it is possible that kainic acid-lesioned rats sustained damage to both the septum and the hippocampus, resulting in an additive effect on passive avoidance behavior. In the absence of histological evidence of hippocampal damage, however, this idea is merely speculative.

Incentive Motivation. Studies have indicated that septal lesions produce increases in incentive motivation. For example, septal-lesioned animals run faster than controls for food and especially sucrose reward (Clody & Carlton, 1969; Henke, 1975), respond at higher rates for rewards in operant situations (Henke, 1979; Hothersall, Johnson, & Collen, 1970), and bar-press at higher rates for food reward (Carlson, & Wielkiewicz, 1972), or for rewarding brain stimulation (Keesey & Powley, 1968). While the elevated plus-maze and the shock-probe burying paradigm are not confounded with food or fluid reward, they may nonetheless involve other types of reward whose incentive value might be increased in septal-lesioned animals. While there hardly appears to be anything rewarding about the shock-probe burying paradigm, exploration of the vast array of visual stimuli present in the plus-maze testing room may have 'rewarding' properties (cf. Widgiz & Beck, in press). If exploratory behavior is rewarding, and perhaps enhanced in septal-lesioned rats, it might explain the observed 'preference' that these rats appear to have for the open

arms in Experiment 3. While it might be possible that open-arm exploration in the plus-maze may be enhanced by an increase in incentive motivation, it would, however, be difficult to explain septal-lesioned rats' reduced burying behavior in terms of an increase in 'incentive motivation'.

Behavioral Sequencing Impairment. There have been reports in the literature that limbic system structures, including the septum, are involved in the sequential control of behavior (Pribram, 1960). Lesions of the septum disturb the sequencing of maternal behaviors (Fleischer & Slotnick, 1978), as well as courtship behaviors in rats (Michal, 1973). However, these studies also revealed that while the pattern of these complex behaviors was disrupted, the individual components of behavior were still present (e.g., decreased successful mounting for male rats despite an increase in pursuit activities toward females). These data suggest that septal-lesioned animals should have no difficulty performing individual behaviors of a complex sequence of behaviors, while their performance of the sequence should be impaired.

While it seems unlikely that an increase in open arm activity on the plus-maze could be a result of a disruption in behavioral sequencing, suppression of a complex, sequenced behavior such as the burying response could certainly lend itself to this type of interpretation. However, septal-lesioned rats did not appear to engage in any of the individual behavioral components of burying (e.g., pushing bedding material with their forepaws or snout), that should have been exhibited if septal-lesioned rats were not less fearful, but simply disrupted in their ability to perform sequenced

behavior.

In summary, while alternative behavioral interpretations of the results of the present experiments are possible, they may not be as parsimonious as an anti-fear interpretation.

Alternative Interpretations: Anatomical

The results of these experiments are at least consistent with the hypothesis that the septum, and more specifically the posterior septum, is involved in the modulation of "anxiety"-related behaviors. However, these behavioral results must be interpreted in light of the limitations of the anatomical procedures employed to investigate this hypothesis. While lesion data have provided much useful information about the involvement of different brain structures in certain functions, lesion data are subject to some limitations. It is difficult to produce a lesion so discrete that it does not interfere in any way with other structures in the vicinity of the lesion, or more distant structures.

Even if it were possible to produce a discrete, restricted lesion, it would not yield conclusive information about the function of a particular brain structure since these structures usually have afferent and efferent connections to other brain areas, in combination with which they may produce their effects. The septum, in this respect, with all of its afferent and efferent connections with other areas of the limbic system, can almost be regarded as a relay station for limbic system information.

The use of neurotoxic-lesion techniques such as kainic acid can improve the specificity of lesions. Since kainic acid and other excitatory amino acids primarily destroy cell bodies leaving fibers of passage intact, behavioral effects of neurotoxic lesions that are comparable to those of electrolytic lesions provide stronger evidence that the structure in question is modulating these behavioral effects. However, this type of lesion technique still cannot provide us with information about the exact role of a structure.

Most behaviors are not controlled by a single brain structure, but by the combination of many brain areas that function together in concert, to produce behavior. Lesioning different areas that work together to produce a behavior might therefore be expected to produce similar results. Emotion-related behaviors such as fear and aggression are assumed to be controlled by the limbic system (Papez, 1937), which is composed of many interconnected structures, including the hippocampus, hypothalamus, habenula, thalamus, cingulate, orbitofrontal, parahippocampal cortex, and the septum. The septum contains many nuclei, each of which have numerous connections with these and other brain regions. The notion that the septum might work in concert with these other limbic system structures to produce their effects is supported by the observation that some of the behavioral effects of septal lesions can also be observed following lesions of other limbic system structures. For instance, hyperreactivity following septal lesions, which is often interpreted as aggressiveness, has also been observed following stimulation of the lateral hypothalamus (Wasman & Flynn, 1962), which has connections to the ventral septum. In addition, the effects

of septal lesions on consummatory behaviors may be attributed to destruction of its connections with the hypothalamus, since the hypothalamus has been implicated in the control of appetite and satiation (Leibowitz, 1980). Anti-fear effects have been observed in the social interaction test, following lesions of the dorsal raphe, which has serotonergic projections to the lateral septal nucleus (File, Hyde, & MacLeod, 1979), as well as on the elevated plus-maze, following neurotoxic lesions of central serotonergic neurons (Briley, Chopin, & Moret, 1990). A reduction in defensive burying has also been observed following decortication in rats (Kolb & Wishaw, 1981), as well as caudate-putamen lesions in mice (Cigrang, Vogel, & Misslin, 1986). Conditioned fear responses in the potentiated startle paradigm were found to be blocked following lesions of the amygdala in rats (Hitchcock & Davis, 1986). Furthermore, many of the behavioral effects of septal lesions in traditional aversive learning paradigms, have also been observed following lesions of the hippocampus (Gray & McNaughton, 1983). In summary, these results underline the importance of viewing the septum as a part of a neural 'system' which controls a variety of behaviors, rather than as a unitary structure with a single, specific function.

Even within the septum, behavioral effects may be anatomically dissociated. The major subdivisions of the septum are the medial and lateral septal nuclei rostral to the fornix, and the triangular septal nucleus, caudal to the fornix. Lesion studies that have attempted to differentiate the behavioral functions of different areas of the septum have generally compared either medial with lateral septal lesions, or anterior with posterior septal lesions. Studies that have compared the effects of medial and

lateral septal lesions on "anxiety"-related behaviors have generali found that lesions of the lateral septal nuclei reduce 'fear', whereas lesions of the lial septal nuclei do not (Hamilton, Kelsey, & Grossman, 1970). The results of the present experiments suggest that the posterior, but not the anterior septum modulates "anxiety"-related behaviors, since lesions of the posterior, but not anterior septum reduced 'fear' reactions. However, the anterior septum is primarily composed of the medial septal nuclei, and the diagonal band of Broca, as well as comprising the anterior portion of the lateral septal nuclei. At the posterior portion of the septum, the medial septal nuclei becomes very small and disappears fairly quickly, behind which the lateral septal nuclei join the midline. Therefore, the posterior portion of the septum is primarily composed of lateral septal nuclei, and the anterior portion of the septum consists primarily of medial septal nuclei. Perhaps the behavioral differences following posterior and anterior septal lesions, found in the present experiments, were actually a reflection of the differential role of lateral and medial septal nuclei in the control of anxiety.

The results of the present experiment are partially consistent with Gray's (1982) Behavioral inhibition hypothesis (i.e., septal lesions disinhibit behavior). In the elevated plus-maze, open-arm avoidance was 'disinhibited' in the septal-lesioned rats. However, this disinhibition of septal-lesioned rats was not observed with respect to the burying response. Burying behavior in septal-lesioned rats was completely suppressed. Furthermore, electrolytic septal-lesioned rats did not show a

'disinhibition' toward the shock-probe, taking no more shocks than sham-lesioned controls. While septal lesions produce a variety of behavioral effects, an anti-fear effect appears to be the most parsimonious interpretation of the results of the present experiments.

References

Beardslee, S.L., Papadakis, E., Fontana, D.J., & Commissaris, R.L. (1990). Anti-panic drug treatments: Failure to exhibit anxiolytic-like effects on defensive burying behavior. Pharmacology, Biochemistry, and Behavior, 35, 451-45.

Beatty, W.W., Beatty, B.A., O'Brian, D.H., Gregoire, K.C., & Dahl, B.L. (1973). Factors underlying deficient passive avoidance behavior by rats with septal lesions. Journal of Comparative and Physiological Psychology, 85, 502-514.

Ben-Ari, Y., Tremblay, E., Ottersen, O.P., & Naquet, R. (1979). Evidence suggesting epileptogenic lesions after kainic acid: pretreatment with diazepam reduces distant but not local brain damage. Brain Research, 165, 362-365.

Bengelloun, W.A., Burright, R.G., Donovick, P.J. (1977). Septal lesions, cue availability, and passive avoidance acquisition by hooded male rats of two ages. Physiology and Behavior, 18, 1033-1037.

Bignami, G., De Acetis, L., & Gatti, G.L. (1971). Facilitation and impairment in avoidance responding by phenobarbital sodium, chlordiazepoxide and diazepam - the role on performance baselines. Pharmacology and Experimental Therapeutics, 176, 725-732.

Blanchard, D.C., Blanchard, R.J., Lee, E.M.C., & Nakamura, S. (1979). Defensive behaviors in rats following septal and septal-amygdala lesions. Journal of Comparative and Physiological Psychology, 93, 378-390.

Blatt, R.C. (1976). Facilitation and non-facilitation of avoidance behavior of rats with septal lesions in the shuttle box and running wheel. Journal of Comparative and Physiological Psychology, 90, 704-713.

Boast, C.A., Zornetzer, S.F., & Hamrick, M.R. (1975). Electrolytic lesions of various hippocampal subfields in the mouse: differential effects on short- and long-term memory. Behavioral Biology, 14, 855-94.

Bolles, R.C. (1970). Species-specific defence reactions and avoidance learning. Psychological Reviews, 77, 32-48.

Brady, J.V., & Nauta, W.J.H. (1955). Subcortical mechanisms in emotional behavior: the duration of affective changes following septal and

habenular lesions in the albino rat. Journal of Comparative and Physiological Psychology, 48, 412-420.

Briley, M., Chopin, P., & Moret, C. (1990). Effect of serotonergic lesion on "anxious" behavior measured in the elevated plus-maze in the rat. Psychopharmacology, 101, 187-189.

Broadhurst, P.L. (1975). The Maudsley reactive and non-reactive strain of rats: A survey. Behavioral Genetics, 5, 299-319.

Bunnell, B.N., & Smith, M.H. (1966). Septal lesions and aggressiveness in the cotton rat. Psychonomic Society, 6, 443-444.

Calhoun, J.B. (1962). [illegible]logy and Sociology of the Norway Rat. Bethesda, MD: U.S. [illegible]artment of Health, Education, and Welfare.

Capell, H., Ginsberg, [illegible] Webster, C.D. (1972). Attenuation of conditioned emotional responding by amphetamine. British Journal of Pharmacology, 45, 525-531.

Caplan M. (1973). An analysis of the effects of septal lesions on negatively reinforced behavior. Behavioral Biology, 9, 129-167.

Carlson, J.G., & Wielkiewick, R.M. (1972). Delay of reinforcement in instrumental discrimination learning in rats. Journal of Comparative and Physiological Psychology, 81, 365-370.

Carlson, N.R. (1970). Two-way avoidance behavior of mice with limbic lesions. Journal of Comparative and Physiological Psychology, 70, 73-78.

Cigrang, M., Vogel, E., & Misslin, R. (1986). Reduction of neophobia in mice following lesions of the caudate-putamen. Physiology and Behavior, 36, 25-28.

Clarke, A., & File, S.E. (1982). Selective neurotoxin lesions of the lateral septum: Changes in social and aggressive behaviours. Pharmacology, Biochemistry, and Behavior, 17, 623-628.

Clody, D.E., & Carlson, P.L. (1969). Behavioral effects of lesions of the medial septum of rats. Journal of Comparative and Physiological Psychology, 67, 344-351.

Craft, R.M., Howard, J.L., & Pollard, G.T. (1988). Conditioned defensive burying as a model for identifying anxiolytics. Pharmacology, Biochemistry, and Behavior, 30, 775-780.

Davidson, A.B. (1970). Facilitory effects of chlordiazepoxide on behavior of rats. Proceedings of the 78th Annual Convention of the American Psychological Association, 807-808.

Dickinson, A. (1975). Suppressive and enhancing effects of footshock on food-reinforced operant responding following septal lesions in rats. Journal of Comparative and Physiological Psychology, 88, 851-861.

Donovick, P.J., Burright, R.G., & Bengelloun, W.A. (1979). The septal region and behavior: An example of the importance of genetic and experiential factors in determining effects of brain damage. Neuroscience and Biobehavioral Reviews, 3, 83-96.

Donovick, P.J., Burright, R.G., & Gittelson, P.L. (1969). Body-weight and food and water consumption in septal lesioned and operated control rats. Psychology Report, 25, 303-310.

Douglas, R.J., & Raphelson, A.C. (1966). Septal lesions and activity. Journal of Comparative and Physiological Psychology, 62, 465-467.

Dunnett, S.B. (1985). Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria-fornix on delayed matching in rats. Psychopharmacology, 87, 357-363.

Estes, W.K., & Skinner, B.F. (1941). Some quantitative properties of anxiety. Journal of Experimental Psychology, 29, 390-400.

Feldon, J., Rawlins, J.N.P., & Gray, J.A. (1982). Discrimination of response-contingent and response-independent shock by rats: effects of medial and lateral septal lesions and chlordiazepoxide. Behavioral Neural Biology, 35, 121-138.

File, S.E., Hyde, J.R.G., & MacLeod, N.K. (1979). 5,7-dihydroxytriptamine lesions of the dorsal and medial raphe nuclei and performance in the Social Interaction test of anxiety and in a home-cage aggression test. Journal of Affective Disorders, 1, 115-122.

Fleischer, S., & Slotnick, B.M. (1978). Disruption of maternal behavior in rats with lesions of the septal area. Physiology and Behavior, 21, 189-200.

Frank, L.H., & Beatty, W.W. (1974). Effects of septal lesions on passive avoidance behavior using ice water as the aversive stimulus. Physiology and Behavior, 12, 321-323.

Fried, P.A. (1969). Effects of septal lesions on conflict resolution in rats. Journal of Comparative and Physiological Psychology, 69, 375-380.

Fried, P.A. (1973). The septum and hyperreactivity: A review. British Journal of Psychology, 64, 267-275.

Garber, E.E., & Simmons, H.J. (1968). Facilitation of two-way avoidance performance by septal lesions in rats. Journal of Comparative and Physiological Psychology, 66, 559-562.

Gluckman, M.I. (1965). Pharmacology of oxazepam (Serax), a new anti-anxiety agent. Current Therapeutic Research, 7, 721-740.

Gray, D.S., Terlecki, L.J., Treit, D., & Pinel, J.P.J. (1981). Effect of septal lesions on conditioned defensive burying. Physiology and Behavior, 27, 1051-1056.

Gray, J.A. (1971). The Psychology of Fear and Stress. London: Weidenfeld and Nicolson.

Gray, J.A. (1979). Emotionality in male and female rodents: a reply to Archer. British Journal of Psychology, 70, 425-440.

Gray, J.A. (1982). The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System. New York: Oxford University Press.

Gray, J.A., & McNaughton, N. (1983). Comparison between the behavioral effects of septal and hippocampal lesions: A review. Neuroscience & Biobehavioral Reviews, 7, 119-188.

Haefely, W., Pieri, L., Polc, P., & Schaffner, R. (1981). General pharmacology and neuropharmacology of benzodiazepine derivatives. In F. Hoffmeister, & G., Stille, Handbook of Experimental Pharmacology, Vol /II. Berlin: Springer-Verlag.

Hamilton, L.W., Kelsey, J.E., & Grossman, S.P. (1970). Variations in behavioral inhibition following different septal lesions in rats. Journal of Comparative and Physiological Psychology, 70, 79-86.

Harvey, J.A., Lints, C.E., Jacobson, L.E., & Hunt, H.F. (1965). Effects of lesions in the septal area on conditioned fear and discriminated instrumental punishment in the albino rat. Journal of Comparative and Physiological Psychology, 59, 37-48.

Henke, P.G. (1975). Septal lesions and the extinction of incentive motivation. Physiology and Behavior, 15, 537-542.

Henke, P.G. (1979). Limbic lesions and the energizing, aversive, and inhibitory effects of nonreward in rats. Canadian Journal of Psychology, 33, 133-140.

Hill, H.E., Pescor, F.T., Belleville, R.E., & Wikler, A. (1957). Use of differential bar-pressing rates of rats for screening analgesic drugs I. Techniques and effects of morphine. Journal of Pharmacology and Experimental Therapy, 120, 388.

Hitchcock, J., & Davis, M. (1986). Lesions of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. Behavioral Neuroscience, 100, 11-22.

Hothersall, D., Johnson, D.A., & Collen, A. (1970). Fixed ratio responding following septal lesions in the rat. Journal of Comparative and Physiological Psychology, 73, 470-476.

Huppert, F.A., & Iversen, S.D. (1975). Response suppression in rats: a comparison of response-contingent and non-contingent punishment, and the effect of the minor tranquilizer, chlordiazepoxide. Psychopharmacologia, 44, 67-75.

Iwahara, S. (1971). Effects of drug-state changes upon two-way shuttle avoidance responses in rats treated with chlordiazepoxide or placebo. Japanese Psychological Research, 13, 207-218.

Jonason, K.R., & Enloe, L.J. (1971). Alterations in social behavior following septal and amygdaloid lesions in rats. Journal of Comparative and Physiological Psychology, 75, 286-301.

Keesey, R.E., & Powley, T.L. (1968). Enhanced lateral hypothalamic reward sensitivity following septal lesions in the rat. Physiology and Behavior, 3, 557-562.

Kelsey, J.E., & Landry, B.A. (1988). Medial septal lesions disrupt spatial mapping ability in rats. Behavioral Neuroscience, 102, 289-293.

Kesner, R.P., Crutcher, K., & Beers, D.R. (1988). Serial position curves for item (spatial location) information: role of the dorsal hippocampal formation and medial septum. Brain Research, 454, 219-226.

Kolb, B., & Whishaw, I.Q. (1981). Decortication of rats in infancy or adulthood produced comparable functional losses on learned and species-typical behaviors. Journal of Comparative and Physiological Psychology, 95, 468-483.

Kumar, R. (1971). Extinction of fear. II. Effects of chlordiazepoxide and chlorpromazine on fear and exploratory behavior in rats. Psychopharmacologia, 19, 61-66.

Lauener, H. (1963). Conditioned suppression in rats and the effects of [illegible]logical agents theron. Psychopharmacologia, 19, 163-187.

Lee, E.H.Y., Lin, Y.P., & Yin, T.H. (1988). Effects of lateral and medial septal lesions on various activity and reactivity measures in the rat. Physiology and Behavior, 42, 97-102.

Leibowitz, S.F. (1980). Neurochemical system of the hypothalamus: Control of feeding and drinking behavior and water electrolyte secretion. In P.J. Morgane and J. Panksepp, Handbook of the Hypothalamus, Vol 3, Part A, New York: Marcel Dekker.

Lints, C.E., & Harvey, J.A. (1969). Alterred sensitivity to footshock and decreased brain content of serotonin following brain lesions in the rat. Journal of Comparative and Physiological Psychology, 67, 23-32.

Lubar, J.F., Schaefer, C.F., & Wells, D.G. (1969). The role of the septal area in the regulation of water intake and associated motivational behavior. Annals of the New York Academy of Science, 157, 875-893.

McDaniel, J.R., Donovick, P.J., Burright R.G., & Fanelli, R.J. (1980). Genetics, septal lesions and avoidance behavior in mice. Behavioral Neural Biology, 28, 285-299.

McMillan, D.E., & Leander, J.D. (1976). Effects of drugs on schedule controlled behavior. In S.D. Glick, & J. Goldfarb (Eds.), Behavioral Pharmacology, Saint-Louis: Mosby.

Michal, E.K. (1973). Effects of limbic lesions on behavior sequences and courtship behavior of male rats. Behaviour, 44, 264-285.

Miczek, K.A. (1973). Effects of scopolamine, amphetamine and benzodiazepines on conditioned suppression. Pharmacology, Biochemistry, and Behavior, 1, 401-411.

Miyamoto, M., Kato, J., Narumi, S., & Nagaoka, A. (1987). Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Research, 419, 19-31.

Morgan, J.M., & Mitchekk, J.C. (1969). Septal lesions enhance delay of responding on a free operant avoidance schedule. Psychonomic Science, 16, 10-11.

Morrison, C.F., & Stephensen, J.A. (1973). Effects of stimulants on observed behavior of rats on six operant schedules. Neuropharmacology, 12, 297-310.

Myhrer, T. (1989). Exploratory behavior and reaction to novelty in rats: Effects of medial and lateral septal lesions. Behavioral Neuroscience, 103, 1226-1233.

Nielson, H.C., McIver, A.H., & Boswell, R.S. (1965). Effects of septal lesions on learning, emotionality, activity, and exploratory behavior in rats. Experimental Neurology, 11, 147-157.

O'Kelly, L.I. (1963). The psychophysiology of motivation. Annual Review of Psychology, 14, 57-92.

Oishi, H., Iwahara, S., Yang, K.-M., & Yogi, A. (1972). Effects of chlordiazepoxide on avoidance response in rats. Psychopharmacologia, 23, 373-385.

Owings, D.H., & Coss, R.G. (1978). Snake mobbing by California ground squirrels: Adaptive variation and ontogeny. Behavior, 62, 50-69.

Papez, J.W. (1937). A proposed mechanism of emotion. Archives of Neurological Psychiatry, 38, 725-743.

Paxinos, G., & Watson, C. (1986). The Rat Brain in Stereotaxic Coordinates (second edition). New York: Academic Press.

Pellow, S. (1986). Anxiolytic and anxiogenic drug effects in a novel test of anxiety: Are exploratory models of anxiety in rodents valid? Methods & Findings in Experimental & Clinical Pharmacology, 8, 557-65.

Pellow, S., Chopin, P., File, S.E., & Briley, M. (1985). Validation of open:closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. Journal of Neuroscience Methods, 14, 149-167.

Pellow, S., & File, S.E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in the elevated plus-maze: a novel test of anxiety in the rat. Pharmacology, Biochemistry, and Behavior, 24, 525-529.

Pinel, J.P.J., Symons, L.A., Christensen, B.K., & Tees R.C. (1989). Development of defensive burying in Rattus norvegicus: Experience and defensive responses. Journal of Comparative Psychology, 103, 359-365.

Pinel, J.P.J., Gorzalka, B.B., & Ladak, F. (1981). Cadaverine and putrescine initiate the burial of dead conspecifics by rats. Physiology and Behavior, 27, 819-824.

Pinel, J.P.J., & Treit, D. (1978). Burying as a defensive response in rats. Journal of Comparative and Physiological Psychology, 92, 708-712.

Pinel, J.P.J., & Treit, D. (1979). Conditioned defensive burying in rats: Availability of burying materials. Animal Learning and Behavior, 8, 447-451.

Poplawsky, A. (1978). Long term maintenance of shuttlebox avoidance behavior before and after septal lesions. Physiological Psychology, 6, 294-299.

Poplawsky, A., & Johnson, D.A. (1973). Open-field social behavior of rats following lateral or medial septal lesions. Physiology and Behavior, 11, 845-854.

Poucet, B., & Herrmann, T. (1990). Septum and medial frontal cortex contribution to spatial problem-solving. Behavioral Brain Research, 37, 269-280.

Pribram, K.H. (1960). A review of theory in physiological psychology. Annual Review of Psychology, 11, 1-40.

Rawlins, J.N.P., Feldon, J., & Gray, J.A. (1980). Discrimination of response-contingent and response-independent shock by rats: effects of chlordiazepoxide HCl and sodium amylobarbitone. Quarterly Journal of Experimental Psychology, 32, 215-232.

Robichaud, R.C., Sledge, K.L., Hafner, M.A., & Goldberg, M.E. (1973). Propranolol and chlordiazepoxide on experimentally induced conflict and shuttle box performance in rats. Psychopharmacologia, 32, 157-160.

Ross, J.F., Grossman, L., & Grossman, S.P. (1975). Some behavioral effects of transecting ventral or dorsal fiber connections of the septum in rats. Journal of Comparative and Physiological Psychology, 89, 523-536.

Sagvolden, T. (1976). Free-operant avoidance behavior in rats with lateral septal lesions: effect of shock intensity. Brain Research, 110, 559-574.

Sansone, M., Renzi, P., & Amposta, B. (1972). Effects of chlorpromazine and chlordiazepoxide on discriminated lever-press avoidance behavior and intertrial responding in mice. Psychopharmacologia, 27, 313-318.

Seigel, A., & Skog, D. (1970). Effects of electrical stimulation of the septum upon attack behavior elicited from the hypothalamus in the cat. Brain Research, 23, 371-380.

Simon, P., & Soubrie, P. (1979). Behavioral studies to differentiate anxiolytics and sedative activities of the tranquilizing drugs. In J.R. Boissier (Ed.), Differential Pharmacology of Anxiolytics and Sedatives: Modern Problems of Pharmacopsychiatry, Vol 14. Basel; Karger.

Sodetz, F.J. (1970). Septal ablation and free-operant avoidance behavior in the rat. Physiology and Behavior, 55, 773-777.

Sodetz, F.J. (1972). Sidman avoidance performance and response suppression in rats with septal lesions. Journal of Comparative and Physiological Psychology, 79, 142-150.

Soubrie, P., de Angelis, L., Simon, P., & Boissier, J.R. (1976). Effets des anxiolytiques sur la prise de boisson en situation nouvelle et familiere. Psychopharmacologia, 50, 41-45.

Soubrie, P., Kulkarmi, S., Simon, P., & Boissier, J.R. (1975). Effets des anxiolytiques sur la prise de nourriture de rats et de souris places en situation nouvelle ou familiere. Psychopharmacologia, 45, 203-210.

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

Swanson, L.W., & Cowan, W.M. (1979). The connections of the septal region in the rat. Journal of Comparative Neurology, 186, 621-656.

Terlecki, L.J., Pinel, J.P.J., & Treit, D. (1979). Conditioned and unconditioned defensive burying in the rat. Learning and Motivation, 10, 337-350.

Thomas, E. (1988). Forebrain mechanisms in the relief of fear: The role of the lateral septum. Psychobiology, 16, 36-44.

Thomas, G.J., Moore, R.Y., Harvey, J.A., & Hunt, H.F. (1959). Relations between the behavioral syndrome produced by lesions in the septal region of the forebrain and maze learning of the rat. Journal of Comparative and Physiological Psychology, 52, 527-532.

Thomas, J.B., & McCleary, R.A. (1974). One-way avoidance behavior and septal lesions in the rat. Journal of Comparative and Physiological Psychology, 86, 751-759.

Thomas, J.B., & Thomas, K.A. (1972). Running-wheel avoidance behavior following septal area lesions in the rat. Journal of Comparative and Physiological Psychology, 81, 143-148.

Thompson, R. (1978). Localization of a 'passive avoidance memory system' in the white rat. Physiological Psychology, 6, 263-274.

Trafton, C.L. (1967). Effects of lesions in the septal area and cingulate cortical areas on conditioned suppression of activity and avoidance behavior in rats. Journal of Comparative and Physiological Psychology, 63, 191-197.

Treit, D. (1985). Animal models for the study of anti-anxiety agents: A review. Neuroscience & Biobehavioral Reviews, 9, 203-222.

Treit, D. (1987). Ro 15-1788, CGS 8216, picrotoxin, and pentylenetetrazole: Do they antagonize anxiolytic drug effects through anxiogenic action? Brain Research Bulletin, 19, 401-405.

Treit, D. (1990). A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics. Pharmacology, Biochemistry, and Behavior, 36, 203-205.

Treit, D., Pinel, J.P.J., & Fibiger, H.C. (1981). Conditioned defensive burying: A new paradigm for the study of anxiolytic agents. Pharmacology, Biochemistry, & Behavior, 15, 619-626.

Treit, D., Pinel, J.P.J., & Fibiger, H.C. (1982). The inhibitory effect of diazepam on defensive burying is reversed by picrotoxin. Pharmacology, Biochemistry, & Behavior, 17, 359-361.

Tsuda, A., Ida, Y., Nishimura, H., & Tanaka, M. (1989). Anxiogenic effects of B-CCE as measured in two different conditioning paradigms. Psychobiology, 17, 202-206.

Tsuda, A., Yoshishige, I., & Tanaka, M. (1988). The contrasting effects of diazepam and yohimbine on conditioned defensive burying in rats. Psychobiology, 16, 213-217.

Waddington, J.L., & Olley, J.E. (1977). Dissociation of the anti-punishment activities of chlordiazepoxide and atropine using two heterogeneous passive avoidance tasks. Psychopharmacology, 51, 93-96.

Wasman, M., & Flynn, J.P. (1962). Directed attack elicited from the hypothalamus. Archives of Neurology, 6, 220-227.

Widgiz, S.L.M., & Beck, C.H.M. (in press). Diazepam effects on behavior in an elevated runway: Evidence for reward-like effects of benzodiazepines. Behavioral Brain Research.

Wilkie, D.M., MacLennan, A.J., & Pinel, J.P.J. (1979). Rat defensive behavior: burying noxious food. Journal of the Experimental Analysis of Behavior, 31, 299-306.

Zucker, I. (1965). Effect of lesions of the septal-limbic area on the behavior of cats. Journal of Comparative and Physiological Psychology, 60, 344-352.

APPENDIX

Hypotheses of Septal Function

Author	Hypothesis
Caplan (1973)	The septum is part of the neural substrate for mediation and expression of behaviors' emotional or motivational component
Dickinson (1974)	The septum functions in a response suppression system sensitive primarily to aversive stimuli
Donovick, Burright & Bengelloun (1979)	The septal region plays an important role in modulating hypothalamic function, by integrating the organism's need state signals with environmental influences and past experience
Gray (1982)	Inputs such as signals of punishment, signals of non-reward, or novelty trigger the behavioral inhibition system to produce either behavioral inhibition, an increment in arousal, and/or increased attention. This system is dependent at least in part on the septo-hippocampal system
Miyamoto, Kato Narumi, & Nagaoka (1987)	The septohippocampal cholinergic mechanism plays an important role in spatial memory
Myhrer (1989)	The hippocampal function integrates septal and entorhinal inputs permitting it to respond differentially to sensory stimuli. This "matching" system functions to discriminate the degree of novelty or familiarity of any sensory system
O'Kelly (1963)	The septum serves as a comparative/integrative mechanism which weighs shifting evidence reflecting its internal and external environment in light of its own capabilities and past experience
Papez (1937)	The septum serves as a relay station in the circuitry of "emotion" or "affect"
Siegel & Skog (1970)	The septum plays a role in the regulation of aggressive behavior by inhibiting the lateral hypothalamic nucleus

Hypotheses of Septal Function (continued)

Sutherland, Kolb Whishaw (1982)	The frontal cortex and the septo-hippocampal system form a functionally integrated system for learning and using spatial representation of aspects of the environment
Thomas (1988)	The septal region is a central link in a major forebrain inhibition system. The septal inhibition suppresses aversive emotional states. Lateral septal region mediates relief from fear or anxiety, and is an important structure in providing goal direction for aversively motivated behavior reinforced by relief from fear or anxiety
Vinogradova (1975)	The septo-hippocampal-entorhinal system mediates habituation to environmental stimuli, that is "matching" input signals to determine their novelty or their presence in memory stores.