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AN INVESTIGATION OF A POSSIBLE SITE OF ACTION
OF CHLORPROMAZINE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

JACOB ROTHWACKS RAHLT, B.S., M.Sc.

The Ohio State University

1960

Approved by:

[Signature]
Adviser
Department of Pharmacy
DEDICATION

To those who have believed in me—to those who have aided me to attain the Degree Doctor of Philosophy—to my teachers for untiring aid—to my colleagues for encouragement—to my friends for advice and enlightenment—to my wife and daughter for inspiration—this is dedicated.
"Our censure should be reserved for those who will close all doors but one. The surest way to lose truth is to pretend that one already wholly possesses it."

Gordon W. Allport
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INTRODUCTION

Part 1

The brain is a mass of correlation, a storehouse of potential, a myriad of unknowns. Areas which are in control of a function are in themselves under the influence of other areas.

It is reasonable to assume that a specific function is under direct control of a chain of impulses, or a chain of events which result from these impulses (functional localization), and that by breaking this chain at any of its connections, the function can be obliterated. Why then will destruction of specific cranial area "X" destroy the single function "X_a"?

An assumption must be made, on the basis of what is known and what is to be hypothesized. If nerve fibers A, B, C and D all pass through a specific locale, "X," then destruction of "X" will remove the functions of nerve fibers A, B, C, and D. With the knowledge that these fibers are involved in the function "X_a," it is apparent that this function will be removed or, at best, impaired.

It is possible that area "X" may contain only fibers with the function "X_a." In this case it would
be shown that area "X" is an area with localization of function, while being an integral portion of a chain possessing functional localization.

Anatomically, the theory of localization of function is sound, and physiologically the theory of functional localization is sound. With a basic, seemingly rational compromise theory, agreement may be reached that these theories are mutually compatible rather than mutually antagonistic.

The occurrence of a "release phenomenon" will be basically described in this dissertation. The explanation of a release phenomena may be

1) the destruction of an area specifically involved (localization of function);

2) the recovery through re-routing of impulses (functional localization).

Again, the theories are compatible, for re-routing of impulses to correct a functional disorder is dependent on the absence of a single controlling area which may have been destroyed.

Part 2

The cerebral cortex controls most of the conscious activities of the human. Herrick (1) in 1929 said that "the cerebral cortex is the dominant part"...of the somatic nervous system. "It is an overlord that governs the somatic nervous system and...our adjustments to the
environment and the people in it." The cerebral cortex as an overlord controls many functions attributable only to the higher animals, among which are the ability to reason and to control reflexes. As an overlord the cerebral cortex is subject to the same internal stress and strain that any ruler would have. In order to dominate, the cerebrum must control the sub-cortical regions, and it is in these areas that many psychic troubles are localized and initiated.

The cerebral cortex has been phylogenetically derived over a period of time which history is unable to record. Through this time the neo-cortex, the highest center, developed, and possession of the neo-cortex has made man the most formidable intellectual animal on earth.

This paper concerns itself with some of the "baser" functions which are often suppressed by the neo-cortex by presenting a treatment of the activity of the septal nuclei of the forebrain, the temporal amygdaloid complex, and the effect of chlorpromazine on them. Through this investigation, an attempt has been made to localize the site of action of chlorpromazine, and to correlate the theories of "functional localization," and "localization of function."
LITERATURE SURVEY

As early as 1898 the area of the brain with which this research is concerned had been recognized as highly important (2). Jackson (2) recognized that the temporal area (uncinate gyrus) performed a function in stimulating the organism, which when disrupted causes an epileptic automatism.

The major portion of the research that has been done in the rhinencephalic area (limbic lobe of Broca (3)) has occurred since the publication of a paper by Papez (4) in which an important theory was propounded. Prior to this paper, it had been commonly believed that the rhinencephalon dealt with some phase of olfactory function, despite the publication of a paper modifying this belief. Herrick (5) in 1933 had said that the rhinencephalon in addition to some function in olfaction, served as a non-specific activator for all cortical activities influencing memory, learning, and affective behavior. Papez (4) felt that there was no evidence to support the belief of rhinencephalic control of the olfactory function.

In his "A Proposed Mechanism of Emotion," Papez advanced the theory "that the hypothalamus, the anterior
thalamic nuclei, the gyrus cinguli, the hippocampus and their connections constitute a harmonious mechanism which may elaborate the functions of central emotion, as well as participate in emotional expression."

"The central emotive process of cortical origin may...be conceived as being built up in the hippocampal formation, and as being transferred to the mammillary body and thence through the anterior thalamic nuclei to the cortex of the gyrus cinguli. The cortex of the cingular gyrus may be looked on as the receptive region for the experiencing of emotion." Papez went on to describe degrees of emotion by theorizing that...

"Radiation of the emotive process from the gyrus cinguli to other regions in the cerebral cortex would add emotional colouring to psychic processes."

The Papez theory spurred investigations dealing with many of the major structures of the limbic lobe. In addition to the two areas that the present author is investigating, the forebrain septal nuclei and the temporal amygdaloid complex, many other areas have been explored. These areas are diagrammatically summarized by MacLean (6), and include the structures lying in the brain stem, and those between the subcallosal striae and the hippocampal gyrus, caudad from the frontal lobe, laterad from the temporal lobes, and as far caudad as the mesencephalon. These structures are the (a) corpus callosum,
(b) fornix, (c) stria medullaris, (d) stria terminalis, (e) medial forebrain bundle, (f) dentate gyrus, (g) hippocampus, (h) mammillary body, (i) diagonal band of Broca, (j) anterior thalamic nucleus, (k) interpeduncular nucleus, (l) habenula, (m) basal ganglia and possibly the medial olfactory stria, the lateral olfactory stria, the olfactory tubercle and the olfactory bulb. The preceding areas together constitute the main subcortical structures and/or connections of the rhinencephalon (limbic lobe), and as this paper continues, many of these areas will be implicated in functional and/or anatomical relationship to the amygdaloid body and septal nuclei.

A. The Septal Nuclei

The septal area as defined anatomically, is a region extending from the bed of the anterior commissure (which passes transversely through the septum) and descending columns of the fornix (which descends vertically through the septum) forward to the caudal boundary of the anterior olfactory nucleus, including the subcallosal gyrus and parolfactory area (7). It is laterally bounded by the anterior horns of the lateral ventricle, ventrally by the olfactory tubercle and dorsally by the corpus callosum. At various times this area has
been called the "parolfactory area" (8), the "corpus precommissurale" (9) and the "corpus paraterminale" (10).

The septal region is divided into medial and lateral nuclear groups, which although anatomically related, appear to possess different functions. The medial group of nuclei has been considered to generally possess afferent or receptor functions and the lateral group to possess efferent or emitter functions (11). Both groups of nuclei probably have mixed functions, with one being predominant in each group. The medial septal nucleus is located mainly in the precommissural septum, and its anterior cells merge with those of the anterior hippocampus. The lateral septal nucleus is the largest nuclear mass of the septal region. It borders the medial wall of the lateral ventricle, surrounding the pre- and post-commissural fornix fibers. It reaches from the olfactory tubercle to the membrane of the septum pellucidum (septum lucidum).

Neuronal Pathways of the Septal Area

The septal area receives afferent fibers from various sources in the brain. The hippocampus in particular (12, 13) projects fibers directly to the lateral septal nucleus through the column of the fornix. Both the lateral and medial nuclei of the septum will demonstrate degeneration on sectioning the fornix (14).
The septal area is innervated by radiations from the stria terminalis, a major efferent pathway of the amygdaloid complex, and obtains specific projections to the medial septal nucleus (15). There also seem to be direct connections between the amygdaloid and the lateral basal septum through the stria terminalis, as well as secondary and tertiary connections (16, 17, 18). The medial olfactory stria innervates the septal area with impulses from the olfactory tubercle (19) and olfactory bulb (20). The diagonal band of Broca functions partly as a secondary efferent pathway from the amygdaloid to the septal nuclei (7, 18), and is a portion of what is known as the Radiation of Zuckerkandl (19). The medial forebrain bundle sends fibers both to the septal area and the amygdaloid complex (7), while the septal area receives innervation from the frontal lobe (21, 22, 23, 24, 25, 26), and from the globus pallidus (27) to form part of the olfacto-somato-visceral circuit.

The septal nuclei transmit efferent impulses to cortical and subcortical structures. It has been postulated (28, 29, 30) that the septal nuclei act as a "way-station" for both ascending and descending impulses, the medial nucleus transmitting ascending impulses, and the lateral nucleus transmitting descending impulses. It has also been suggested (18) that the
hippocampus may be activated by stimulation of the amygdala by way of the septal area corticopetal fornix fibers.

The diagonal band of Broca is a septal-amygdaloid connection composed of a cellular continuity between the medial septal nucleus and the anterior amygdaloid area (27). Therefore, the septal-amygdaloid circuit is from amygdaloid to septal area via the stria terminalis, and from the septal to amygdaloid nucleus via the diagonal band of Broca.

The lateral septal nucleus transmits hippocampal and hypothalamic impulses to the nucleus accumbens and the caudate nucleus-putamen complex. The globus pallidus, which is in direct connection with the caudate (31), discharges into the medial septal nucleus, providing for the olfacto-somato-visceral circuit. Through the amygdaloid complex and the pyriform lobe, the septal area may also receive modified olfactory impulses.

The septal nucleus is also in efferent connection with the habenula (32) and the hypothalamus. The latter connection is mainly via the medial forebrain bundle (11, 32). There are also direct connections with the mammillary bodies (29), mesencephalic interpeduncular nucleus and pontine nuclear complex (32). The pontine complex has been identified as a region which is important in diffuse cortical activation (33), thus indirectly implicating
the septal nuclei, amygdaloid complex and medial forebrain bundle in the integration of conscious behavior.

Descriptions have also been given of efferent septal connections with the olfactory tubercle and the lateral olfactory areas (19, 30, 34). More important are its connections with the preoptic nuclei (35) involving the septum in control of pituitary function via the tractus preoptico hypophysis (36), the neuro-secretory pathway.

There are also septal connections with the thalamus (37) passing from the anterior septum to the anterior thalamic nucleus, and medial gray of the thalamus.

B. The Amygdaloid Complex

A major portion of the discussion of the anatomy of the amygdaloid nuclei has been taken from the standard reference by Ranson and Clark (38). All other references have been cited.

The amygdaloid body is a nuclear group which lies on the floor of the tip of the inferior horn of the lateral ventricle. (In higher mammals it occupies the rostral wall.) The amygdaloid body is continuous with the temporal lobe of the cortex (uncus, (uncinate gyrus),) anterior to the anterior perforate substance.

The amygdala is in close anatomical relationship with the rostral end of the tail of the caudate nucleus.
Therefore, it is anatomically related to the lentiform (putamen and globus pallidus) body. (The lentiform body plus the caudate nucleus compose the corpus striatum.)

The amygdaloid lies ventral to the globus pallidus, and is separated from it by the anterior commissure (commissure anterior cerebri). The claustrum is in anatomical relationship with the anterior pole of the amygdaloid body.

The amygdaloid nuclei are subdivided into a primitive and a more recently acquired portion. The phylogenetically older, more primitive divisions are the central, medial and cortical nuclei, plus a nucleus of the lateral olfactory tract. The nuclei of more recent phylogenetic origin are the lateral, basal and the accessory basal nuclei.

Neuronal Pathways of the Amygdaloid Complex

The amygdaloid complex may be grossly divided into two major portions (39, 40), the cortico-medial group and the baso-lateral group of nuclei (41). Both anatomical and physiological studies have shown that the cortico-medial (phylogenetically older) groups of nuclei are closely related to the olfactory function (42). The baso-lateral group does not appear to be specifically related to olfaction, and is more likely concerned with other amygdaloid effects. For this reason, attention
will be focused mainly on the neuronal pathways of the baso-lateral groups of nuclei.

General agreement among anatomists represents the stria terminalis as the main efferent amygdaloid pathway. This pathway has been described by various workers using a variety of animals and techniques (12, 15, 27, 39). With the exception of the inter-connecting commissural component of the bilateral lateral olfactory tracts, all components of the stria terminalis are efferent projection pathways. These pathways communicate with the telencephalic sub-cortical structures, and the phylogenetically older diencephalon (12, 19, 27, 39, 41, 43). The fibers of the stria terminalis end in the septal nuclei, nucleus accumbens, preoptic area, habenula and the anterior hypothalamus. The fibers may run as far caudal as the hypothalamic ventromedial nucleus (44), and possibly as far caudal as the pre-mammillary nuclei (7).

There are alternate pathways which may by-pass the stria terminalis. There is an amygdalo-hypothalamic tract connecting the cortico-medial nuclei of the amygdala to the hypothalamus (34, 45). There are also fibers running diffusely from the amygdaloid to the hypothalamus which may join the medial forebrain bundle (39). Other fibers run in the anterior commissure to the preoptic nuclei (41).
A large number of fibers originating in the baso-lateral nuclei do not contribute to the stria terminalis (12, 27). These fibers seem to run into the longitudinal association bundle which runs toward the basal portion of the telencephalon medium. This bundle may merge with the diagonal band of Broca (39, 43) or may continue and reach the nucleus accumbens (18). Other preparations indicate that the longitudinal association bundle joins the medial forebrain bundle and has fibers ending in the preoptic area (27).

Gloor (18) states that the lateral amygdaloid nucleus contributes no fibers to the stria terminalis, while the basal nucleus contributes only a small number. It has also been suggested (7) that the diagonal band of Broca carries amygdaloid impulses to the pyriform cortex, olfactory tubercle and septal nuclei.

There are fibers originating in the baso-lateral amygdala which innervate the entopeduncular nucleus (27), and fibers of the dorso-medial amygdala innervating the pulvinar and posterior lateral nucleus of the thalamus (46, 47).

Amygdalo-mesencephalic connections have not been demonstrated in the mammalian brain, although they have been shown in the avian brain (archi-striato-mesencephalic system) (48, 49). It has also been shown that the avian stria terminalis reaches the mesencephalon,
and even the rhombencephalon (50, 51). Pathways similar to these also may appear in the reptilian brain (7).

It is possible that a tract physiologically delineated by Wall and Davis (52) may carry amygdaloid fibers to the brain stem (18).

Lastly, there is an intra-amygdaloid association system (18) which relays impulses within the various amygdaloid nuclei. These pathways may provide outlets for nuclei which do not demonstrate direct neuronal pathways. They may also provide a means of integrating an impulse throughout the entire extent of the amygdaloid complex.

C. Possible Function of the Septal Nuclei and Amygdaloid Complex

Approximately forty years after Jackson (2) recognized the importance of temporal function in epileptic automatism, Jasper (53) arrived at a similar conclusion. With the use of electrographic techniques, deep probes of the temporal area were made by Jasper and other workers (54, 55, 56). These investigations implicated the rhinencephalic formations as the possible locus of discharge causing psychomotor seizures. Later investigations (57) suggested that the discharge originated in the region of the amygdaloid complex, and that the amygdaloid complex "stores" most of the potential signs of psychomotor symptomology (58).
The evidence that has been presented to support the theory that the amygdaloid complex may be the site of psychomotor seizures led this author to the hypothesis that the amygdaloid may be the site of action of one or more of the drugs acting to depress the central nervous system. The action of some of the tranquilizers, and chlorpromazine in particular, on the amygdaloid has been the subject of conflicting reports.

In 1956 (59) it was reported that chlorpromazine will stimulate the amygdaloid body, with the resultant action being tranquilization. This report has been variously contradicted by the works of other authors (60, 61, 62, 63) who have shown that the amygdaloid complex is stimulatory in nature. Ablation of the amygdala will cause profound depression. In face of the evidence pointing to a stimulatory function of the amygdala, it seems unlikely that chlorpromazine stimulates the amygdaloid complex to cause tranquilization, as suggested by Preston (59). In fact, MacLean in 1958 (64) stated that no localization of chlorpromazine activity on the amygdaloid complex could be demonstrated by means of electro-encephalographic changes.

It was suggested by Olds and Travis (65) that chlorpromazine acts against a forebrain system, while leaving intact a midbrain system. This suggestion is compatible with the hypothesis that chlorpromazine
activity is localized within the limbic lobe, and that chlorpromazine activity may be on the amygdaloid complex.

King and Meyer (66) have shown that septal destruction will counteract any depression caused by amygdaloid ablation. Conversely, they demonstrated that amygdaloid ablation will reduce the hyperactivity normally associated with septal ablation. It then seems likely that the amygdaloid complex and septal nuclei are in functional balance with one another.

A marked increase in emotional behavior of animals in which the septal region of the forebrain has been experimentally ablated has been reported (66, 67, 68). This hyper-emotionality is manifested by violent attack or flight reactions in response to previously neutral or innocuous stimuli. Therefore, it is possible that the septal nuclei possess an important function in the "physiological tranquilization" of rats. Destruction of the septal area may result in an excited animal because the septal nuclei normally counterbalance a stimulatory area. King and Meyer (66) suggest that this latter area is the amygdaloid complex.

In 1957, Hunt (69) demonstrated that chlorpromazine will cause an activity shift in the opposite direction to that produced by septal destruction. This activity of chlorpromazine led the author to hypothesize that chlorpromazine activity is on the amygdaloid complex.
D. **Purpose**

A study of the activity of chlorpromazine in animals with surgically destroyed septal nuclei, amygdaloid nuclei and septal-amygdaloid nuclei was made with a view to testing the hypothesis that the septal and amygdaloid nuclei functionally counter balance each other. To determine whether the amygdaloid complex may be the site of action of chlorpromazine, the extent of the drug's activity on operant animals was determined and compared with the effect of chlorpromazine on normal and operant control animals.
EXPERIMENTAL

Basic Techniques

1. Animals

In January of 1959 four male rats of the Lashley strain were crossed with twelve female rats of the Sprague-Dawley strain. In early May of 1959 the animals of the first generation of this cross that possessed the hooded features of the Lashley strain were again bred. By mid-August of 1959, there was a sufficient number of hooded animals of the second generation of this cross available for experimental use. These animals were then compared to their albino and hooded forebears as to reaction to handling and their speed of acquiring conditioned responses.

The Sprague-Dawley albino animals that had been used had been bred from albino animals received from the Maxfield Animal farms. The Lashley strain hooded animals had been bred for several generations at the Ohio State University Research Center, Department of Physiological Psychology.

It was determined that the hybrid animals were somewhat more active than the albino animals, although
there was very little difference in the docility of the animals, but slightly less active than the pure Lashley animals. They acquired responses as rapidly as the Lashley animals, and much more rapidly than the albino rats.

During the initial operations performed, it was found that the hybrid group of animals had a much higher survival rate than the albino animals, although not quite as great as the hooded rats. Based upon results found during previous research, the hybrid animals were placed in an aquarium and forced to swim. The same was done with albino animals of equivalent weight. It was found that the hybrid animals could initially swim more than twice as long as the albino animals.

For the aforementioned reasons, the hybrid group of animals were chosen as the experimental animals for this procedure, and will henceforth be referred to as the LSD#2 animals.¹

The animals were housed in community cages, approximately 18 inches long, 8 inches wide and 8 inches deep. Between six and eight animals were kept in each cage until the experimentation was begun. At this time, all animals were separated and kept in individual

¹LSD#2 represents Lashley-Sprague Dawley rats of the Second Generation.
cages. The feed was given ad lib (Purina Lab Chow), and the animals were permitted to drink as much tap water as they desired.

An equal number of animals of both sexes between the ages of 60 and 90 days, weighing between 120 to 175 grams at the beginning of the experiments were used.

All animals used in the experimental procedure, both operant and non-operant were housed in a room separate from the community animal room. In this manner, they were all subject to equivalent environmental conditions, and were shielded from all outside influences. Prior to the experimental procedure, all animals were lightly anesthetized and were numbered by holes punched in their ears.

2. Preparation of Electrodes

The electrolytic current that was used to destroy the localized areas in the brain was passed through a stainless steel electrode. This electrode consisted of a stainless steel sewing needle which was electrically insulated except for 3/4 mm. at the tip. The electrical insulation was an epoxy resin (The Epoxylite Company) and was applied to the electrode in the following manner:

1. The needles were scrubbed in acetone and absolute alcohol using cotton as the abrasive;
2. The cleaned needles were then dipped slowly and evenly into the epoxy resin;

3. The needles were then baked in a dry air oven for one hour at a temperature of 75°C;

4. The temperature of the oven was then raised rapidly to 175°C, and the electrodes baked for a minimum of 4 hours;

5. Steps 2, 3, and 4 were then repeated four or five times;

6. The electrodes were then placed in saline solution, and an electric current passed through them to test for any leaks;

7. Before being inserted into the brain, 3/4 mm. of the insulating material was shaved from the tip of the electrode.

The electrodes that were prepared were found to be adequate for nine or ten operations. Therefore, one dozen electrodes were prepared simultaneously.

3. Method of Stereotaxic Operation (71)²

The instrument that was used to place the prepared electrode was a stereotaxic device manufactured by the Baltimore Instrument Company. The lesion maker which

²Fenestration Device by the Foredom Electric Company consists of Model 1, Series EE, Handpiece #5D with Floor Rheostat. The Lesion Maker of the Grass Instrument Company is model LM-1.
delivered the electrolytic current was manufactured by
the Grass Instrument Company (see picture on page 23).
The operation was performed in the following manner:

1. The animal was weighed, anesthetized\(^3\) and
given atropine sulfate;

2. The head of the animal was shaved from the
base of the neck forward to the snout;

3. The area of the incision was cleaned with
70% ethyl alcohol;

4. A medial incision was made, extending from
the rostral portion of the nose caudad to a position
slightly posterior to the ears;

5. Using blunt dissection the connective tissue
of the scalp was removed and the skin retracted;

6. The head muscle was removed by cutting and
scraping, and the head washed with sterile saline
solution;

7. The ears were clipped, and the cartilage
removed;

8. The teeth were fixed on the mouth bar;

9. The ears were equilaterally fixed by the ear
bars;

10. The mouth bar was pushed forward to tighten
the teeth;

\(^3\)The anesthetic will be described following this
method.
FIGURE 1

PHOTOGRAPH OF THE STEREOTAXIC DEVICE AND THE LESION MAKER USED IN THE STEREOTAXIC OPERATION
11. The horizontal position of the head was then checked and assured;

12. The nose bar was then clamped on;

13. The skin was then clamped back with hemostats, if necessary;

14. Epinephrine (1:1000) was then applied to the skull to prevent surface oozing of blood;

15. The skull was then dried, and the landmarks identified;

16. The coordinates for the particular operation were then determined and recorded;

17. The coordinates for the lesion were then set, and the skull marked with a pencil;

18. The skull was then fenestrated with a dental type drill (see page 23), using constant lubrication;

19. After cleaning the electrode, they were reset for the proper coordinates;

20. The cortex was then dried, the electrode lowered to the surface of the cortex, the depth recorded, and the rectal cathode inserted;

21. The electrode was then lowered to the proper depth;

22. The current was then delivered for the proper interval of time, and the proper amperage for the operation;
23. The electrode was then removed and washed before being used again; 

24. The animal was then removed from the stereotaxic device in the reverse order from which it had been placed there; 

25. Procaine Penicillin was administered intramuscularly to each operant animal; 

26. The incision was then sutured. 

The anesthetic that was used for this procedure throughout the entire experiment consisted of 1 part veterinary pentobarbital sodium (Nembutal Sodium, Abbott Laboratories) in 8.5 parts 10% ethyl alcohol. The anesthetic was administered via the intraperitoneal route in the dose of 0.01 ml./1 gram body weight. The atropine sulfate used to prevent excess secretion was manufactured by Eli Lilly and Co., Inc. The penicillin procaine used at the termination of the operative procedure was manufactured by Charles Pfizer & Co., Inc. 

The technique of stereotaxic operation was practiced until no single operation took in excess of forty minutes to complete, not including the time for suturing the scalp. 

All lesions were made in reference to landmarks on the skull of the animal. The landmarks which are used are the cross-over points of the sutures in the skull. These points are referred to as lambda and
bregma (see diagram). The coordinates for the lesions were made during the stereotaxic operation were measured from the bregma or the lambda. For the septal operation, the coordinates used were as follows:

0.5 mm. bilateral from the midline (sagittal suture),

2.5 mm. anterior to the bregma,

6.38 mm. deep from the surface of the cerebrum.

For the amygdaloid operations, the coordinates used were as follows:

4.13 mm. bilateral from the midline,

2.0, 4.0 and 6.0 mm. anterior to the lambda,

9.25 mm. deep from the surface of the cerebrum.

These coordinates were used in all the animals whose reactions will be reported in this dissertation. In the septal-amygdaloid animals, lesions were placed using both sets of coordinates in the same animal. The current that was delivered in all cases was calibrated to deliver electrolytic destruction equivalent to 2 ma.
(milliamperes), and was applied for 20 seconds at each locus. When current is mentioned henceforth, it will refer to 2 mA delivered for 20 seconds, unless otherwise specified.

4. Activity_Rating Scale

Septal, amygdaloid, septal-amygdaloid and control operant animals were compared to normal animals on the basis of an activity rating scale. The use of an emotionality rating scale for subjective analysis of animal reactions was first proposed in 1941 by Tryon (70). In 1953 Brady and Nauta devised an emotionality rating scale (67) which was later used and modified by King (68). The author has taken the emotionality rating scale of King and has again modified it. Its title has been altered to Activity Rating Scale because of the author's opinion that what is being observed and measured is activity. This activity may be the end result of emotional processes in the animal, but emotion can then be measured only indirectly. As a result the term activity has been substituted for emotionality. The only other changes have been the additions of points 4 and 5 under sub-heading F (urination and defecation), and changing of point 3 sub-heading F from loose stools to constant urination. In all other aspects this scale is identical to that of King (68).
A. Reaction_to_Object_Presentation--Probe Presented

Close to the Snout:

0. The animal ignores the probe. 4
1. The animal is alert and attentive and is slightly tense.
2. The legs and body are tense and immobile; the vibrissae point forward.
3. The animal scurries away, or makes occasional mild biting attack.
4. Intermediate between 3 and 4 (question of subjective judgement).
5. The animal goes into an aggressive attack, dis-organized panic or violent flight.

B. Response_To A Tap On-the_Back: With the Probe That Had Been Presented:

0. No reaction.
1. The animal twitches, or is restless.
2. The twitching becomes more violent, or the animal scurries away.
3. The animal jumps or hops a bit, and then settles down.

4The numbers 0 through 5 indicate the point score that each animal may attain during each single test of the rating scale. Therefore, the minimum possible score is 0, and the maximum score is 30 when all six points are totalled.
4. The animal leaps in the air and runs about in fright; or there is a big hop followed by movement.

5. The animal goes into a series of violent leaps, or dashes off in panic, or frantically rebounds from side to side in the cage.

C. **Resistance to Capture:** Glove is extended toward the animal slowly and the rat is grasped firmly but not roughly:

0. The animal remains calm; does not move when approached; does not struggle when grasped.

1. The animal remains calm when approached; scurries away and tugs a bit when grasped.

2. The animal avoids on approach; struggles when grasped.

3. The animal retreats on approach; struggles vigorously when grasped.

4. The animal makes a strong attempt to escape when approached; struggles strongly (with some biting) and is disorganized when grasped.

5. The animal leaps violently when grasped; bites frantically, and is exceedingly difficult to capture.

D. **Resistance to Handling**:

0. The animal relaxes in your hand and makes no attempt to escape.
1. The animal is restless, and squirms feebly.
2. The animal makes sporadic attempts to pull out of your hand.
3. The animal struggles fairly continuously and vigorously in his attempt to escape.
4. Same as 3, adding attempts to bite.
5. The animal bites frantically, tugs powerfully and twists in a disorganized manner.

E. Vocalization_In Response_to Capture and Handling:
0. None.
1. The animal gives voice to a few squeaks.
2. The animal squeaks frequently.
3. The animal squeaks frequently, and sometimes squawks.
4. The animal squeaks continuously.
5. The animal screeches loudly, frantically, and continuously.

F. Urination and Defecation_In Response_to Handling:
0. None.
1. The animal urinates slightly.
2. The animal defecates a few stools.
3. The animal urinates constantly.
4. The animal defecates constantly.
5. The animal has continuous loose stools.

It was found that the rating of the animals on the basis of this scale could be carried out simply and
rapidly, and gave highly accurate results which were easily repeated.

5. **Removal of Intact Brains** (71)

In order to perform the histological studies on the brain tissue, the brain was removed intact from one of three (1/3) operant animals and studied for extent and locus of electrolytic lesion. The method used to remove the brain with the minimal amount of tissue destruction was as follows:

1. A lethal dose of an anesthetic was administered to the animal. The anesthetic used was pentobarbital sodium, and was administered intraperitoneally in the dose of 30 mg./animal.

2. From this point until 10% formalin is administered intracardially, all work must be done rapidly, so that the formalin is administered before the animal dies and the heart stops beating.

3. Rapidly cut through the abdominal muscle, anteriorly through the rib case slightly laterad to the sternum, and into the thoracic cavity.

4. Isolate the heart, and identify the left ventricle.

5. Inject 12 to 18 ml. of saline solution directly into the left ventricle.
6. Immediately inject 20 to 30 ml. of 10% formalin into the same area of the left ventricle that the saline was administered.

7. From this point until the brain is removed, care must be taken not to damage the neural material.

8. Cut off the head of the animal, and remove the lower jaw. Skin the remaining portion of the head and remove as much of the head muscle as possible.

9. Remove the skull slowly, piece by piece, using spoon-shaped bone rongeurs.

10. Store the brain, or the required portion thereof in 10% formalin.

The injection of the 10% formalin directly into the left ventricle of the heart preserves the brain tissue in situ, and prevents any biological, enzymatic reaction from destroying the tissue. This allows the surgeon to remove the brain more slowly and more carefully.

6. Preparation of Brain for Embedding (Paraffin)

After the brain has been saturated with formalin to preserve it, it must undergo a series of treatments to remove formalin and water before it can be adequately embedded, mounted, sliced and stained. The procedure used is as follows:

1. Remove the brain from the formalin solution
in which it had been stored, and place it in a new container which is adequately marked to insure adequate identification of the brain section.

2. Place the brain in a bath, and wash it in running tap water for at least 24 hours, or until there is no odor of formalin.

3. Place the brain in 50% ethyl alcohol for 24 hours.¹

4. Place the brain in 70% ethyl alcohol for 24 hours.

5. Repeat #4 using absolute alcohol.

6. Repeat #4 using xylene.

7. Repeat #6, and store the brain in xylene until it is to be used.

7. **Preparation of Brain for Embedding (Celloidin):**
   1. Wash the brain in tap water for 24 hours, or until necessary to remove all traces of formalin.
   2. To dehydrate and clear the brain, place it in pyridine for 24 hours.

8. **Paraffin Embedding:**
   1. Remove the brain from the xylene and place it in molten paraffin.
   2. Repeat #1 twice to remove all traces of xylene.

¹Steps 3 to 6 are for dehydration and clearing the brain material.
3. Pour fresh molten paraffin into a mold, and place the brain in it with the cutting section down.

4. Add molten paraffin until the entire section is immersed.

5. Allow to cool and harden.

6. Reheat the sides of the mold gently, to facilitate removal of the paraffin block from the mold.

9. Pyridine_and_Celloidin_Embedding:

1. Transfer the brain from pyridine to a mixture of 50% pyridine and 50% celloidin (4% concentration) for 24 hours.

2. Repeat #1 twice.

3. Place the brain in 8% celloidin for 24 hours.

4. Place the brain in fresh 8% celloidin.

5. Drying:
   a) Put the tissue in a dish; cover the tissue with about 3 times the thickness of 8% celloidin;
   b) Cover the dish; mark the level, and allow to dry until 50% is evaporated;
   c) Put the tissue block in a dessicator under chloroform fumes, and allow it to dry until hard;
   d) Cut the tissue out of the celloidin;
   e) Place the tissue in 70% ethyl alcohol until ready for use.
6. Mounting:
   a) Trim the edges of the tissue block;
   b) Pour 8% cellloidin on the block (or paraffin, as the case may be), and allow it to dry for 15 minutes;
   c) If cellloidin, place in 70% ethyl alcohol for 2 hours.6

10. Preparation of 8% Cellloidin:
   80.0 Grams Parlodion Strips (Mallinckrodt Chemical)
   460.0 ml. C.P. Ether
   460.0 ml. Absolute Alcohol

   Break the parlodion into small strips, and add it to the anhydrous solvent. Solution will normally be effected within 48 hours unless water is present. In the latter case, the cellloidin will become milky in color, and is to be discarded.

11. Histology:

   The brain of one animal of every three (1/3 the animals) was removed and treated in preliminary procedures for the histology. All tissue was sliced on a sliding blade microtome, and was sectioned at 10 micra. Each tenth section was removed and stained for microscopic study. In this manner the extent and accuracy

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6In celluloidin embedding, the tissue need not be stored in pyridine if its thickness is less than 1 cm.
of the lesion was determined. Staining was done with cresyl violet (a cell stain), and the sections were bleached to remove any over-coloring.

After staining, all tissues were preserved in xylene until ready to be mounted on the slides and observed.
PROCEDURE

1. **Animals and Drugs:**

Before the recorded experimentation was begun preliminary tests and operations were performed to test the resistance of the animals to the operative procedure and to the anesthetic used. As the operative technique employed in all operations was the same, it was concluded that any variation in survival rate would be due to two factors. These factors were difference in animal resistance, and difference in anesthetic effect.

The two groups of animals that were used were Sprague-Dawley albino rats, and rats of the LSD#2 strain. The anesthetics that were used were pentobarbital sodium in the doses of 60 mg./kg. and 45 mg./kg., and the hydroalcoholic mixture of pentobarbital sodium. When the amount of pentobarbital in the latter mixture is calculated, it is found that the dose administered was 63.05 mg./kg. The tests performed with these drugs followed by the operations were in effect Lethal Dose tests.

The animals were divided into six groups of six animals each. Each of the groups had three albino and three LSD#2 rats in it. Three of the groups had septal
operations performed on them, while the other three were subject to amygdaloidectomy. The results, in number of deaths are tabulated on Table 1.

It can be seen from Table 1 that the least amount of deaths occurred in the LSD#2 groups receiving the hydro-alcoholic anesthetic. From this tabulation, it was then decided that the new anesthetic was the anesthetic of choice, and that the animals to be used would be the LSD#2 animals. It should be noted that the survival rate of the LSD#2 animals in all cases was at least twice as great as that of the albino animals. The survival rate of the septal animals given Pent-Al was twice as great in the LSD#2 animals as in the albinos, and in the amygdaloid operants was 2.5 times as great.

It was also noted, although it was not calculated, that animals given Pent-Al must be well fed and well watered. If the animals were in a starved, or deprived condition, the effect of Pent-Al was invariably fatal.

Atropine sulfate was routinely administered to each operant animal in the amount of 2 mg./animal to prevent any mucous secretion in the respiratory tract. Previous work had shown that some animals that had not been pre-treated with atropine had demonstrated

\[1\text{From this point, this anesthetic will be termed the Pent-Al anesthetic.}\]
TABLE 1

SURVIVAL OF ANIMALS OF DIFFERENT STRAINS UNDER OPERANT CONDITIONS EMPLOYING VARIED ANESTHETICS

<table>
<thead>
<tr>
<th></th>
<th>SEPTAL OPERANT</th>
<th></th>
<th>AMYGDALOID OPERANT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lived**</td>
<td>Died</td>
<td>Lived**</td>
<td>Died</td>
</tr>
<tr>
<td>ALBINO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Pb* 60 mg/kg</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>2. Pb 45 mg/kg</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>3. Pent-Al</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

| LEAD           |               |                  |                    |                  |
| 1. Pb 60 mg/kg | 2             | 4                | 1                  | 5                |
| 2. Pb 45 mg/kg | 4             | 2                | 2                  | 4                |
| 3. Pent-Al     | 6             | 0                | 5                  | 1                |

*Pb denotes pentobarbital sodium.

**Lived denotes that the animals lived for a minimum of seven days after the operative procedure.
respiratory difficulties during and after the operation. No animal that was treated showed any indications of respiratory failure due to mucus blockage of the respiratory canals. The atropine was administered in a volume of 1 ml. injected via the intraperitoneal route, and was given directly after loss of the righting reflex due to the anesthetic effect.

To prevent any reduction in the field of operation by coagulation of oozing blood, epinephrine (Adrenalin Hydrochloride, Parke, Davis & Co.) 1:1000 was used to clean the area of the scalp incision. This removed any blood that was present, and constricted the surface blood vessels to prevent further oozing. In this manner the area of operation was kept clean and visible. The epinephrine was applied to the surface of the scalp with a clean, sterile gauze pad, until all oozing of blood had stopped.

When the operation was complete, the trephine holes in the skull were loosely packed with powdered Gelfoam (Upjohn Laboratories) to prevent post-operative bleeding at the site of electrode placement. Using this technique, no animal demonstrated post-operative hematoma. Upon subsequent removal of the brains, it was found that the gelfoam had been absorbed, and that the skull in many of the animals was wholly, and in all, partially sutured. This suture was complete with
connective tissue in all cases, and with bony material in animals that had lived for one post-operative month.

After the operative procedure each animal was given a dose of penicillin to prevent any post-operative infection at the site of incision. The amount of penicillin was varied with the type of operation. The longer the duration of the operation (the longer the animal's brain was exposed to the atmosphere) the greater the dose of penicillin administered. The doses normally given for the various operations were 30,000 u. intramuscularly for a septal operation, 50,000 u. I.M. for an amygdaloid operation, and 60,000 to 100,000 u. I.M. for a septal-amygdaloid operation. In the septal-amygdaloid operation the amount of penicillin administered was varied with the duration of the operation, the gross physical appearance of the animal, and the amount of blood lost by the animal during the operation. In this manner, no animal died from the apparent cause of infection.

2. Operation and Activity Rating:

On the day that the operation was done, each animal weighed between 120 and 175 grams, and was between 60 and 90 days of age. All animals were examined for gross physical condition, and any animal that appeared to be unhealthy or weak was discarded. Five groups of
animals were formed, each group containing 12 male and 12 female members. The five groups that were formed were: 1) septal operant animals; 2) amygdaloid operant animals; 3) septal-amygdaloid operant animals; 4) operant control animals; 5) normal control animals.

Five or six operations were performed in a single day, and usually five days of operations were required to complete one group. This was the case if any of the operant animals died. When death occurred within seven days, the results from this animal were discarded. Overall, one operant animal of the septal group died and was replaced. In the amygdaloid group, three died and were replaced, and in the septal-amygdaloid group four died and were replaced. None of the operant control animals or normal control animals died within the seven day period of testing.

The operant control animals were subject to sham operations, in which the scalp was incised, the skull trephined, and a needle (electrode) inserted at the coordinates of septal-amygdaloid animals. The animals were given the same drugs as the septal-amygdaloid operants, and received the same doses.

As it took five days to complete a single group of animals, the activity ratings were staggered over a five day period. That is to say, that the post-operative day 6, or activity rating 6 of the first operant animals
was post-operative day 1 for the last operant animals.
In this manner, the activity ratings of all the animals were kept on an equivalent day-to-day basis.

Exceptions to the five-day operation schedule were the normal animals, which were all chosen on the same day, and the control operant animals. The operations on the latter groups of animals were done over a two day period of time. As a result, the normal animals were all tested on the same day, while the operant control animals were tested on two separate days according to the day upon which they had been operated.

Activity ratings were performed on all animals for a period not less than ten days. All animals used were rated for three days pre-operatively, and at least seven days post-operatively. It was determined statistically that there was no significant difference between animals prior to the operations.

After the operations had been completed the animals were given a minimum of 24 hours to recover from post-operative trauma. At that point the activity ratings were begun.

The activity rating of each animal was determined and recorded, and then the animal was treated with chlorpromazine hydrochloride. Thirty minutes following the injection, the activity ratings of the animals were again taken. Previous work had shown (72) that a thirty
minute duration between administration of chlorpromazine (Thorazine Hydrochloride, Smith, Kline & French Laboratories) and testing of its effect was optimal to note the effect of the drug.

An extra group of septal animals was made to determine the graded effect of chlorpromazine, if any, in graded doses. This group also consisted of 12 male and 12 female LSD#2 rats. These animals were divided into sub-groups of 4 male and 4 female rats each, and were given 2 mg/kg., 4 mg./kg., and 8 mg./kg. of chlorpromazine. As in previous work (72), it was found that 8 mg./kg. was the optimal dose.

After the activity ratings had been completed, one-third of the animals from each group were sacrificed. The brains of these animals were then studied histologically to determine the locus and the extent of the lesion that had been made. The animals that were sacrificed were four males and four females of each group. The slides that best demonstrated the extent of lesion were photographed, and will be found under results.

3. Statistics

The data obtained were analyzed statistically using a design designated by Lindquist (73) as a
"Type I 'Mixed' Design." It was decided that if the F ratios obtained for variance between groups, within groups, and interaction were significant, the \( t \) test for critical difference '\( d \)' (73) would be applied to indicate the significance of differences between means.
RESULTS

I. Design

The statistical design followed the experimental procedure in that it was subdivided into three parts; Part 1, the septal group, Part 2, the amygdaloid group, and Part 3, the septal-amygdaloid group. The three parts are distinguished solely by the difference in operative procedure employed. These parts all include an analysis of the control operant and normal groups. Each group was subjected to the same treatments. The treatments included no drug administration followed in one-half hour by the administration of 8 mg./kg. chlorpromazine hydrochloride. Following these three parts, an additional section was included to further analyze the differences between the three operant groups.

The criterion measure employed was a value from the activity rating scale adapted from King's (68) emotionality rating scale.

II. Activity

Initially, the activity rating for a group of normal LSD#2 animals was determined without chlorpromazine, and under the influence of 8 mg./kg. chlorprom-
mazine I.P. It was found that the normal rating without chlorpromazine was approximately eight. This rating was a constant throughout the entire rating period, with only very slight non-significant fluctuations. When given chlorpromazine the activity ratings of the normal animals dropped to three, and was a constant throughout the period of testing.

All other groups of animals were given three days of pre-operative testing, and it was found that the ratings obtained were not significantly different from the ratings of the animals in the normal group.

In animals in which the area of the septal nuclei had been destroyed, the activity rose sharply to twenty on post-operative day 1. This extreme activity gradually decreased with the passage of time, until on post-operative day 7 the activity was only slightly above the normal activity of 8. (After post-operative day 7, the activity ratings of the septal animals were continued for a period of six weeks. It was found that the activity level of septal animals remained at 9, or slightly above the normal average.)

17.89. 22.99.

3The numerals 20 and 3, and all other numbers noted for activity ratings are averages for the entire group of 24 animals being discussed, and are rounded off to the nearest whole number.
Graph I

Activity of Normal and Opioidt Control Animals

Days 1-10

Notes:
- Normal (baseline level)
- Post-operative
- Pre-operative
- Post-operative with opioid treatment
- Post-operative - no opioid treatment

Legend:
- Drug 1
- Drug 2
On the first post-operative day, the activity of the septal animals was reduced from 20 to 3 when the animals were treated with 8 mg./kg. of chlorpromazine. On all subsequent days of rating, the activity of the septal animals was reduced to a level of three.\(^4\)

In animals that had been subject to destruction of the amygdaloid complex, the activity rating dropped from normal to 3 on the first post-operative day. This rating remained fairly constant during the entire 7 day testing period, with only slight, insignificant fluctuations.\(^5\) Chlorpromazine (8 mg./kg., I.P.) was unable to reduce the activity of the animal to any significant degree. There is no significant difference between the amygdaloid animals and any animals that were treated with 8 mg./kg. chlorpromazine. It is also noted that the activity rating of the amygdaloid animals is very low (Graph 3 and 4) and that there is no change of amygdaloid activity when treated with 8 mg./kg. chlorpromazine (Graph 3).\(^6\)

Graph 2 demonstrates the difference between septal animals and normal animals, and the similarity of the two groups after treatment with 8 mg./kg. chlorpromazine. Although it is not demonstrated pictorially, it was found that 2 mg./kg. chlorpromazine reduces septal
GRAPH 2

ACTIVITY OF SEPTAL AND NORMAL ANIMALS
(DAYS 1 - 10)

- Pre-operative - No Chlorpromazine
- Pre-operative - With Chlorpromazine
- Post-operative - No Chlorpromazine
- Post-operative - With Chlorpromazine

SEPTAL

NORMAL

NORMAL BASE-LINE
NORMAL CHLORPROMAZINE BASE-LINE
activity to about 8, 4 mg./kg. to about 5, and 11 mg./kg. to about 3. Therefore it seemed that 8 mg./kg. was an optimal dose of chlorpromazine, for it was the smallest dose that gave the greatest measurable response. This result agrees very favorably with previous work done (72).

Graph 3 is a comparison of amygdaloid and septal-amygdaloid activity under like conditions. Septal-amygdaloid animals were found to have an activity rating of six\(^7\) before treatment with chlorpromazine, and a rating of three\(^8\) after treatment. The slight difference in activity of the untreated septal-amygdaloids from the normals remained at a constant level throughout the period of activity measurement. This difference was found to be statistically significant.

It can readily be seen from Graph 1, that there is no difference between the group of normal rats, and that of the operant control rats. Without chlorpromazine the normal rats gave an average rating of 7.89, while the operant control group gave a rating of 7.93. When under the influence of 8 mg./kg. chlorpromazine, the normal rats gave a rating of 2.99, while the operant control group gave a rating of 3.05. Statistically there is no difference.

Graph 4 depicts the activity rating of all groups

76.09.  83.05.
GRAPH 3
ACTIVITY OF AMYGDALOID AND SEPTAL-AMYGDALOID ANIMALS
DAYS 1-10

- Pre-operative - no Chlorpromazine
- Pre-operative - with Chlorpromazine
- Post-operative - no Chlorpromazine
- Post-operative - with Chlorpromazine

NORMAL BASE-LINE

AMYGDALOID
NORMAL CHLORPROMAZINE BASE-LINE

SEPTAL-AMYGDALOID

DAYS 1-10
SUMMARY OF ACTIVITY OF NON-CHLORPROMAZINE TREATED ANIMALS

(DAYS 3-10)

GRAPH 4

SEPTAL

NORMAL

AMYGDALOID

OPERANT CONTROL

SEPTAL-AMYGDALOID

NORMAL BASELINE
of animals on post-operative day 1 through 7. (These
days are equal to testing days 3 to 10.) It may be noted
that the only group of animals that show any change in
activity from day to day is the septal group, whose
activity declines through post-operative day 7. The
other groups remain constant in activity throughout the
tenure of the experiment.

Graph 5 demonstrates the activity of all groups
of animals on post-operative day 1 through 7 under the
influence of 8 mg./kg. chlorpromazine. It is apparent
from these results that chlorpromazine will reduce the
activity level of all groups of animals to a base-line
of approximately 3. The only apparent variation in
these results are in the amygdaloid group of animals.
Although there is no statistical difference in the
amygdaloid group of animals before and after treatment
with chlorpromazine, the graphical tabulation on Graphs
3 and 5 seems to indicate that there may be some slight
reduction of activity in the amygdaloid group after
chlorpromazine administration. As a greater dose of
chlorpromazine did not give a greater depression of
activity, it may be possible that subjective measurement
with an activity rating scale is not fine enough to
allow for gradations in activity below 3.

The application of the statistical method demon-
strated that the means of the groups treated with
GRAPH 5
SUMMARY OF ACTIVITY OF CHLORPROMAZINE TREATED ANIMALS
(DAYS 3-10)

SEPTAL  NORMAL  AMYGDALOID  OPERANT  CONTROL  SEPTAL-AMYGDALOID

NORMAL CHLORPROMAZINE BASE-LINE

3
2
1

chlorpromazine were not significantly different from each other. The same evaluation showed that the operant control animals were essentially equivalent in activity to the normal group of animal. The septal operant group, the amygdaloid operant group and the septal-amygdaloid operant group behaved in a manner different enough from normal to be statistically significant.

III. Statistical Analysis

A. Septal Group--Hypotheses

1. There are no significant differences in activity among septal operant animals, control operant animals, and normal animals.

2. There is no significant difference between animals after treatment with chlorpromazine and animals before having been treated with chlorpromazine, as determined by activity.

3. There are no significant differences among the septal group, the control operant group and the normal group before being treated with chlorpromazine.

4. There are no significant differences among the septal group, the control operant group and the normal group after being treated with 8 mg./kg. chlorpromazine hydrochloride.

The hypotheses were tested by an analysis of
variance. The data were analyzed with a design designated by Lindquist (73) as a Type I 'Mixed' Design.

a) Hypothesis 1: The variance for the between-subjects effect (groups) to determine differences between group means was tested for significance by the between-subjects error variance. The F-ratio with 2 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 2. Since the F-ratio was significant, the _t_ test for "critical difference" (73) was applied for indicating differences between group means. The "critical difference" corresponding to a particular level of significance was computed from the following formula:

\[
d = t \sqrt{\frac{2 MS(V)}{N}}
\]

where \(d\), the "critical difference," is computed by multiplying \(t\), representing the value of \(t\) which is significant only at the selected level for the degrees of freedom, times the square-root of twice the mean square variance, or more correctly, the error variance, divided by \(N\), the number of subjects. The "critical differences" were computed for the 5% and 1% levels of confidence.

Significant differences were found between the means of the septal and operant control groups, and between the means of the septal and normal groups,
### TABLE 2

**SUMMARY OF A TYPE I 'MIXED' DESIGN ANALYSIS OF VARIANCE FOR SEPTAL, OPERANT CONTROL AND NORMAL ANIMALS**

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>df</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARES (Variance)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>367.29</td>
<td>183.65*</td>
<td>***</td>
</tr>
<tr>
<td>Error (b)</td>
<td>69</td>
<td>1.50</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>1835.77</td>
<td>1835.77*</td>
<td>***</td>
</tr>
<tr>
<td>AB</td>
<td>2</td>
<td>366.18</td>
<td>183.09*</td>
<td>***</td>
</tr>
<tr>
<td>Error (w)</td>
<td>69</td>
<td>90.77</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>2661.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*—Value is significant at the 1% and 5% level of confidence.

***—Value is significant at the 1% and 5% level of confidence.
at the 5% and 1% level of confidence. Based on these findings, the first hypothesis was rejected.

b) Hypothesis 2: The variance for the within-subjects treatments (no chlorpromazine—chlorpromazine 8 mg./kg.) to determine differences between treatment means was tested for significance by the within-subjects error variance. The F-ratio with 1 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 2. Since the F-ratio was significant, the t test for "critical difference" was applied for indicating differences between treatment means. The "critical difference" was computed for the 5% and 1% level of confidence. As shown in Table 3, significant differences were found between treatments. These differences were found between the septal non-treated group and the control operant group and normal group, both treated and untreated and the septal treated animals; between the septal treated group and the normal and control operant non-treated groups; within the operant control group before and after treatment; between the non-treated control group and the treated normal group; and within the normal group before and after treatment. These differences were significant at the 5% and 1% level of confidence. Based on the above findings, the second hypothesis was rejected.
TABLE 3

MEAN SCORES AND DIFFERENCES BETWEEN MEAN SCORES FOR TREATMENTS OF SEPTAL, OPERANT CONTROL AND NORMAL GROUPS OF ANIMALS

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENTS</th>
<th>TREATMENTS DIFFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1-Septal</td>
<td>a1-no drug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a2-drug</td>
<td></td>
</tr>
<tr>
<td>B2-Operant Control</td>
<td>a1-no drug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a2-drug</td>
<td></td>
</tr>
<tr>
<td>B3-Normal</td>
<td>a1-no drug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a2-drug</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MEANS</th>
<th>TREATMENTS</th>
<th>DIFFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B_1A_1) --- 14.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B_1A_2) --- 3.03</td>
<td>(B_1a_1)</td>
<td>11.65*</td>
</tr>
<tr>
<td>(B_2A_1) --- 7.93</td>
<td>(B_1a_2)</td>
<td>4.90*</td>
</tr>
<tr>
<td>(B_2A_2) --- 3.05</td>
<td>(B_2a_1)</td>
<td>4.86*</td>
</tr>
<tr>
<td>(B_3A_1) --- 7.89</td>
<td>(B_3a_1)</td>
<td>4.84*</td>
</tr>
<tr>
<td>(B_3A_2) --- 2.99</td>
<td>(B_3a_2)</td>
<td></td>
</tr>
</tbody>
</table>

*---Value significant at the 5% and 1% levels of confidence.
c) Hypothesis 3: The variance for the **between-subjects treatments** (non-chlorpromazine treated) to determine difference between means was tested for significance in the same manner as Hypothesis 2. As shown in Table 3, significant differences were found between means. The differences were found between the untreated septal animals and the untreated normal and operant control animals. Based on this finding, the third hypothesis was rejected.

d) Hypothesis 4: The variance for the **between-subjects treatments** (chlorpromazine treated) to determine differences between means was tested for significance in the same manner as Hypotheses 2 and 3. As shown in Table 3, no significant differences were found between means. Based on these findings, the fourth hypothesis was not rejected.

The **interaction variance** between treatments and groups was tested for significance by the **error variance** for **within-subjects**, and was found to be significant at the 5% and 1% level of confidence.

B. **Amygdaloid Group--Hypotheses**

1. There are no significant differences in activity among amygdaloid operant animals, control operant animals, and normal animals.

2. There is no significant difference between
animals after treatment with chlorpromazine (8 mg./kg.) and animals before having been treated with chlorpromazine, as determined by activity.

3. There are no significant differences among the amygdaloid group, the control operant group and the normal group before being treated with chlorpromazine.

4. There are no significant differences among the amygdaloid group, the operant control group and the normal group after being treated with chlorpromazine.

The hypotheses were tested as under Septal Group.

a) Hypothesis 1: The variance for the between-subjects effect (groups) to determine differences between group means was tested for significance by the between-subjects error variance. The F-ratio with 2 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 4. Since the F-ratio was significant, the t test for "critical difference" was applied for indicating differences between group means.

Significant differences were found between the means of the amygdaloid and operant control groups, and the amygdaloid and normal groups, at the 5% and 1% level of confidence. Based on the above findings, the first hypothesis was rejected.

b) Hypothesis 2: The variance for the within-subjects treatments (no chlorpromazine--chlorpromazine
### TABLE 4

**SUMMARY OF A TYPE I 'MIXED' DESIGN ANALYSIS OF VARIANCE FOR AMYGDALOID, OPERANT CONTROL AND NORMAL ANIMALS**

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>df</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARES (Variance)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>71</td>
<td>160.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>204.06</td>
<td>102.03*</td>
<td>***</td>
</tr>
<tr>
<td>Error (b)</td>
<td>69</td>
<td>33.13</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td>72</td>
<td>634.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>391.08</td>
<td>391.08*</td>
<td>***</td>
</tr>
<tr>
<td>AB</td>
<td>2</td>
<td>181.96</td>
<td>90.98*</td>
<td>***</td>
</tr>
<tr>
<td>Error (w)</td>
<td>69</td>
<td>61.37</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>795.34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*...Value is significant at the 5% and 1% level of confidence.*

***.Value is significant at the 5% and 1% level of confidence.*
8 mg./kg.) to determine differences between treatment means was tested for significance by the *within-subjects* error variance. The F-ratio with 1 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 4. As the F-ratio was significant, the *t* test for "critical difference" was applied for indicating differences between treatment means, at the 5% and 1% level of confidence. As shown in Table 5, significant differences were found between treatment means. These differences were found between the non-treated amygdaloid group, and the non-treated normal and operant control groups; the treated amygdaloid group and the non-treated normal and operant control groups, the non-treated operant control group and the treated operant control group; the non-treated operant control group and the treated normal group; the treated operant control group and the non-treated normal group, and the treated and non-treated normal group. Based on these preceding findings, the second hypothesis was rejected.

c) Hypothesis 3: The variance for the *between-subjects* treatments (non-chlorpromazine treated) to determine differences between means was tested for significance in the same manner as Hypothesis 2. As shown in Table 5, significant differences were found between means. The differences found were between the
### TABLE 5

**MEAN SCORES AND DIFFERENCES BETWEEN MEAN SCORES FOR TREATMENTS OF AMYGDALOID, OPERANT CONTROL AND NORMAL GROUPS OF ANIMALS**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Treatments Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁—Amygdaloid</td>
<td>a₁—no drug</td>
<td>B₁a₁ B₁a₂ B₂a₁ B₂a₂ B₃a₁ B₃a₂</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
<td>0.12 4.93* 0.05 4.89* 0.01</td>
</tr>
<tr>
<td>B₂—Operant Control</td>
<td>a₁—no drug</td>
<td>B₂a₁ B₂a₂ B₃a₁ B₃a₂</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
<td>5.05* 0.17 5.01* 0.11</td>
</tr>
<tr>
<td>B₃—Normal</td>
<td>a₁—no drug</td>
<td>B₃a₁ B₃a₂</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
<td>4.84* 0.06</td>
</tr>
</tbody>
</table>

*...Value significant at the 5% and 1% level of confidence.*
untreated amygdaloid group, and the untreated operant control and normal groups. Based on this finding, the third hypothesis was rejected.

d) Hypothesis 4: The variance for the between-subjects treatments (chlorpromazine treated) to determine differences between means was tested for significance in the same manner as were Hypotheses 2 and 3. As shown in Table 5, no significant differences were found between means. Based on these findings, the fourth hypothesis was not rejected.

The interaction variance between treatments and groups was tested for significance by the error variance for within-subjects, and was found to be significant at the 5% and 1% level of confidence.

C. Septal-Amygdaloid Group--Hypotheses

1. There are no significant differences in activity among septal-amygadaloid animals, control operant animals and normal animals.

2. There is no significant difference between animals before treatment with 8 mg./kg. chlorpromazine, and animals after treatment with 8 mg./kg. chlorpromazine.

3. There are no significant differences among the septal-amygadaloid group, the control operant group and the normal group before being treated with chlorpromazine.
4. There are no significant differences among the septal-amygdaloid group, the operant control group and the normal group after being treated with chlorpromazine.

The hypotheses were tested as under Septal Group and Amygdaloid Group.

a) Hypothesis 1: The variance for the between-subjects effect (groups) to determine differences between group means was tested for significance by the between-subjects error variance. The F-ratio with 2 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 6. Since the F-ratio was significant, the t test for "critical difference" was applied for indicating differences between group means.

Significant differences were found between the means of the septal-amygdaloid and operant control groups, and between the means of the septal-amygdaloid and normal groups, at the 5% and 1% level of confidence. Based on the above findings, the first hypothesis was rejected.

b) Hypothesis 2: The variance for within-subjects treatments (no-chlorpromazine—chlorpromazine 8 mg./kg.) to determine differences between treatment means was tested for significance by the within-subjects error variance. The F-ratio with 1 and 69 degrees of
TABLE 6
SUMMARY OF A TYPE I 'MIXED' DESIGN ANALYSIS OF VARIANCE FOR SEPTAL-AMYGDALOID, OPERANT CONTROL AND NORMAL ANIMALS

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>df</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARES (Variance)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>71</td>
<td>7.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>25.64</td>
<td>12.82*</td>
<td>***</td>
</tr>
<tr>
<td>Error (b)</td>
<td>69</td>
<td>18.10</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td>72</td>
<td>719.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1</td>
<td>657.03</td>
<td>657.03*</td>
<td>***</td>
</tr>
<tr>
<td>AB</td>
<td>2</td>
<td>27.11</td>
<td>13.56*</td>
<td>***</td>
</tr>
<tr>
<td>Error (w)</td>
<td>69</td>
<td>35.59</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>727.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*...Value is significant at the 5% and 1% level of confidence.

***.Value is significant at the 5% and 1% level of confidence.
freedom was significant at the 5% and 1% level of confidence, as shown in Table 6. Since the F-ratio was significant, the t test for "critical difference" was applied for indicating differences between treatment means. As shown in Table 7, significant differences were found between treatment means, at the 5% and 1% level of confidence. The differences were found between the septal-amygdaloid non-treated and the septal-amygdaloid treated group; between the septal-amygdaloid non-treated group, and the non-treated and treated operant control and normal groups; between the treated septal-amygdaloid group and the non-treated control operant and normal groups; between the non-treated operant control group and the treated operant control and normal groups; between the treated operant control group and the non-treated normal group, and between the non-treated and treated normal group. Based on these findings, the second hypothesis was rejected.

c) Hypothesis 3: The variance for the between-subjects treatments (non-chlorpromazine treated) to determine differences between means was tested for significance in the same manner as was Hypothesis 2. As shown in Table 7, significant differences were found between the means of the untreated septal-amygdaloid group, and the untreated operant control and normal
### TABLE 7

**MEAN SCORES AND DIFFERENCES BETWEEN MEAN SCORES FOR TREATMENTS OF SEPTAL-AMYGDALOID, OPERANT CONTROL AND NORMAL GROUPS OF ANIMALS**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁—Septal Amygdaloid</td>
<td>a₁—no drug</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
</tr>
<tr>
<td>B₂—Operant Control</td>
<td>a₁—no drug</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
</tr>
<tr>
<td>B₃—Normal</td>
<td>a₁—no drug</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MEANS</th>
<th>TREATMENTS DIFFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁A₁—6.09</td>
<td>B₁a₁   3.04* 1.84* 3.04* 1.80* 3.10*</td>
</tr>
<tr>
<td>B₁A₂—3.05</td>
<td>B₁a₂   4.88* 0.00 4.84* 0.06</td>
</tr>
<tr>
<td>B₂A₁—7.93</td>
<td>B₂a₁   4.88* 0.04 4.94*</td>
</tr>
<tr>
<td>B₂A₂—3.05</td>
<td>B₂a₂   4.84* 0.06</td>
</tr>
<tr>
<td>B₃A₁—7.89</td>
<td>B₃a₁   4.90*</td>
</tr>
<tr>
<td>B₃A₂—2.99</td>
<td>B₃a₂   4.90*</td>
</tr>
</tbody>
</table>

*—Value significant at the 5% and 1% level of confidence.
groups. Based on this finding, the third hypothesis was rejected.

d) Hypothesis 4: The variance for the between-subjects treatments (chlorpromazine treated) to determine differences between means was tested for significance in the same manner as were Hypotheses 2 and 3. As shown in Table 7, no significant differences were found between means. Based on the above findings, the fourth hypothesis was not rejected.

The interaction variance between treatments and groups was tested for significance by the error variance for within-subjects, and was found to be significant at the 5% and 1% level of confidence.

D. Septal, Amygdaloid, and Septal-Amygdaloid Groups—Hypotheses

1. There are no significant differences in activity among septal animals, amygdaloid animals, and septal-amgdaloid animals.

2. There is no significant difference between animals before treatment with 8 mg./kg. chlorpromazine hydrochloride, and animals after treatment with 8 mg./kg. chlorpromazine hydrochloride.

3. There are no significant differences among the septal group, the amygdaloid group and the septal-amgdaloid group before being treated with chlorpromazine.

4. There are no significant differences among
the septal group, the amygdaloid group and the septal-amygdaloid group after being treated with chlorpromazine.

The hypotheses were tested as under Septal Group, Amygdaloid Group and Septal-Amygdaloid Group.

a) Hypothesis 1: The variance for the between-subjects effect (groups) to determine differences between group means was tested for significance by the between-subjects error variance. The F-ratio with 2 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 8. Since the F-ratio was significant, the $t$ test for "critical difference" was applied for indicating differences between group means.

Significant differences were found between the means of the septal group and the amygdaloid and septal-amygdaloid groups, and between the means of the amygdaloid and septal-amygdaloid group at the 5% and 1% level of confidence. Based on the above findings, the first hypothesis was rejected.

b) Hypothesis 2: The variance for the within-subjects treatments (no chlorpromazine—chlorpromazine 8 mg./kg.) to determine differences between treatment means was tested for significance by the within-subjects error variance. The F-ratio with 1 and 69 degrees of freedom was significant at the 5% and 1% level of confidence, as shown in Table 8. Since the F-ratio
<table>
<thead>
<tr>
<th>SOURCE</th>
<th>df</th>
<th>SUM OF SQUARES</th>
<th>MEAN SQUARES</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Subjects</td>
<td>71</td>
<td>951.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>895.20</td>
<td>447.60*</td>
<td>***</td>
</tr>
<tr>
<td>Error (b)</td>
<td>69</td>
<td>56.74</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Within Subjects</td>
<td></td>
<td>1776.79</td>
<td></td>
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<td>877.68*</td>
<td>877.68*</td>
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</tr>
<tr>
<td>AB</td>
<td>2</td>
<td>862.83</td>
<td>431.42*</td>
<td>***</td>
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<td>Error (w)</td>
<td>69</td>
<td>36.28</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>143</td>
<td>2728.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*....Value is significant at the 5% and 1% level of confidence.

***...Value is significant at the 5% and 1% level of confidence.
was significant, the t test for "critical difference" was applied for indicating differences between treatment means. As shown in Table 9, significant differences were found between treatment means at the 5\% and 1\% level of confidence. The differences were found between the non-treated septal group and the treated septal group; the non-treated septal group and the amygdaloid and septal-amygdaloid groups, both treated and non-treated; the treated septal group and the non-treated septal-amygdaloid group; the non-treated amygdaloid group and the non-treated septal-amygdaloid group; the treated amygdaloid group and the non-treated septal-amygdaloid group; and the treated and non-treated septal amygdaloid group. Based on the above findings, the second hypothesis was rejected.

c) Hypothesis 3: The variance for the between-subjects treatments (non-chlorpromazine treated) to determine differences between means was tested for significance in the same manner as was Hypothesis 2. As shown in Table 9, significant differences were found between the untreated septal group and the untreated amygdaloid and septal-amygdaloid groups, and between the untreated amygdaloid and untreated septal-amygdaloid groups. Based on these findings, the third hypothesis was rejected.

d) Hypothesis 4: The variance for the between-
### TABLE 9

**MEAN SCORES AND DIFFERENCES BETWEEN MEAN SCORES FOR TREATMENTS OF SEPTAL, AMYGDALOID AND SEPTAL-AMYGDALOID GROUPS OF ANIMALS**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>TREATMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁—Septal</td>
<td>a₁—no drug</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
</tr>
<tr>
<td>B₂—Amygdaloid</td>
<td>a₁—no drug</td>
</tr>
<tr>
<td></td>
<td>a₂—drug</td>
</tr>
<tr>
<td>B₃—Septal-Amygdaloid</td>
<td>a₁—no drug</td>
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<tr>
<td></td>
<td>a₂—drug</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>MEANS</th>
<th>TREATMENT DIFFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁a₁</td>
<td>B₁a₂ B₂a₁ B₂a₂ B₃a₁ B₃a₂</td>
</tr>
<tr>
<td>B₁a₂</td>
<td>3.03</td>
</tr>
<tr>
<td>B₂a₁</td>
<td>11.68</td>
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<tr>
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<td>2.88</td>
</tr>
<tr>
<td>B₃a₂</td>
<td>2.09</td>
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</tbody>
</table>

*...Value significant at the 5% and 1% level of confidence.*
subjects treatments (chlorpromazine treated) to determine differences between means was tested for significance in the same manner as were Hypotheses 2 and 3. As shown in Table 9, no significant differences were found between means. Based on the above findings, the fourth hypothesis was not rejected.

The interaction variance between treatments and groups was tested for significance by the error variance for within-subjects, and was found to be significant at the 5% and 1% level of confidence.

Tables 10 and 11 contain the raw weekly scores for each animal that was used in the procedure. These scores have been tabulated, and show the ratings that were used in determining the statistical analysis that has been presented. The mean for each group is also listed to facilitate interpretation and calculation of the statistical data.

IV. Lesions

The brains of those animals designated as septal were found on microscopic examination to have sustained extensive damage to the septal region. Massive destruction of the precommissural and supracommissural septum was evident in all cases, indicating disruption of the fornix system. In many instances, however, the postcommissural fornix was intact, and the anterior commissure was always
undamaged. Figure 3 is a photomicrograph of a brain section with typical septal damage.

The subjects designated amygdaloid suffered bilateral damage to one or more nuclei of the amygdaloid complex. Most frequently this damage was limited to the lateral and basal nuclei, although occasional animals showed partial destruction of the central, medial, and/or cortical nuclei. Figure 4 is a photomicrograph of damage to the amygdaloid nuclei.

V. Addendum

After gathering the results that have been described in the preceding portion of this dissertation, a basic psychological study was attempted on septal, amygdaloid, septal-amygdaloid and normal animals.

Two types of studies were attempted. These studies were (1) food deprivation, involving the speed in which an animal could learn to press a bar for a food reward, and the number of bar presses per hour, and (2) an avoidance schedule, using electric shock as the noxious stimulus.

Data was gathered for only two days. This data was obtained only from the food deprivation schedule. Septal animals learned to press a bar very rapidly, as compared to normal and septal-amygdaloid animals, while amygdaloid animals did not learn as rapidly as normals.
These results are subjective, for the operant animals did not survive more than three days on a deprivation schedule.

In an attempt to avoid death due to deprivation, animals were trained, and then subjected to operative procedure. Approximately 86% of animals on a deprivation schedule did not survive the operative procedure. As a result of this, the animals were trained, and then given free access to food for three days prior to the operative procedure. The operations were successful, but when the animals were returned to a deprivation schedule death normally followed within three days.

The following results are an average of two days of psychological testing on the animals that had been trained, fed, subjected to surgery and then placed on a deprivation schedule.

Septal animals showed 1,436 bar presses per hour. After treatment with 8 mg./kg. chlorpromazine, the same animals averaged 123 presses per hour.

Amygdaloid animals pressed the bar for their food reward 118.5 times per hour before chlorpromazine treatment. After treatment they pressed the bar at a rate of 91 times per hour.

Septal-amygdaloid animals pressed the bar 576 times per hour, while with chlorpromazine they pressed the bar 117 times per hour.
Normal animals demonstrated 808 bar presses per hour without chlorpromazine, and 119 times per hour when treated with 8 mg./kg. chlorpromazine.

No avoidance schedule could be devised which was suitable to all groups of animals. The amount of electrical shock needed to stimulate an amygdaloid animal invariably led to a convulsive death in the septal animal. Convulsions in the septal animals were always the case at a level of stimulation which was minimal for normal or septal-amygdaloid animals.

The minimal level of stimulation for septal animals gave no response in the other groups of animals. Therefore, no conclusions could be drawn from studies of avoidance reaction.
FIGURE 3
UNRETouched PHOTOMICROGRAPHS OF BRAIN SECTIONS WITH TYPICAL SEPTAL DAMAGE
FIGURE 4
UNRETouched PHOTOMICROGRAPHS OF BRAIN SECTIONS WITH TYPICAL AMYGDALOID DAMAGE
### TABLE 10

**TABLE OF MEAN WEEKLY SCORES FOR NORMAL AND OPERANT CONTROL ANIMALS**

<table>
<thead>
<tr>
<th>B₁</th>
<th>A₁</th>
<th>A₂</th>
<th>B₂</th>
<th>A₁</th>
<th>A₂</th>
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<td>Operant Control</td>
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</tbody>
</table>

**A₁** = Activity of the animal in the absence of chlorpromazine.

**A₂** = Activity of the animal under the influence of 8 mg./kg. chlorpromazine

**MEANS**

\[
\begin{align*}
A₁B₁ &= 7.89 \\
A₁B₂ &= 7.93 \\
A₂B₁ &= 2.99 \\
A₂B₂ &= 3.05 \\
\end{align*}
\]
TABLE 11

TABLE OF MEAN WEEKLY SCORES FOR SEPTAL, AMYGDALOID AND SEPTAL-AMYGDALOID ANIMALS

<table>
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<th>B3</th>
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<td>A1</td>
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<td>24</td>
<td>15.57</td>
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</tr>
</tbody>
</table>

A1 = The Activity of the animal in the absence of chlorpromazine.

A2 = The Activity of the animal under the influence of 8 mg./kg. chlorpromazine.

MEANS

\[ A_1B_1 = 14.68 \]
\[ A_1B_2 = 3.00 \]
\[ A_1B_3 = 6.09 \]
\[ A_2B_1 = 3.03 \]
\[ A_3B_2 = 2.88 \]
\[ A_2B_3 = 3.05 \]
INTERPRETATION OF DATA (DISCUSSION)

The results of this experiment indicate that extensive damage to the septal region of the forebrain increases overt activity. This increased activity agrees with previous reports (66, 67, 68) that septal destruction causes an increase in affective behavior and emotionality. The results are also in agreement with the observations of Hunt (69), who concluded that chlorpromazine depresses the activity of both septal and normal animals.

Extensive destruction of the temporal amygdaloid complex resulted in a decrease in activity, as determined by the activity rating scale. This decrease in activity is in agreement with work indicating that the destruction of the amygdaloid complex will cause a reduction in affective behavior and emotionality (60, 61, 62, 63).

If it is assumed that the septal area functions as a "quiescent" area, or is related to the function of calming, two questions arise in relation to (a) the basic anatomical design of the septal area, and (b) the area with which it is in functional balance.

Throughout this paper, the septal nuclei have been variously referred to as 'the septal region' or
the 'septal area.' The results obtained seem to contradict any statement which may assign to the septal nuclei any localization of (emotional activity) function. However, the septal nuclei exist as a discrete, accurately localized histological entity. This work indicates that the function of depression is not localized within this group of nuclei.

The septal area has as its function, or one of its functions, a type of depression usually termed emotional depression. With destruction of the septal nuclei, there is an immediate and violent increase in activity, possibly due to a mechanism in the rat which parallels emotionality in the human. With the passage of time, a release phenomenon seems to occur, and the animal gradually returns to a near-normal state. This reaction would preclude any attempt to localize the site of depressant 'emotion' within the septal nuclei.

The animals used do not return to a completely normal state of activity during the period observed, so it is highly probable that the septal nuclei do play a part in 'emotional quiescence,' but it appears that the role played is not very important. The results obtained suggest that the septal nuclei are a 'bridge' over which emotional impulses normally pass. With destruction of this 'bridge,' the impulses are gradually re-routed until near-normalcy is restored.
When the septal nuclei are destroyed, a drastic increase in activity is the result. This activity could be due to a functional imbalance in the mechanism of the animal paralleling emotionality. It would seem then that there is a discrete area, or groups of areas which are concerned with the function of stimulation of the animal. In recent years many workers have postulated that the amygdaloid complex of nuclei is most likely to be the area that is in functional balance with the septal nuclei (62, 60, 63, 61, 66). If it is assumed that the amygdaloid complex is in functional balance with the septal nuclei, and its various supplementary areas, it then follows that the former area is highly stimulatory in function, and that its ablation would cause profound depression.

Depression due to the destruction of the amygdaloid complex has been demonstrated (60, 61, 62, 63). Depression in affective behavior due to destruction of the amygdaloid complex indicates that the amygdala is involved in the production, or control, of flight and aggressive behavior. It is then quite possible that this area is in functional balance with the septal nuclei of the forebrain. Depression of the amygdaloid complex by chlorpromazine should then be an effective means to combat septal hyperactivity.

By employing the assumption that chlorpromazine
acts directly upon the amygdaloid complex it is possible to explain the lack of significant difference between all the groups treated with chlorpromazine.

By depressing the amygdaloid complex to a constant degree in all animals, a certain percentage of its stimulatory function would be disrupted. In all animals, this disturbance of function would be constant. In the normal and operant control animals the depressant portion of the emotional cycle is functioning normally, so that the animals are at a rating level of 8. The balance is gone in the septal animals, with the resulting rating of 20. Both levels of emotional activity are apparently being caused by constant stimulation from the amygdaloid complex. The explanation for septal hyperactivity is simply a lack of functional balance. With depression of the amygdaloid complex to a constant degree in all animals, emotional activity will drop to a constant level, for stimulatory activity is being equally suppressed in all animals.

If the amygdaloid body is the site of action of chlorpromazine, its destruction would remove the activity of the drug. This hypothesis is borne out by the results. It seems quite probable that the major site of action of chlorpromazine is on the amygdaloid. The results lead to the belief that the suggestion of Olds and Travis
(65), that chlorpromazine acts on a forebrain system, is correct.

These results indicate that there is a localization of function within the amygdaloid complex. With destruction of this area, depression of activity is the result. There occurs no recovery of function during the tenure of this experiment, so it is highly possible that the function is centered within the amygdaloid complex, or that the neuronal pathways concerned with stimulatory function all pass through the amygdaloid area. In either case, the amygdala may be considered to be an area of localization of function.

The basic psychological experiments that have been described in the addendum add some weight to the conclusions that have been reached. Septal animals show a much greater activity in performing a learned function, while the activity of amygdaloid animals is greatly reduced from the norm. Septal-amygdaloid animals are reduced in activity, but not to a great extent.

Upon treatment with 8 mg./kg. chlorpromazine, all groups of animals are reduced in activity, although the percentage of reduction of the amygdaloid animals is quite small when compared to the other animals. There is not enough weight of evidence in the psychological experiments for the results to stand alone, but the data
that was gathered does support the evidence from the activity rating scale.

The results of all the experiments indicate that destruction of the amygdaloid complex results in a state similar to that of the chlorpromazine treated animal. It was also noted, that chlorpromazine could not significantly reduce the activity of an amygdaloid animal. It would appear from this result that chlorpromazine exerts its effects on the amygdaloid complex, or on areas which are directly controlled by the amygdaloid complex.

Destruction of the septal nuclei and the amygdaloid complex at the same time results in a slight decrease in activity from the norm. The slight reduction of activity that results could be due to a preponderance of septal, or septal type activity in the 'functional balance' between the septum and amygdala.

In the septal-amygdaloid animals, there is no evidence of a release phenomenon taking place, for the animals behave equally from day to day, and activity neither increases nor decreases significantly during the post-operative testing period. The lack of release phenomenon suggests that there is an area other than the amygdaloid which is in 'functional balance' with the septal nuclei. It is most likely that both the amygdaloid area, and this other area act in a supplementary
manner to one another, and can, in emergencies, perform a function which its supplemental area is no longer capable of performing. It seems that the supplemental area is capable of preventing the overt demonstration of a release phenomenon.

There is further evidence that there is another area with an amygdaloid-type function. This work has shown that the activity rating of septal-amygdaloid animals is 6, prior to treatment with chlorpromazine. It is also shown that chlorpromazine will reduce the activity of septal-amygdaloid animals to 3, as is the case in all other groups of animals. If the amygdaloid complex is the sole site of action of chlorpromazine, the septal-amygdaloid animal would not be depressed. The observation that chlorpromazine will depress the septal-amygdaloid animal suggests that chlorpromazine has a depressant action on a stimulatory area other than the amygdaloid complex, and that this secondary area is depressed to a degree closely approximating the depression of the amygdaloid complex.

The data then indicate that chlorpromazine does not have a single site of action in the brain, but rather that the sites of chlorpromazine activity are multiple.
SUMMARY AND CONCLUSIONS

1. The activity ratings of the five groups of animals before being treated with chlorpromazine were as follows:

- Septal .................. 14.68
- Amygdaloid .............. 3.00
- Septal-Amygdaloid ..... 6.09
- Operant Control ....... 7.93
- Normal .................. 7.89.

2. The activity ratings of the five groups of animals after being treated with chlorpromazine were as follows:

- Septal .................. 3.03
- Amygdaloid .............. 2.88
- Septal-Amygdaloid ..... 3.05
- Operant Control ....... 3.05
- Normal .................. 2.99.

3. Chlorpromazine hydrochloride, at the dose level of 8 mg./kg., is unable to depress LSD#2 rats below the activity level of 3.

4. The activity level of 3 is equivalent to the activity level of animals that have had the amygdaloid complex destroyed.
5. LSD#2 rats are stronger and more resistant to operative procedure than are their albino forebears.

6. The septal nuclei of the forebrain do not seem to possess a localization of emotional activity, due to an ensuing release phenomenon.

7. The temporal amygdaloid complex seems to possess a localization of emotional activity, for there is no return to normalcy after destruction.

8. There is evidence of an additional cranial area which is supplemental in activity to the amygdaloid complex.

9. The amygdaloid does not appear to be the sole site of chlorpromazine activity, although evidence suggests that chlorpromazine does act on the amygdaloid complex.
BIBLIOGRAPHY


AUTOBIOGRAPHY

I, Jacob Rothwacks Raitt, was born Jacob Rothwacks in New York City on May 2, 1936. I received my primary-school education at P.S. 79 in the Bronx, New York, and my secondary-school education at the Bronx High School of Science. I entered the Columbia University College of Pharmacy in 1952, and subsequently received my Bachelor of Science Degree in Pharmacy in 1956, when I entered the Graduate School of The Ohio State University.

In 1957 I was granted an assistantship in Pharmacology at The Ohio State University College of Pharmacy which I held until 1959. At that time I was granted a pre-doctoral research fellowship by the National Institutes of Health (National Institute of Mental Health), division of the Public Health Service of the Department of Health, Education, and Welfare. I held this fellowship until I received the Degree Doctor of Philosophy.