ABSTRACT

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The role of isolation and GABA in the development of muricidal behavior has never been clearly elucidated. In this study one hundred rats were randomly divided into five groups which were either isolated or aggregated for seven days prior to muricidal testing. Upon sacrifice the following brain areas were tested for GABA content: hypothalamus, left and right amygdaloid areas and olfactory bulbs. Fifty-three percent of isolated animals developed muricidal behavior whereas twenty-three percent of the aggregated rats became muricidal. In all brain areas tested GABA was significantly lower in isolated animals. In all regions of brain taken from muricidal animals, GABA was significantly lower than those of their non-muricidal counterparts. Administration of amino-oxyacetic acid, a GABA transaminase inhibitor, resulted in a preferential elevation of GABA levels in the olfactory bulbs. The drug significantly inhibited muricidal behavior in all groups. It is concluded from this study that isolation is a dominant component in the induction of muricidal behavior and that low GABA levels are associated with both muricidal behavior and the isolation process.*

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THE EFFECTS OF AMINO-OXYACETIC ACID AND ISOLATION ON GABA LEVELS IN THE HYPOTHALAMUS, LEFT AMYGDALOID, RIGHT AMYGDALOID AND OLFACTORY BULBS OF MURICIDAL AND NON-MURICIDAL RATS

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Master of Science in Biology

by

Paul Bolin, Jr.

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For those who, for just a short time,
were fellow travelers to diverse places.

Joy at the start
Fear in the journey
Joy in the coming home
A part of the heart
Gets lost in the learning
Somewhere along the road.

- Fogelberg

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ABBREVIATIONS

AOAA	- amino-oxyacetic acid
B_6	- vitamin B_6
C	- control group
CHAT	- choline acetyltransferase
CNS	- central nervous system
CT	- behaviorally tested control group
DPA	- n-dipropylacetate
G	- behaviorally tested experimental group
GABA	- γ-aminobutyric acid
GABA-T	- γ-aminobutyric acid - α-ketoglutarate transaminase
GAD	- glutamic acid decarboxylase
5-HT	- serotonin
HYP	- hypothalamus
i.p.	- intraperitoneal
α-KG	- α-ketoglutarate
LA	- left amygdaloid
OB	- olfactory bulbs
RA	- right amygdaloid
SSDH	- succinic semialdehyde dehydrogenase

INTRODUCTION

Muricidal behavior and intraspecies aggression have both served as indices of aggressive behavior in small laboratory animals. While the role of isolation in intraspecies aggression has been thoroughly examined, the role of isolation in muricidal behavior has never been clearly elucidated. One objective of this research was to study the effects of isolation on muricidal behavior. The effect of age on muricidal behavior was also examined.

The putative neurotransmitter γ-amino butyric acid (GABA) is thought to play an important role in several types of aggressive behavior. Attempts to locate the anatomical sites associated with muricidal behavior have suggested that the olfactory bulbs, amygdala, and hypothalamus are three structures that may be linked to these behaviors. This thesis investigated whether muricidal rats differed from non-muricidal rats in regards to levels of endogenous GABA in regions of the brain thought to be associated with aggressive behavior. Brain areas that were examined were the left amygdaloid, right amygdaloid, olfactory bulbs and hypothalamus because they all have implicated roles in both types of aggressive behavior and they all receive input from GABAergic neurons.

The non-specific GABA-α-ketoglutarate transaminase inhibitor, amino-oxyacetic acid (AOAA), has been shown to raise whole brain GABA levels (Wallach, 1961). AOAA, a once clinically studied anticonvulsant has also been shown to lower the incidence of intraspecies

aggression in laboratory mice (DaVanzo and Sydow, 1979). However, the effects of AOAA on particulate GABA levels within the brain has not been studied. Also, the effectiveness of AOAA as an anti-muricidal agent has not been determined. The following study examined the anti-muricidal activity of AOAA and correlated this behavioral parameter with relative elevation of GABA within the four structures studied.

Recently, it has been suggested that functional laterality exists within the limbic system. By assaying the left and right amygdaloid areas separately, it was possible to test for behavioral laterality in regard to GABA.

REVIEW OF LITERATURE

Within the past decade the formation of a new branch of science, the neurosciences, has come about. This discipline was formed by the hybridization of pharmacology, biochemistry, psychology and electrophysiology. From this new science has come voluminous material on nervous system function; specifically, on the mediators of this function - the neurotransmitters.

GABA was first isolated in central nervous systems in 1950 (Roberts and Frankel, 1950; Awapara, 1950). With the advent of an accurate GABA assay (Scott and Jakoby, 1959), GABA came under extensive study, especially as it relates to changes in behavior. GABA is now considered the major inhibitory neurotransmitter in the CNS.

Even though this thesis will focus on GABA, it must be remembered that there are many synaptically active substances within the CNS. Therefore, the properties of GABA must always be considered in relation to the total chemical environment of the CNS.

Intravenous and intraperitoneal injections of GABA have been shown to effect no change in GABA levels in whole brain (Roberts et al., 1958). These results suggest that GABA does not cross the blood brain barrier. Instead, the amount of GABA in the CNS is the product of enzymatic reactions that reside chiefly within the CNS.

Five amino acids found in the CNS: glutamic acid, aspartic acid, alanine, glutamate and GABA have intimate ties with the Kreb's cycle and thus general brain metabolism (See FIGURE 1).

There are 2 main reactions involving GABA in the CNS. Glutamic

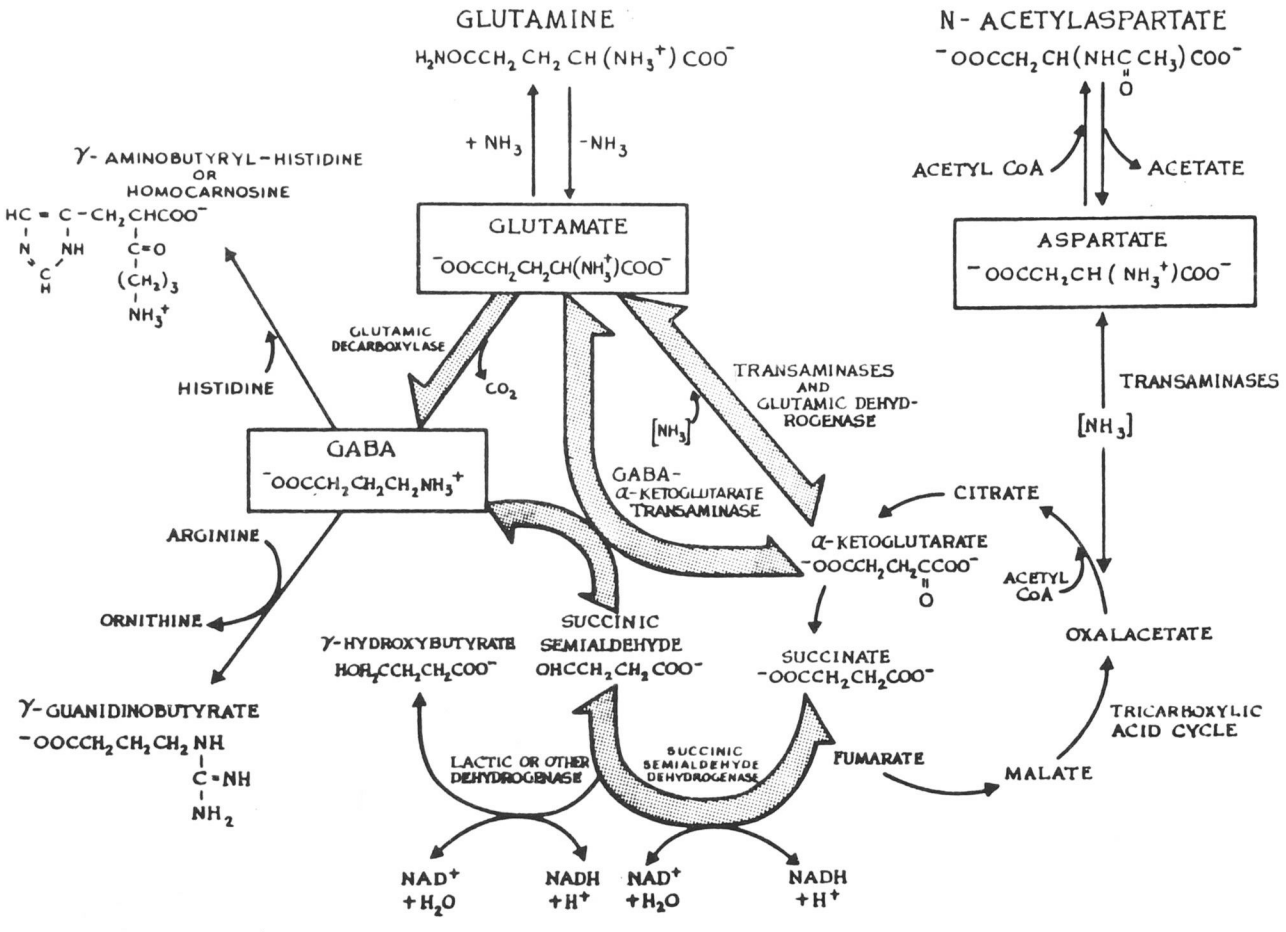
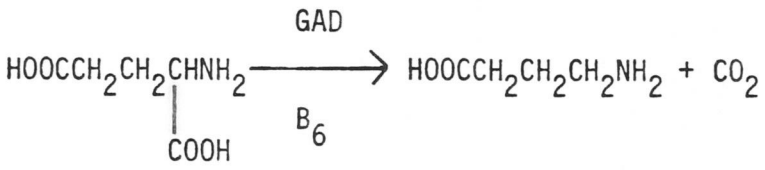


FIG. 1. Outline of chief known reactions of GABA, glutamate and aspartate in the nervous system. The reactions pertinent to GABA metabolism are emphasized by the large arrows.

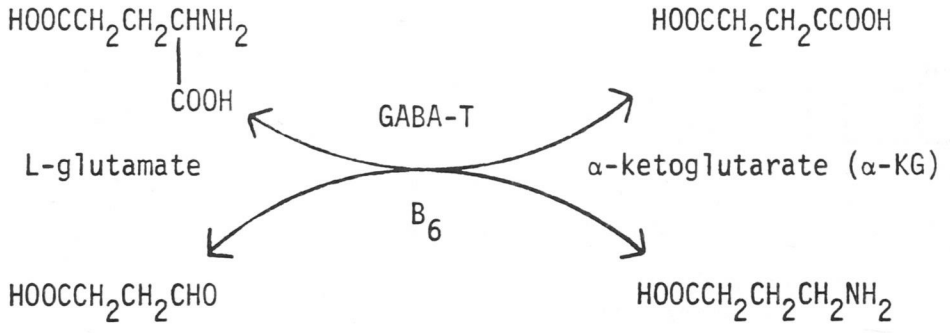
acid decarboxylase (GAD), a B_6 - dependent enzyme is chiefly responsible for the synthesis of GABA, as shown below.



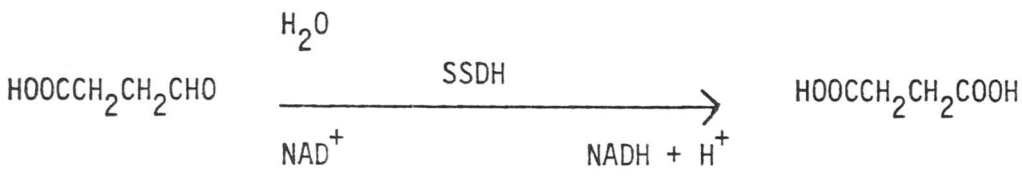
L-glutamate GABA

This decarboxylation is essentially irreversible.

The major catabolic pathway involves GABA-α-ketoglutarate transaminase (GABA-T), also a B_6 - dependent enzyme. This pathway is shown below.



If a continuous source of succinic semialdehyde were available, GABA could be formed by the reversal of the catabolic reaction. However, there is probably no occurrence of this <u>in vivo</u> due to succinic semialdehyde being shunted directly into the Kreb's cycle by succinic semialdehyde dehydrogenase (SSDH).



succinic semialdehyde succinate

Wide variations in GAD and GABA-T activity have been found within the CNS. These variations may be due to the enzyme's degree of association with membranes. Hammond et al. (1970) using computer simulations, estimated that 72% of CNS GABA is located in glial cells, leaving only 28% associated with GABAminergic nerve terminals.

There exists a relationship between GABA levels and GAD activity in various brain areas as demonstrated by Sisken et al. (1960). These results suggest that steady state concentrations of GABA in various areas of brain are normally governed by GAD activity and not GABA-T activity.

GAD possesses maximal activity at pH 6.5; GABA-T at 8.2 Therefore, intracellular acidosis might be expected to increase GABA levels whereas an increase in pH might lower GABA levels. Recent evidence attesting to 2 isozymes of GAD (Martin and Miller, 1976) may help explain regional differences in GABA levels also.

Following are reported endogenous GABA levels (μmole GABA/gram tissue) for the brain areas studied:

Hypothalamus -

3.10 Horton et al. (1979)

3.55 Knieriem et al. (1977)

3.76 Balcom et al. (1975)

3.33 Starr and Tanner (1975)

Olfactory bulbs -

3.00 Balcom et al. (1975)

Amygdala

2.25 (+ pyriform cortex) Balcom et al. (1975)

The hypothalamus has been shown to have circadian fluctuation in GABA levels (Cattabeni et al., 1978). GABA levels reached a peak at the fifth hour of the light period and then dropped 40 ηmole GABA/mg protein until leveling off at the tenth hour of the light period. However, pineal GABA is not thought to be influenced by photoperiodism. Also, GABA has been shown not to influence the adrenergic regulation of pineal serotonin N - acetyl transferase (Mata et al., 1976). The mechanism behind circadian fluctuation of hypothalamic GABA is presently unknown.

Both GAD and GABA-T are B_6 (sp. pyridoxal phosphate) dependent enzymes. GABA binds with the aldehydic group on pyridoxal phosphate (see FIGURE 2). The aldehydic group of pyridoxal phosphate can react with a number of reagents rendering itself unavailable for substrate attachment. The carbonyl trapping agents hydroxylamine, semicarbazide, hydrizine, and AOAA are commonly used in this type of inhibition.

Amino-oxyacetic acid, a once clinically studied anticonvulsant elevates GABA in:

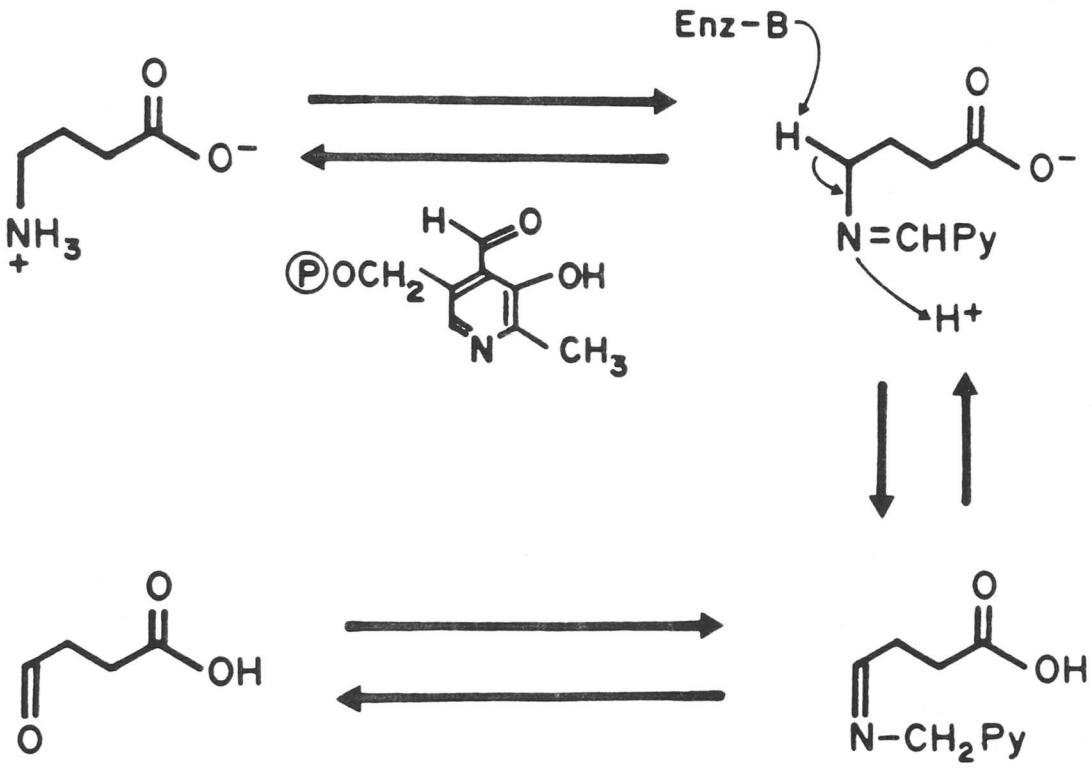


FIGURE 2. The mechanism of pyridoxal phosphate.

whole brain	DaVanzo and Sydow (1979)
hypothalamus	Starr and Tanner (1975)
amygdala	DaVanzo (unpublished data)
olfactory bulbs	DaVanzo (unpublished data)

In whole brain, AOAA elevates GABA to a maximal value in approximately 8 hours, followed by a gradual decline (See FIGURE 3). However, a plot of anticonvulsant activity does not correspond to the graph of GABA elevation with AOAA (Wood and Peesker, 1976) (See FIGURE 4).

Endogenous GABA levels and pharmacological agents that affect GABA levels have been shown to influence other CNS neurotransmitters. The GABA-T inhibitor, n-dipropylacetate (DPA), has been shown to significantly affect norepinephrine turnover rates in the amygdala and medial hypothalamus. DPA also affects serotonin in the amygdala and the lateral hypothalamus (Mandel et al., 1978). Starr and Tanner (1975) found that AOAA could significantly affect noradrenaline in the hypothalamus. Ferkany et al. (1980) demonstrated that AOAA caused a dopamine receptor supersensitivity and a decrease in GABA receptor binding in the corpus striatum. Perez et al. (1977) demonstrated that there may be interaction between GABA, dopamine and acetylcholine in tracts connecting the caudate nucleus to the substantia nigra.

For a number of years, aggressive behavior in lower laboratory animals has served as paradigms for studying various pharmacological agents. Two such models are: (1) isolation-induced intraspecies male aggression (See PLATE 1) and (2) spontaneous interspecies male

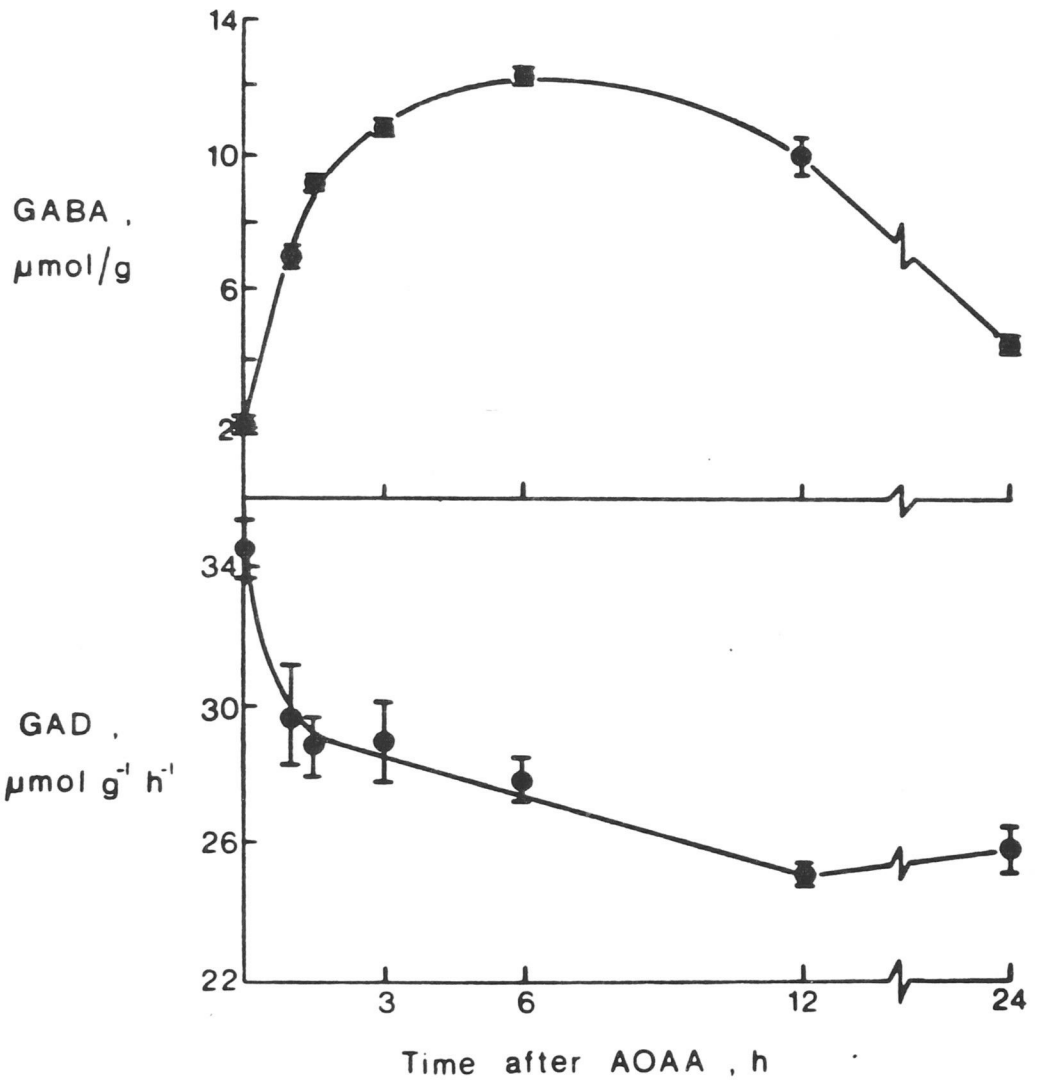


FIGURE 3. The time course of the effect of AOAA on GABA and GAD.

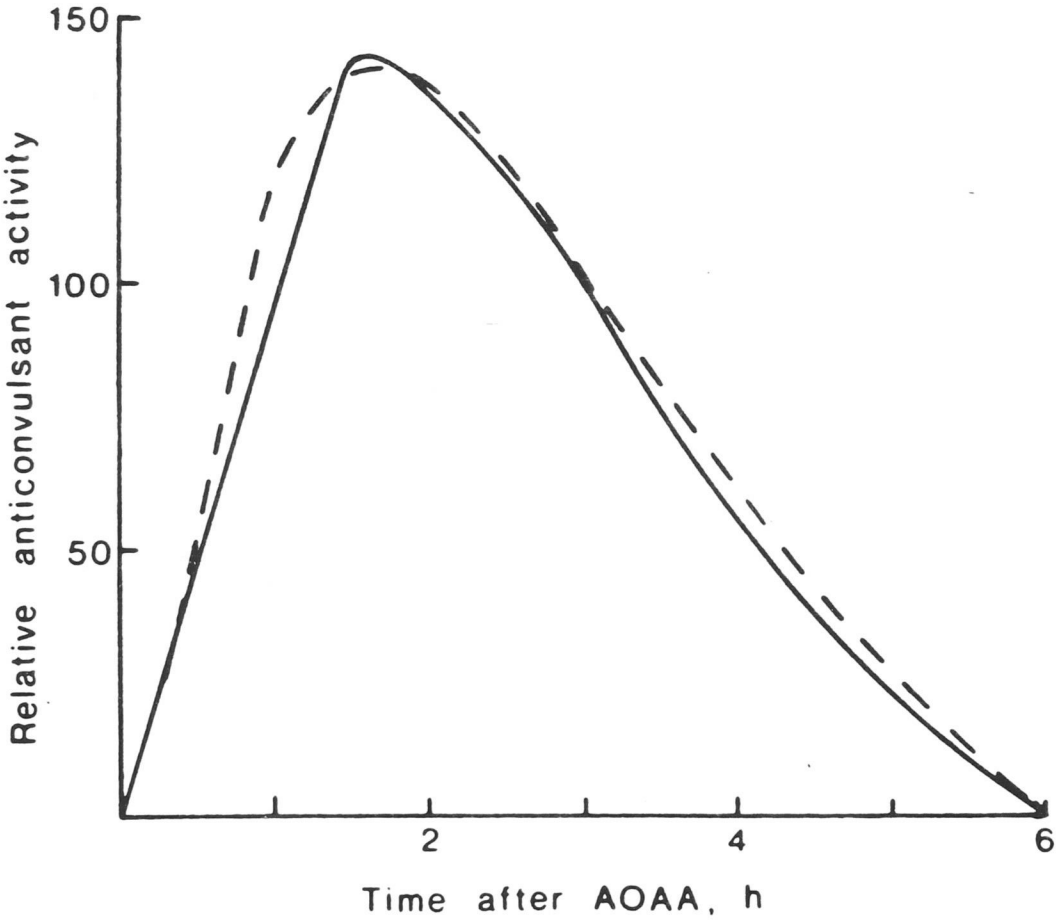


FIGURE 4. The time course of the anticonvulsant activity of AOAA.

PLATE 1

MALE MOUSE INTRASPECIES AGGRESSION

aggression, i.e., muricidal behavior (See PLATE 2 and 3).

Isolation and training constitute the major components of interspecies aggression. In behavioral testing using interspecies aggression, a trained aggressive mouse is exposed to a stimulus animal. The behavioral end point is the latency of attack.

Muricidal behavior is the name given by Horovitz et al. (1966) to direct interspecies aggression of a rat upon a mouse. This behavior was first observed by Karli (1956) and has since enjoyed increasing attention in the literature. Muricidal behavior has four distinct advantages as a pharmacological screen:

(1) No training or warm-up periods are required to potentiate this behavior as is often employed in intraspecies behavioral testing (DaVanzo et al., 1966).

(2) Muricidal behavior is an abberant behavior in the rat.

(3) Convenience is added to a behavioral protocol.

(4) Finally, the main advantage of this model is that it has a clear and precise behavioral end point, namely-interspecies muricide.

Researchers classify muricidal behavior as a lethal interspecies attack by a rat at the upper cervical region of the mouse spinal cord. Muricidal behavior probably involves: (1) a predatory component, (2) an isolation-induced component, and (3) a component of learning. These components will be expanded upon below. Muricidal behavior has been used not only as an index of aggression, but also as a selective model for antidepressant activity (Horovitz, 1966).

PLATE 2

INTERSPECIES AGGRESSION: THE MURICIDAL RAT

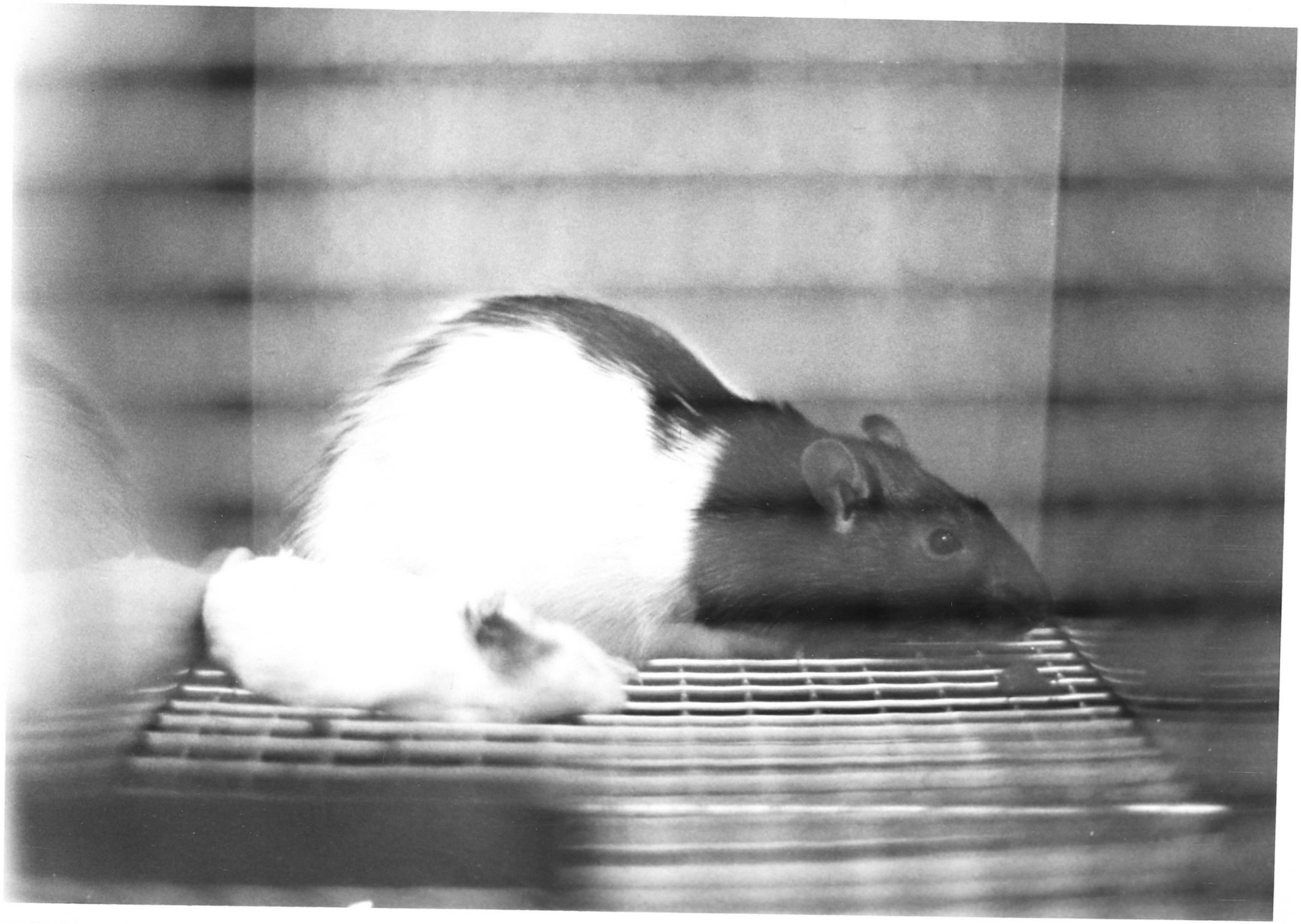
(Note the submissive behavior

displayed by the mouse).

PLATE 3

INTERSPECIES AGGRESSION: THE MURICIDAL RAT

(In most cases, muricidal behavior does not lead to devourment of the mouse)



There are conflicting reports on the effect of isolation on muricidal behavior. Korn and Moyer (1968) and Myer (1969) have reported no significant increases in muricidal behavior with isolation. Gibbons et al. (1979) state that the isolation protocol is merely a convenience. Contrary to the thinking of Gibbons et al. (1979), Broderick (1979) demonstrated that isolation is an important factor in the induction of muricidal behavior. The literature abounds with examples of isolation potentiating muricidal behavior; Karli (1956) (See FIGURE 5) and Broderick (1979) (See FIGURE 6). Janssen et al. (1962) reported that a rat demonstrating muricidal behavior will kill more quickly in an environment to which it is accustomed. Also, Malick induced muricidal behavior in non-killer rats by isolation (quoted in Broderick, 1979). Therefore, isolation can serve several functions in muricidal experimentation:

(1) Acquaintance of the subject to the testing site (Janssen et al., 1962).

(2) Experimental design convenience-individual cage labeling supersedes artificial labeling of the animals themselves.

(3) Potentiation of the muricidal response - in some rat strains with low innate aggressiveness, isolation would be imperative to cost-effective experimentation (Broderick, 1979).

The effects of isolation discussed above are probably comparable to the isolation component in intraspecies aggression described by Scott (1951) and later by Yen (1959). Even though this component adds

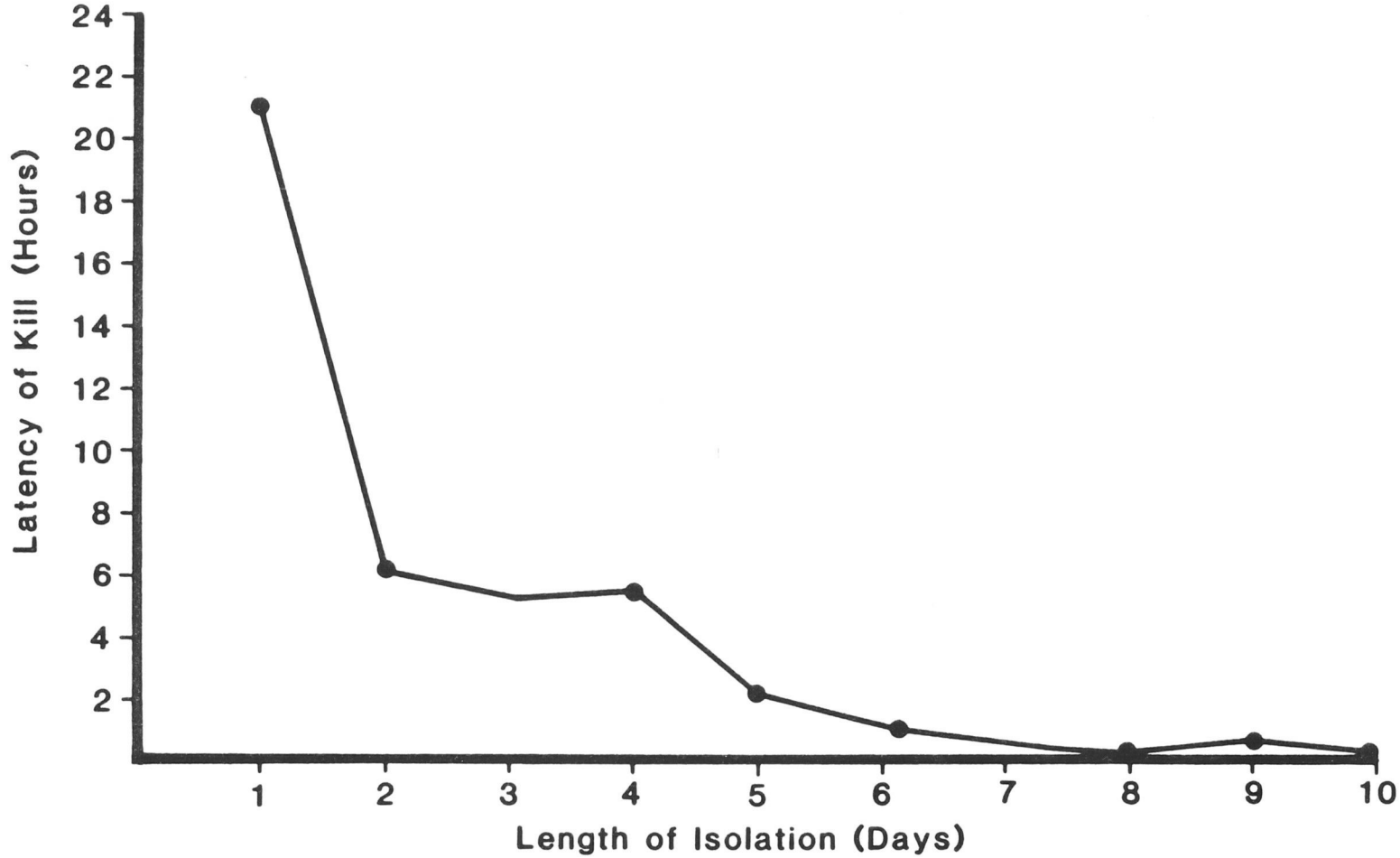


Figure 5. Effects of isolation on latency of muricidal responses
Reproduced from Karli (1956).

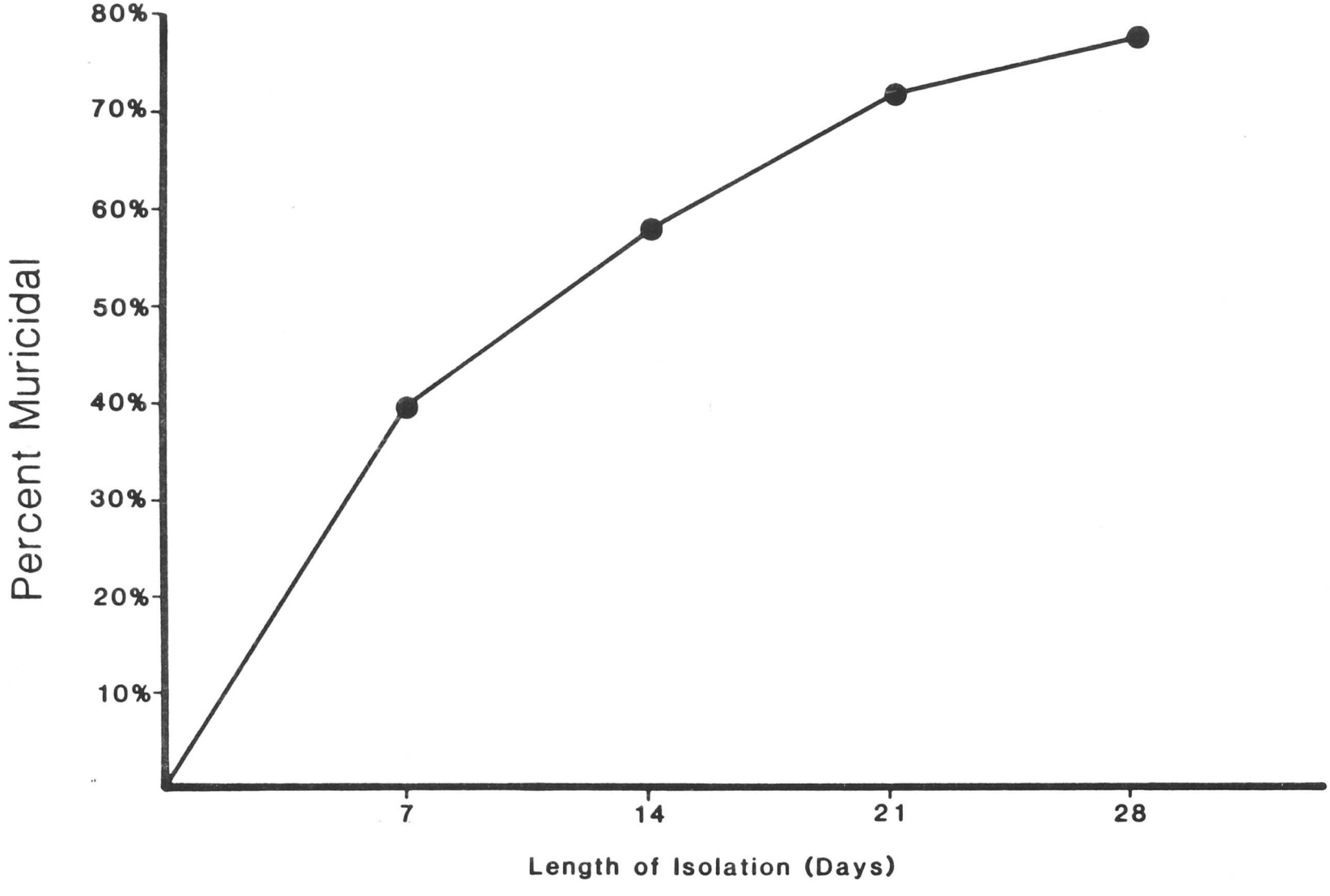


Figure 6. The effects of isolation on muricidal behavior, reproduced from Broderick, (1979).

to the complexity of muricidal testing, many researchers have dealt with this factor by including non-isolated controls in the experimental protocol.

Malick (1975) demonstrated that varying degrees of food deprivation does indeed increase muricidal responses and that eliminating food deprivation, in some cases, does reverse this increase. However, Karli (1956) showed that rats who were non-killers would starve to death rather than kill and devour a mouse which is placed in their home cages. Moyer (1968) described the muricidal response as an instinctive food seeking response. Yet Broderick (1979) observed that muricidal responses, in most cases, did not lead to devourment of the mouse. Karli (1956) demonstrated that a rat could clearly distinguish between a dead mouse, which they devoured almost immediately, and a live one, which they do not kill solely to obtain food. These separate observations suggest that predation does influence muricidal behavior; however, it does not control the behavior.

A distinct dinural rhythm with regard to killing behavior, per se, is present. Seven percent of muricidal rats failed to kill repeatedly during the evening hours (Broderick, 1979). Also, DiChiara (1971) observed that muricidal responses were minimal during the middle of both the light and dark experimental periods. This is quite interesting considering the circadian fluctuations in hypothalamic GABA levels (Cattabeni et al., 1978).

This author has found no specific mention in the literature of the effect of the mouse on muricidal behavior. However, it appears

obvious that the social-emotional responsiveness of the mouse affects the behavior responses of the rat.

Methods for muricidal classification vary. Eichelman and Thoa (1973) and Gibbons et al. (1979) classified rats as muricidal or non-muricidal by allowing the mouse to remain in the test cage for a period of 24 hours. This is contrary to the "spontaneous kill" originally described by Karli (1956). The literature varies greatly with respect to the length of the muricidal test; e.g. 5 sec. exposure (Horovitz, 1966); 5 minutes (Malick, 1975b); 24 hours (Barr, 1978); to 3 weeks (Hull and Homan, 1975). The majority of investigators use the convenient time of 24 hours probably to eliminate any dinural effects. Some investigators see the need for multiple tests (Broderick, 1979) and (Myer, 1969).

Given the same environmental and experimental conditions discussed above, not all rats kill. Several other factors influence the induction of muricidal behavior.

Almost every strain of the rat species, Rattus norvegicus, has demonstrated muricidal behavior, e.g. Holtzman (Horovitz et al., 1966); Long-Evans (Malick, 1975, Gibbons et al., 1978, 1979, Barr et al., 1978, Broderick, 1979); NIH (Mukherjee and Pradhan, 1976); Sprague-Dawley (Marks et al., 1978, Hull and Homan, 1975); Wistar (Karli, 1956, 1960, Vergenes et al., 1974). There is no mention in the literature of a specific strain effect on muricidal behavior. However, barring experimental differences, the strains do vary in behavior. It has been reported that percentages of killers vary

according to rat strain (Bandler and Moyer, 1970). Wild rats, Rattus rattus, have considerably greater percentages of killers in their population than domesticated rats, Rattus norvegicus (Karli, 1956).

The number of rats that developed muricidal behavior in Holtzman was higher than in four other strains tested (Horovitz et al., 1966). Broderick (1979) found a 40% muricidal response in the Long-Evans rats, while Barr (1978) observed a 45% occurrence of muricidal behavior in this strain.

Once the killing behavior has been established, it persists indefinitely (DiChiara et al., 1971; Broderick, 1979). However, Broderick (1979) demonstrated that the latency of kill is subject to change. Karli (1956) demonstrated that repeated testing of a previously muricidal rat will decrease the latency of kill. This observation probably represents the influence of learning on muricidal behavior.

The age of the rat is not a controlling factor as far as the time of muricidal induction is concerned (Myer, 1971). This author found no mention in the literature of a specific effect of age on muricidal behavior.

The central nervous system cannot be viewed as a whole consisting of separate elements, each having its own individual function. Rather, individual structures confer further dimensions upon other structures, the summation of these interactions leading to the unique behavior of an individual. This effect is very pronounced within the limbic system. Therefore, the following central nervous system structures associated with muricidal behavior can only be fully understood when they are

considered against the background to total brain physiology.

Karli and Vergnes (1964) demonstrated suppression of muricidal behavior by placing bilateral lesions within the lateral and posterior areas of the hypothalamus. Bilateral destruction of the dorsomedial thalamic nuclei have been shown to evoke muricidal responses (Eclancher and Karli, 1971). Albert and Brayley (1979) demonstrated that the lateral septum, the region ventral to the anterior septum, and the medial hypothalamus are each important inhibitory areas modulating muricidal behavior. Allikmets and Ditrikh (1965) observed that septal lesions increase the occurrence of spontaneous aggression among male rats. Karli and Vergnes (1965), by placing extensive lesions in various parts of the amygdala (lateral, cortico-basal, and centro-medial lesions) in a series of domesticated mice found that the centro-medial nucleus was the crucial area in facilitating muricidal behavior. A bilateral transection of the amygdalo-hypothalamic fibers passing through the ansa lenticularis abolishes the rat's mouse-killing behavior (Vergnes and Karli, 1964) but does not seem to have any effect on intraspecies aggression (Grossman, 1970).

Vergnes et al. (1974) showed that there may be inhibitory mechanisms for controlling muricidal behavior in both the olfactory bulbs and the raphe nuclei. Vergnes and Karli (1963) increased muricidal responses without affecting intermale behavior in the same species by removing the olfactory bulbs. However, Ropartz (1968) reported that removal of the olfactory bulbs suppresses spontaneous aggression among male mice. Bandler and Vergnes (1979) demonstrated

that the diagonal band of Broca is probably an inhibitory area modulating muricidal behavior. Moreover, it has been suggested that ascending 5-HT (serotonin) pathways between the septum and midbrain raphe may serve an inhibitory role in muricidal behavior (Marks et al., 1977). DaVanzo et al. (1966) enucleated the eyes of aggressive mice and observed no reduction in aggressive behavior. This author has found no mention in the literature of the above experiment being performed with muricidal rats.

Both central and systemic biochemical factors are associated with the induction and maintenance of muricidal behavior. Testosterone has been implicated as playing a major role in the development and maintenance of aggressive behavior in mice (Scott et al., 1951); (Mugford, 1974). However, Earley and Leonard (1977) published data supporting that low testosterone is associated with high aggressiveness and low GABA levels in all areas studied except the amygdala.

Serotonin has been implicated in mediating the inhibition of aggressive behavior (DiChiara et al., 1971). Gibbons et al. (1979) demonstrated that maintaining rats on a tryptophan free diet for 4 to 6 days induced muricidal responses in non-killer rats and potentiated killing in muricidal rats. Muricide induction has been demonstrated in rats placed on a thiamine deficient diet. This induction was then reversed by intraperitoneal (i.p.) injections of 5-hydroxytryptophan, a precursor of serotonin (Onodera et al., 1979). Moreover, Kulkarni (1968) has implicated the amygdala as the site of muricidal inhibition by serotonin.

Kulkarni (1968) demonstrated that norepinephrine may have an inhibitory effect on interspecies aggression. There is evidence that the amygdala plays a central role in this response (Horovitz, 1966; Leaf, 1970). Horovitz et al. (1965) demonstrated that both amphetamine and imipramine are effective antimuricidal agents. Depletion of norepinephrine by alpha-methyl-para-tyrosine has been shown to potentiate muricidal behavior (Leaf et al., 1969). Welch and Welch (1971) demonstrated that the turnover and release of norepinephrine is lower in isolated than aggregated mice.

This author has found no mention in the literature of a link between dopamine and muricidal behavior. However, drugs known to potentiate dopamine availability (e.g. amphetamine, L-DOPA, and monamine oxidase inhibitors), are effective antimuricide agents (Horovitz et al., 1965; Kulkarni, 1968).

This author has found no evidence linking endogenous brain acetylcholine levels with muricide. Moreover, the available evidence is negative (Consolo and Valzelli, 1970). However, some peripherally administered anticholinergics are effective muricidal blockers. McCarthy (1966) reported that cholinergic stimulation with pilocarpine induced muricidal responses in non-killer rats. It should be noted, however, that pilocarpine affects norepinephrine levels also.

GABA has also been shown to have an inhibitory effect on aggressive behavior. DaVanzo and Sydow (1979) demonstrated dose-dependent suppression of intraspecific aggression with AOAA. In previously muricidal rats, baclofen, muscimol and GABA-cetylester inhibited

muricidal responses in a dose-dependent fashion (Delini-Stula and Vassout, 1978). Mack et al. (1975) demonstrated that the GABA content of the olfactory bulbs was much lower (-30%) in muricidal rats than their non-muricidal counterparts. Mandel et al. (1978) blocked muricidal behavior with bilateral intraolfactory bulb injections of n-dipropylacetate (DPA) a competitive inhibitor of GABA-T. Earley and Leonard (1977) demonstrated lower levels of GABA in the striatum, hippocampus, amygdala, and the olfactory bulbs of isolated aggressive mice when compared to aggregated non-aggressive mice.

There may be some link between the GABAergic and cholinergic systems within the amygdala. Killer rats following muricide blockage with DPA demonstrated significantly lower choline acetyltransferase (CHAT), an acetylcholine synthesizing enzyme, activity in the amygdala than non-treated killers (Mandel et al., 1978). This demonstrated a correlation between low olfactory bulb GABA levels and high amygdaloid CHAT activity. This fact is quite interesting considering that it has been suggested that a GABAergic neural tract originating in the olfactory bulbs may modulate inhibitory impulses to the amygdala (Mack and Mandel, 1976).

MATERIALS AND METHODS

Housing

Long Evans rats (male, 200-225 mg)[1] upon receipt were stored in aggregated housing (22 x 38 x 48 cm plastic cages) in groups of six until a given day of each week of the experimental period. On this day, subjects were separated at random into three housing groups for testing. The background control group (C) was housed in an aggregated manner for one week and then sacrificed without muricidal testing. Tested control groups (CT_n) were housed aggregated for one week and then tested for muricidal behavior immediately prior to being sacrificed. After separation, all group G subjects were removed to isolated housing (18 x 18 x 24 cm self-cleaning wire cages) and kept at constant temperature and humidity with twelve hours light (9:00 a.m. to 9:00 p.m.) and twelve hours dark (9:00 p.m. to 9:00 a.m.). Racks of cages were positioned so that the isolated rats had no visual contact with any other rat. Both groups C and CT remained in aggregated housing under conditions described above. All groups received water and Wayne Rat Chow[2] ad libitum.

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[1]Blue Spruce Farms, Altamont, N.Y.

[2]Allied Mills Inc., Peoria, Ill.

Muricidal Testing

After one week of isolation, the animals in group CT were moved to individual wire cages (18 x 18 x 24 cm). At 3:00 p.m. on this day each animal in groups G and CT was exposed to a white mouse (aggregated male, 30-50g)[3] for a period of four hours. Latency of kill was recorded and the subjects sacrificed either immediately upon the death of the mouse or at the end of the exposure period, whichever came first.

Rats that killed mice within four hours were classified as muricidal. Rats not killing within four hours were classified as non-muricidal. Rats that attacked within four hours with the mouse not dying within the four hour period from inflicted wounds were classified as aggressive. Rats that attacked within four hours with the mouse dying within the four hour period from inflicted wounds were classified as muricidal and latency of kill was recorded as the delay of a lethal attack.

AOAA Treatment

Rats treated with AOAA were handled as above. However, after behavioral classification, the subjects were returned to their experimental housing: G remained isolated and CT was returned to aggregated housing. At 2:00 p.m. on the day following classification, G and CT rats received i.p. injections of AOAA.[4] Injections were made with a concentration of 6 mg AOAA/cc 0.9% saline. Injection volume was

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[3]Flow Labs, Inc., Dublin, N.C.

[4]The UpJohn Company, Kalamazoo, Mich.

adjusted according to weight to achieve a dosage of 20 mg/kg. After a one hour recovery period, the subjects were retested for muricidal behavior as above except all subjects were sacrificed at the end of the test period regardless of how they were classified. Subjects were staggered through the above test procedure in five minute intervals to insure equal exposure time to AOAA and allow time for injection, classification, sacrificing, etc.

Dissection of Brain Parts

Upon sacrifice by decapitation, the brain was carefully removed and placed on the ventral surface. The olfactory bulbs were dissected from the overlying frontal cortex by a coronal cut placed as close to the cortex as possible. The brain was then turned with the ventral surface up. Two coronal cuts were made along the width of the brain; first at the caudal end of the optic chiasma and then caudally to the mammillary bodies. This central coronal section was then turned upon the rostral surface. A horizontal section was then made from the left rhinal fissure to the right rhinal fissure. Two oblique sagittal sections were then made along each internal capsule, removing the left and right amygdaloid areas. A transverse section was then made along the mammillary fasciculus to remove the hypothalamus. (See Appendix I for dissection coordinates). The tissues were then frozen in dry ice. The above dissection was performed within 90 to 120 seconds to prevent postmortem GABA elevation. Tissues were then stored at -40°C until being assayed for GABA.

GABA Assay

Individual tissues were weighed on a Mettler PN163 balance[5] and then diluted 1:20 in a solution of 75 parts 95% ethanol to 25 parts H_2O. The diluted tissue was then homogenized for 30 seconds using a glass teflon homogenizer.[6] The homogenate was then contrifuged on a Damon/IEC B-20A centrifuge[7] at 4°C, 7,000 g, for 15 minutes. A 0.2 ml portion of the supernatant was evaporated on a Brinkman Rotovapor-R[8] and then reconstituted in 0.4 ml of a 0.1 M sodium pyrophosphate buffer (pH 8.6).

A Coleman 55 spectrophotometer[9] was used to assay the amount of GABA present in each sample. This assay involves the indirect production of NADPH which is then measured spectrophotometrically at 340 nm. The sequence of reactions is shown diagramatically below.

$$\text{GABA} + \alpha\text{-ketoglutarate} \xrightarrow[\text{transaminase (GABA-T)}]{\text{GABA}/\alpha\text{-ketoglutarate}} \text{succinic semialdehyde} + \text{glutamate}$$

$$\text{succinic semialdehyde} + \text{NADP} \xrightarrow[\text{dehydrogenase (SSDH)}]{\text{succinic semialdehyde}} \text{succinate} + \text{NADPH}$$

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[5]Mettler Instrument Corporation, Hightstown, NJ

[6]Fisher Scientific Co., Raleigh, NC

[7]Damon/IEC Division, Needham Hts, Mass.

[8]Fisher Scientific Co.,

[9]Coleman Instruments Division, Oak Brook, Ill.

The assay for transamination and subsequent dehydrogenase reactions was facilitated by the use of Gabase,[10] an enzyme prepared commercially from Pseudomonas fluorescens. Briefly, 0.3 ml of tissue was combined with 2.3 ml of a 0.1 M sodium pyrophosphate buffer (pH 8.6), 0.15 ml of 0.004M NADP, and 0.1 ml of Gabase (2 units/ml). After determining the initial optical density, tubes were incubated with 0.15 ml of 0.013 M α-ketoglutarate[12] for one hour. At this time, final optical density was determined and GABA concentrations calculated using the difference between the two readings. A reagent blank and standard curve was prepared for each assay. (See FIGURE 7 for assay flowchart).

Statistics

Assay results were pooled and analyzed by use of mean, standard error of the mean, one-way analysis of variance, three-way analysis of variance, analysis of variance with covariance, t-test, and chi-square analysis.

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[10]Sigma Chemical Company, St. Louis, Mo.

[11]Sigma Chemical Company

[12]Sigma Chemical Company

tissue

weigh
dilute 1:20 in 75 parts (95% EtOH) and 25 parts (H_2O)
homogenize
centrifuge

supernatant

evaporate 0.2 ml of supernatant
reconstitute in 0.4 ml of sodium pyrophosphate buffer

0.3 ml of reconstituted tissue

add 0.15 ml of 0.004M NADP
add 0.1 ml of 2 units 1 ml Gabase
add 2.3 ml of sodium pyrophosphate buffer

initial OD reading at 340mm

add 0.15 ml of 0.013M α-KG
allow one hour reaction time

final OD reading at 340nm

FIGURE 7 Flowchart for the spectrophotometric determination of GABA

RESULTS

GABA Levels in Various Brain Regions

Resting GABA levels in a random sample population were found to vary within the four limbic structures studied. The hypothalamus had the highest GABA levels with the olfactory bulbs, right amygdala, and left amygdala having lower levels in decreasing order (see TABLE 1). These values were then compared to the means for all non-drug treated experimental rats as a quality-control check for the dissection and subsequent assay procedures. In no case, did the mean for the structures studied vary statistically from the mean for the random population

Isolation and Muricidal Behavior: GABA Level Correlation

Isolation was shown to be a dominant factor in the induction of muricidal behavior. Rats isolated for 7 days exhibited a greater incidence of muricidal activity (53%) than did their aggregated counterparts (23%) (See FIGURE 8).

Isolated rats also demonstrated significantly lower regional brain GABA levels ($p<0.05$) in all areas studied (See TABLE 2). The same general trend was observed when the data in TABLE 2 were divided by behavioral classification (See TABLE 3). Significantly lower ($p<0.001$) GABA levels due to muricidal influence were only observed in the hypothalamus, however (See FIGURE 9). Also shown in FIGURE 9, muricidal rats have lower GABA levels in all areas studied when compared to non-muricidal rats with the exception of the left

TABLE 1. MEAN GABA LEVELS FOR RANDOM POPULATION AND ALL NON-DRUG TREATED EXPERIMENTAL RATS

Area	Control Group (η=6)	Experimental Group (η=51)
Hypothalamus	4.79 ± 0.33	4.51 ± 0.13
Left Amygdaloid	2.06 ± 0.35	1.97 ± 0.09
Right Amygdaloid	2.08 ± 0.25	2.10 ± 0.08
Olfactory Bulbs	3.06 ± 0.35	2.74 ± 0.13

GABA values in μmoles/gram tissue (Mean ± SEM).
No significant differences, Student's *t*-test.

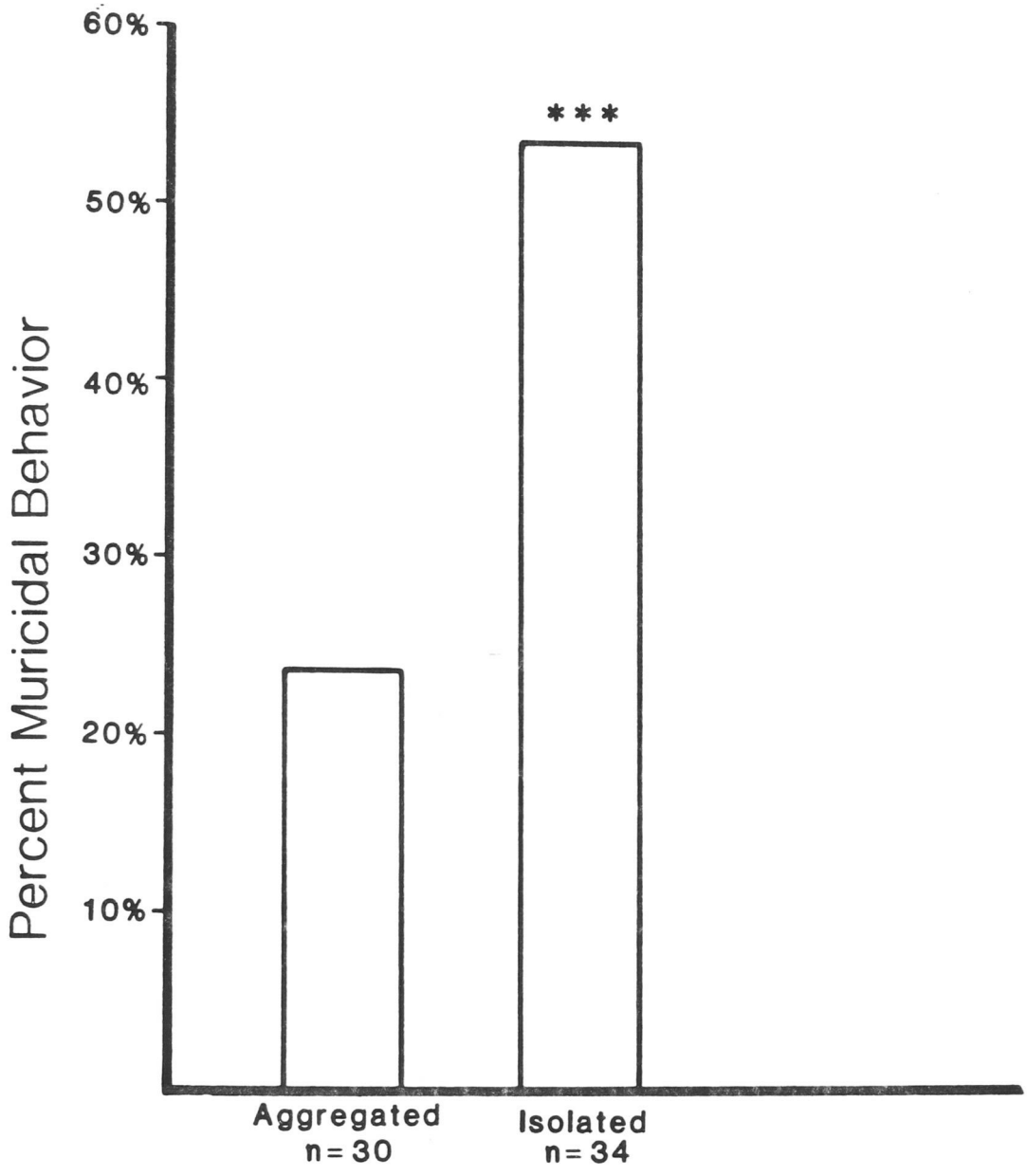

Figure 8. The effect of isolation on the development of muricidal behavior.

***** p<0.001, chi-square analysis**

TABLE 2. PARTICULATE GABA LEVELS IN ISOLATED VS. AGGREGATED RATS

	Aggregated (η=17)	Isolated (η=34)
Hypothalamus	4.93 ± 0.20	4.30 ± 0.16*
Left Amygdaloid	2.32 ± 0.15	1.80 ± 0.09**
Right Amygdaloid	2.34 ± 0.15	1.98 ± 0.10*
Olfactory Bulbs	3.22 ± 0.26	2.50 ± 0.12*

GABA values in μmoles/gram tissue (Mean ± SEM).
** $P<0.01$, Student's *t*-test.
* $P<0.05$, Student's *t*-test.

TABLE 3. PARTICULATE GABA LEVELS IN ISOLATED AND AGGREGATED RATS AS INFLUENCED BY BEHAVIOR

		Aggregated (n)	Isolated (n)
Hypothalamus	Non-Muricidal	5.22 ± 0.29 (10)	4.80 ± 0.17 (16)
	Muricidal	4.53 ± 0.19 (7)	3.85 ± 0.20 (18)*
Left Amygdaloid	Non-Muricidal	2.25 ± 0.17 (10)	1.82 ± 0.15 (16)
	Muricidal	2.42 ± 0.27 (7)	1.78 ± 0.12 (18)
Right Amygdaloid	Non-Muricidal	2.43 ± 0.22 (10)	2.11 ± 0.11 (16)
	Muricidal	2.22 ± 0.19 (7)	1.87 ± 0.15 (18)
Olfactory Bulbs	Non-Muricidal	3.40 ± 0.42 (10)	2.67 ± 0.18 (16)
	Muricidal	2.97 ± 0.21 (7)	2.36 ± 0.17 (18)*

GABA values in μmoles/gram tissue (Mean ± SEM).
* $P<0.05$, Student's *t*-test.

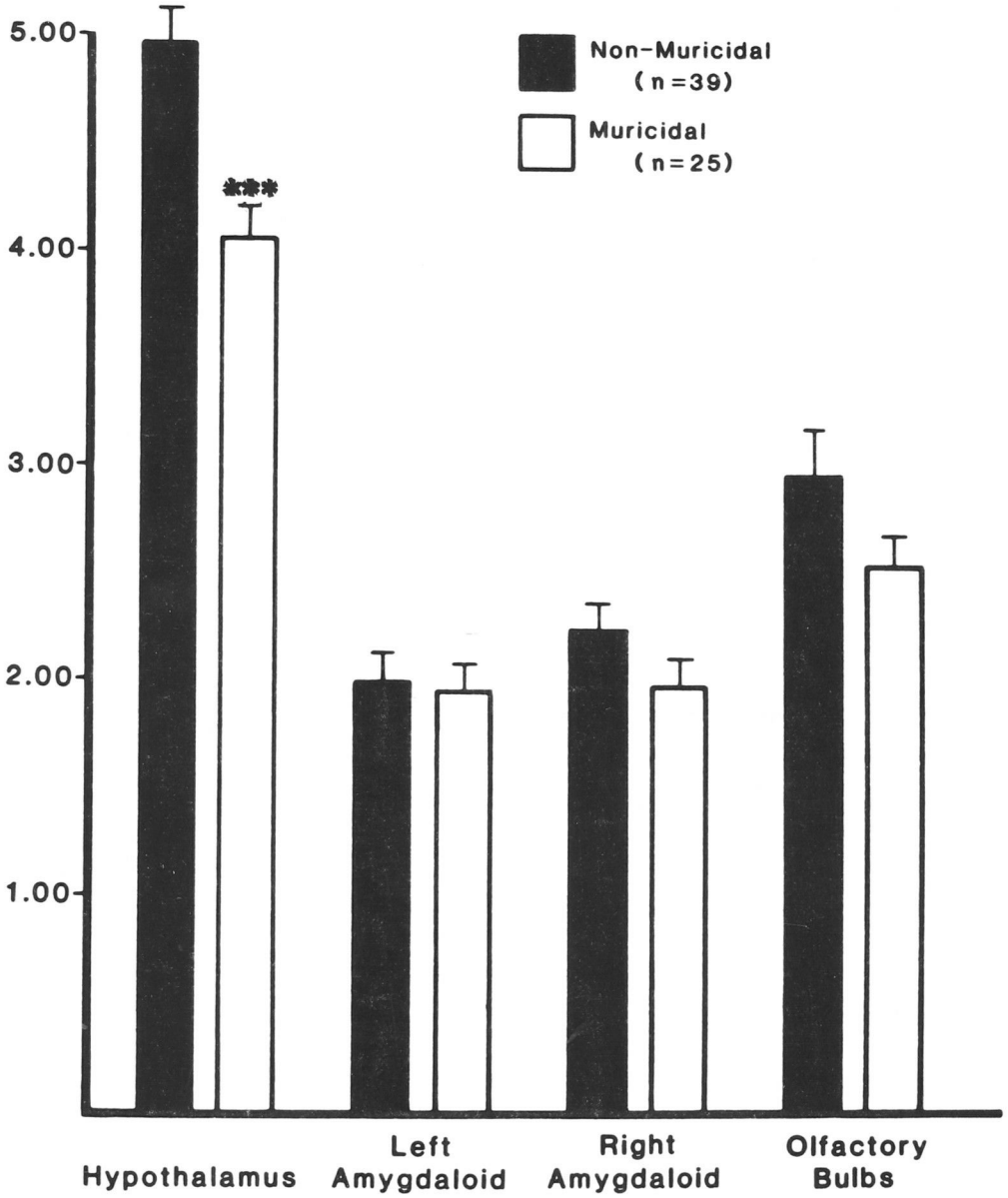

Figure 9. Particulate GABA levels in muricidal and non-muricidal rats.

*** GABA values in μ moles/gram tissue (mean ± SEM)
$p < 0.001$, Students t test.

amygdala. The data in TABLE 4 reflect this same trend when the populations are separated according to pre-test housing experience.

AOAA and Muricidal Behavior

AOAA was shown to be an effective anti-muricidal agent in both isolated and aggregated rats (See FIGURE 10). However, as seen in FIGURE 11, AOAA was a significantly more effective ($p<0.001$) anti-muricidal agent in isolated rats than in aggregated rats. Also, in no case did a previously non-muricidal rat become muricidal after AOAA treatment.

AOAA Effects on Regional GABA

AOAA was shown to significantly elevate GABA levels ($p<0.001$) in all areas studied (See TABLE 5). However, the degree of elevation varied within the four structures studied. The olfactory bulbs displayed a 102% increase, while the hypothalamus exhibited a mere 19% increase. The amygdala experienced an elevation of approximately 81% in both the left and right hemispheres.

AOAA Effects on Particulate GABA Levels: Correlation with Isolation and Muricidal Behavior

Isolated rats demonstrated high GABA elevation due to AOAA in all brain areas studied except the right amygdala (See TABLE 6). However, this trend is not as consistent when the data are divided according to pre-treatment behavioral classification (See TABLE 7).

Following AOAA-treatment, muricidal rats had significantly higher GABA levels than non-treated muricidal rats in all areas studied

TABLE 4 PARTICULATE GABA LEVELS IN MURICIDAL AND NON-MURICIDAL CONTROLS AS INFLUENCED BY HOUSING

		Non-Muricidal (η)	Muricidal (η)
Hypothalamus	Isolated	4.80 ± 0.17 (16)	3.85 ± 0.20 (18)***
	Aggregated	5.22 ± 0.29 (10)	4.53 ± 0.19 (7)
Left Amygdaloid	Isolated	1.82 ± 0.15 (16)	1.78 ± 0.12 (18)
	Aggregated	2.25 ± 0.17 (10)	2.42 ± 0.27 (7)
Right Amygdaloid	Isolated	2.11 ± 0.11 (16)	1.87 ± 0.15 (18)
	Aggregated	2.43 ± 0.22 (10)	2.22 ± 0.19 (7)
Olfactory Bulbs	Isolated	2.67 ± 0.18 (16)	2.36 ± 0.17 (18)
	Aggregated	3.40 ± 0.42 (10)	2.97 ± 0.21 (7)

GABA values in μmoles/gram tissue (Mean ± SEM).
*** $P<0.001$, Student's *t*-test.

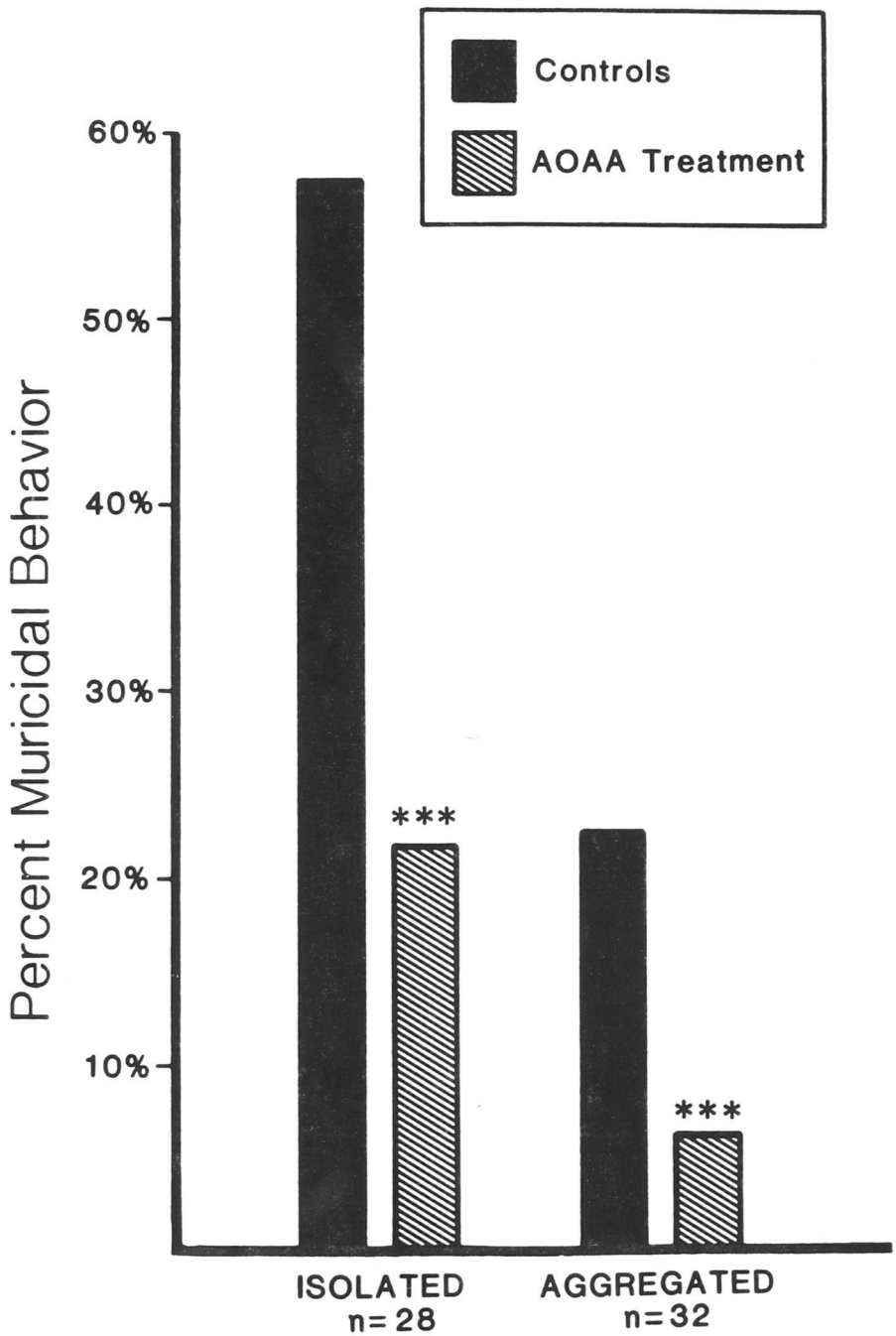

Figure 10 Reversal of muricidal behavior by AOAA in aggregated and isolated rats.

***p<0.001,chi-square analysis

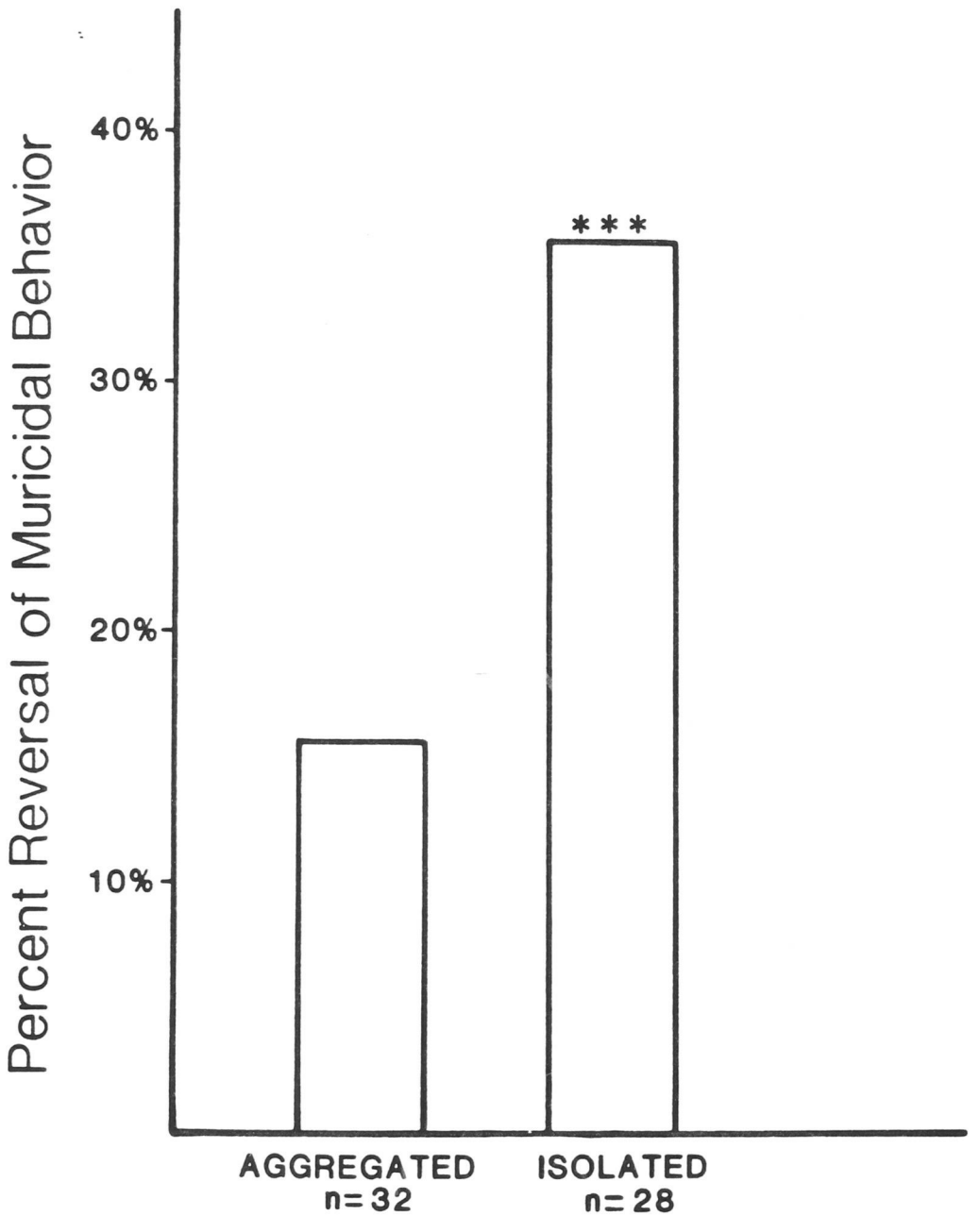

FIGURE 11. The effect of isolation on the percent drop in muricidal behavior due to AOAA treatment.

***p < 0.001, chi-square analysis

TABLE 5 THE EFFECT OF AOAA ON PARTICULATE GABA

	Non-Treated (η=51)	AOAA Treatment (η=43)	Percent GABA Elevation
Hypothalamus	4.51 ± 0.13	5.35 ± 0.15***	18.6%
Left Amygdaloid	1.97 ± 0.09	3.57 ± 0.16***	81.2%
Right Amygdaloid	2.10 ± 0.08	3.81 ± 0.17***	81.4%
Olfactory Bulbs	2.74 ± 0.13	5.54 ± 0.27***	102.2%

GABA values in μmoles/gram tissue (Mean ± SEM).
*** $P<0.001$, Student's t-test.

TABLE 6 GABA ELEVATION DUE TO AOAA TREATMENT IN AGGREGATED AND ISOLATED RATS

	Aggregated	Isolated
Hypothalamus	0.79 (16.0%)	0.86 (20.0%)
Left Amygdaloid	1.84 (79.3%)	1.44 (80.0%)
Right Amygdaloid	2.14 (91.5%)	1.47 (74.2%)
Olfactory Bulbs	3.12 (96.9%)	2.60 (104.0%)

Values are absolute increases (μmoles/gram tissue) with percent elevation when compared to comparable non-drug treated groups.

TABLE 7 GABA ELEVATION DUE TO AOAA TREATMENT IN AGGREGATED AND ISOLATED RATS AS INFLUENCED BY PRE-TREATMENT BEHAVIORAL CLASSIFICATION

	Behavioral Classification Before AOAA Treatment	Aggregated	Isolated
Hypothalamus	Non-Muricidal	0.98 (18.8%)	0.52 (10.8%)
	Muricidal	0.72 (15.9%)	1.07 (27.8%)
Left Amygdaloid	Non-Muricidal	2.17 (96.4%)	2.01 (110.4%)
	Muricidal	0.58 (24.0%)	0.50 (28.1%)
Right Amygdaloid	Non-Muricidal	2.12 (87.2%)	1.72 (81.5%)
	Muricidal	1.09 (49.1%)	0.76 (40.6%)
Olfactory Bulbs	Non-Muricidal	3.69 (108.5%)	3.57 (133.7%)
	Muricidal	1.44 (48.5%)	1.13 (47.9%)

Values are absolute increases (μmoles/gram tissue) with percent elevation when compared to comparable non-drug treated groups.

(See FIGURE 12 and TABLE 8). Non-muricidal rats also demonstrated higher levels of GABA following AOAA treatment (See FIGURE 13). However, it is interesting to note that when the hypothalamus data are divided according to previous housing conditions, all areas showed high significance ($p<0.001$) except the hypothalamus which demonstrated no significance in either housing group (See TABLE 9).

Muricidal rats that demonstrated behavioral changes following AOAA had higher GABA levels than those remaining muricidal in all brain areas studied (See TABLE 10). FIGURE 14 and TABLE 10 demonstrate that the trend associating low GABA with high aggressiveness still holds true when the data are collected with animals treated with AOAA.

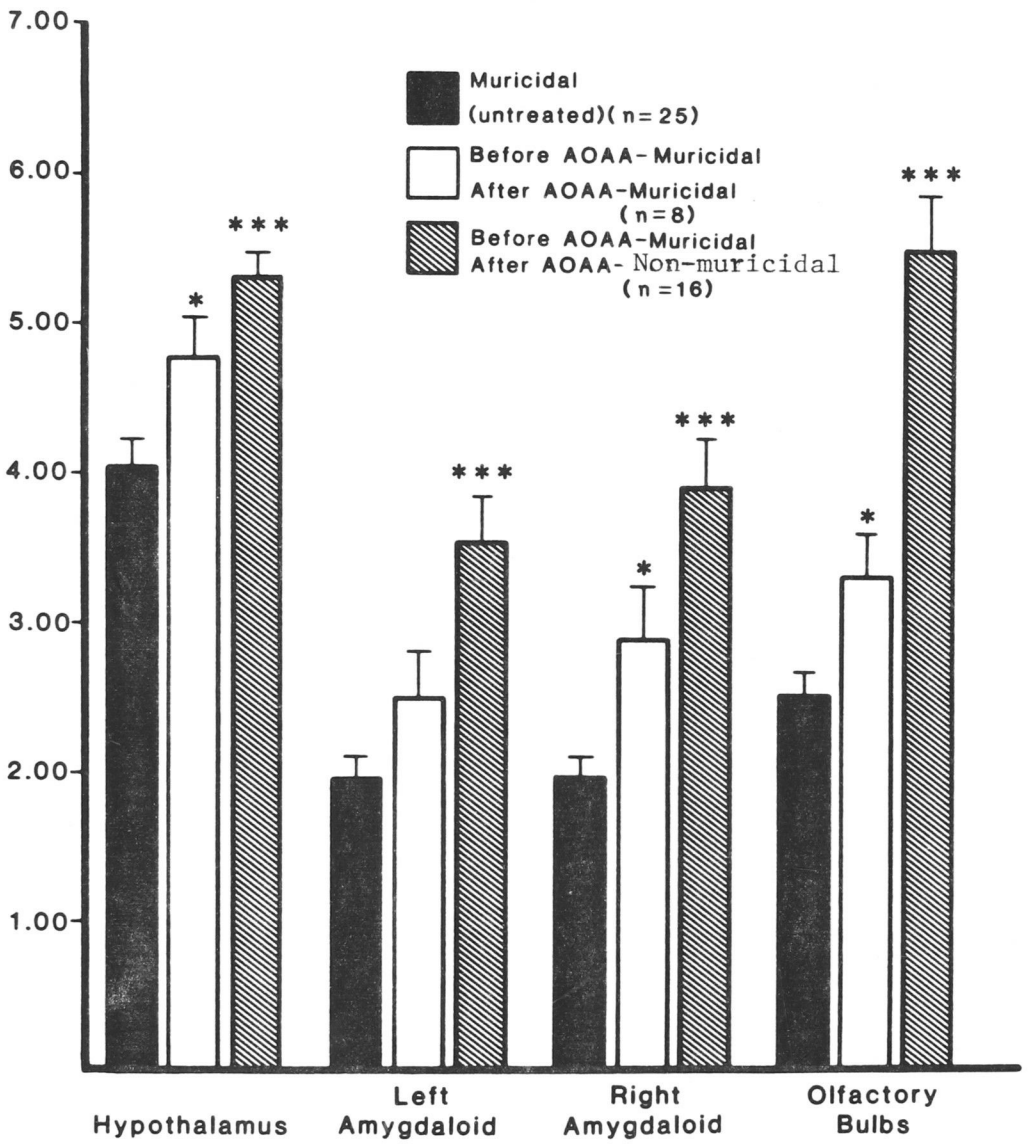

Figure 12 Behavioral alterations and concomitant changes in GABA levels after AOAA treatment in muricidal rats.

GABA values in μ mole/gram tissue (mean ± SEM).

*p < 0.05, Student's t test, compared to untreated controls.

***p < 0.001, Student's t test, compared to untreated controls.

TABLE 8. BEHAVIORAL ALTERATIONS AND CONCOMITANT CHANGES IN GABA LEVELS AFTER AOAA TREATMENT IN MURICIDAL RATS AS INFLUENCED BY HOUSING

		Muricidal (Untreated) (n)	Before AOAA - Muricidal After AOAA - Muricidal (n)	Before AOAA - Muricidal After AOAA - Non-Muricidal (n)
Hypothalamus	Isolated	3.85 ± 0.20 (18)	5.12 ± 0.15 (6)***	4.98 ± 0.16 (10)***
	Aggregated	4.53 ± 0.19 (7)	3.72 ± 0.03 (2)**	5.82 ± 0.26 (6)**
Left Amygdaloid	Isolated	1.78 ± 0.12 (18)	2.45 ± 0.36 (6)	2.98 ± 0.26 (10)***
	Aggregated	2.42 ± 0.27 (7)	2.60 ± 0.45 (2)	4.38 ± 0.50 (6)**
Right Amygdaloid	Isolated	1.87 ± 0.15 (18)	2.97 ± 0.46 (6)	3.28 ± 0.30 (10)***
	Aggregated	2.22 ± 0.19 (7)	2.72 ± 0.13 (2)	4.98 ± 0.41 (6)***
Olfactory Bulbs	Isolated	2.36 ± 0.17 (18)	3.22 ± 0.32 (6)*	4.97 ± 0.41 (10)***
	Aggregated	2.97 ± 0.21 (7)	3.62 ± 0.74 (2)	6.37 ± 0.58 (6)***

GABA values in μmoles/gram tissue (Mean ± SEM).
* $P<0.05$, Student's *t*-test, compared to untreated controls.
** $P<0.01$, Student's *t*-test, compared to untreated controls.
*** $P<0.001$, Student's *t*-test, compared to untreated controls.

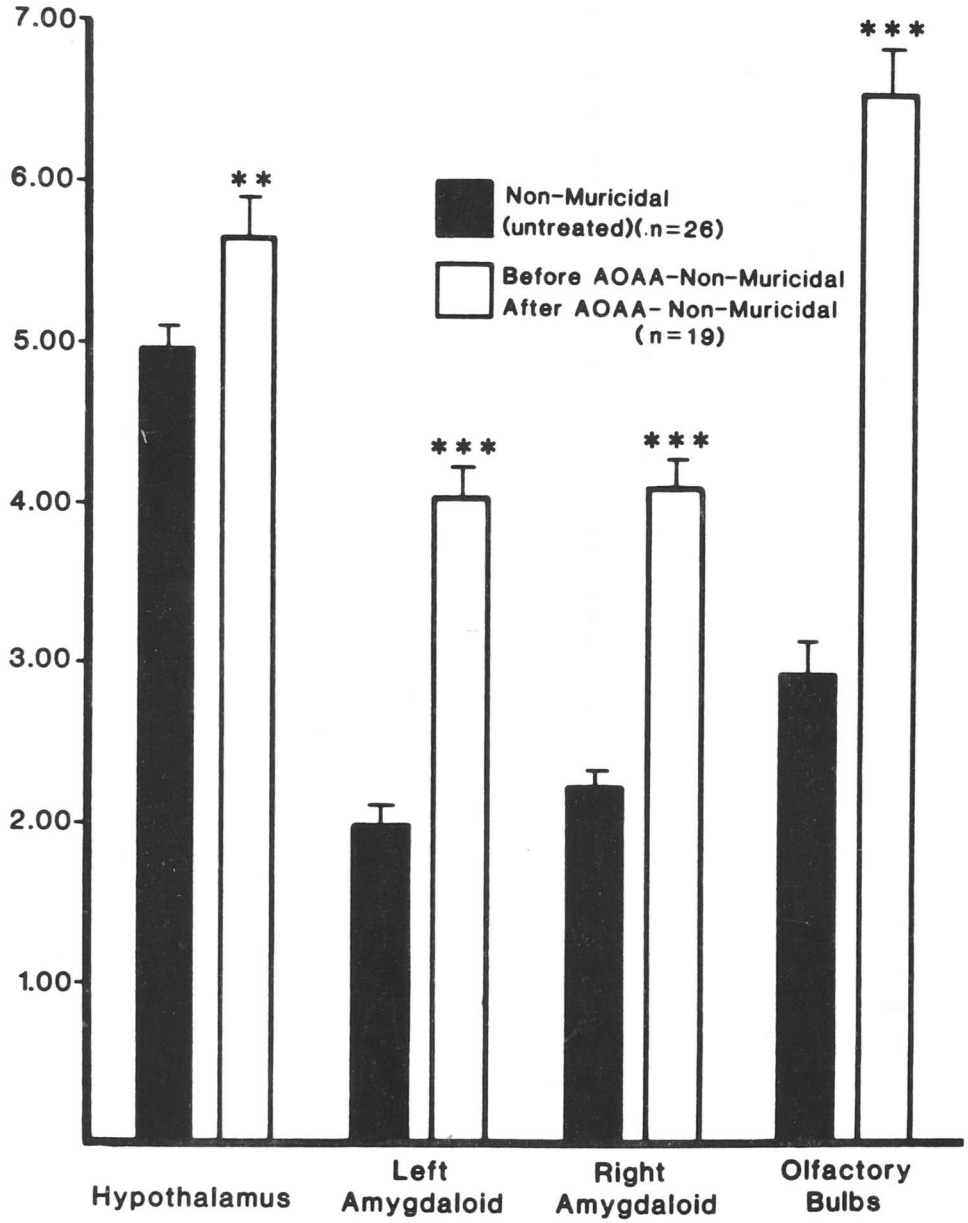

Figure 13 Behavioral alterations and concomitant changes in GABA levels after AOAA treatment in non-muricidal rats.

GABA values in μ moles/gram tissue (mean ± SEM)

**p< 0.01 Student's t test, compared to untreated controls

***p< 0.001, Student's t test, compared to untreated controls

TABLE 9. BEHAVIORAL ALTERATIONS AND CONCOMITANT CHANGES IN GABA LEVELS AFTER AOAA TREATMENT IN NON-MURICIDAL RATS AS INFLUENCED BY HOUSING

		Non-Muricidal (Untreated) (n)	Before AOAA- Non-Muricidal After AOAA- Non-Muricidal (n)
Hypothalamus	Isolated	4.80 ± 0.17 (16)	5.32 ± 0.19 (12)
	Aggregated	5.22 ± 0.29 (10)	6.20 ± 0.59 (7)
Left Amygdaloid	Isolated	1.82 ± 0.15 (16)	3.83 ± 0.19 (12)***
	Aggregated	2.25 ± 0.17 (10)	4.42 ± 0.25 (7)***
Right Amygdaloid	Isolated	2.11 ± 0.11 (16)	3.83 ± 0.26 (12)***
	Aggregated	2.43 ± 0.22 (10)	4.55 ± 0.17 (7)***
Olfactory Bulbs	Isolated	2.67 ± 0.18 (16)	6.24 ± 0.37 (12)***
	Aggregated	3.40 ± 0.42 (10)	7.09 ± 0.48 (7)***

GABA values in μmoles/gram tissue (Mean ± SEM).
*** P<0.001, Student's t-test, compared to untreated controls.

TABLE 10. BEHAVIORAL CHANGES REFLECTING ALTERED GABA LEVELS FOLLOWING AOAA TREATMENT AS INFLUENCED BY HOUSING

		Before AOAA-Muricidal After AOAA-Muricidal (η)	Before AOAA-Muricidal After AOAA-Non-Muricidal (η)	Before AOAA-Non-Muricidal After AOAA-Non-Muricidal (η)
Hypothalamus	Isolated	5.12 ± 0.15 (6)	4.98 ± 0.16 (10)	5.32 ± 0.19 (12)
	Aggregated	3.72 ± 0.03 (2)	5.82 ± 0.26 (6)***	6.20 ± 0.59 (7)
Left Amygdaloid	Isolated	2.45 ± 0.36 (6)	2.98 ± 0.26 (10)	3.83 ± 0.19 (12)***
	Aggregated	2.60 ± 0.45 (2)	4.38 ± 0.50 (6)	4.42 ± 0.25 (7)
Right Amygdaloid	Isolated	2.97 ± 0.46 (6)	3.28 ± 0.30 (10)	3.83 ± 0.26 (12)
	Aggregated	2.72 ± 0.13 (2)	4.98 ± 0.41 (6)**	4.55 ± 0.17 (7)***
Olfactory Bulbs	Isolated	3.22 ± 0.32 (6)	4.97 ± 0.41 (10)**	6.24 ± 0.37 (12)***
	Aggregated	3.62 ± 0.74 (2)	6.37 ± 0.58 (6)	7.09 ± 0.48 (7)

GABA values in μmoles/gram tissue (Mean ± SEM).
Control group was MURICIDAL before and after AOAA.
** $P<0.01$, Student's *t*-test.
*** $P<0.001$, Student's *t*-test.

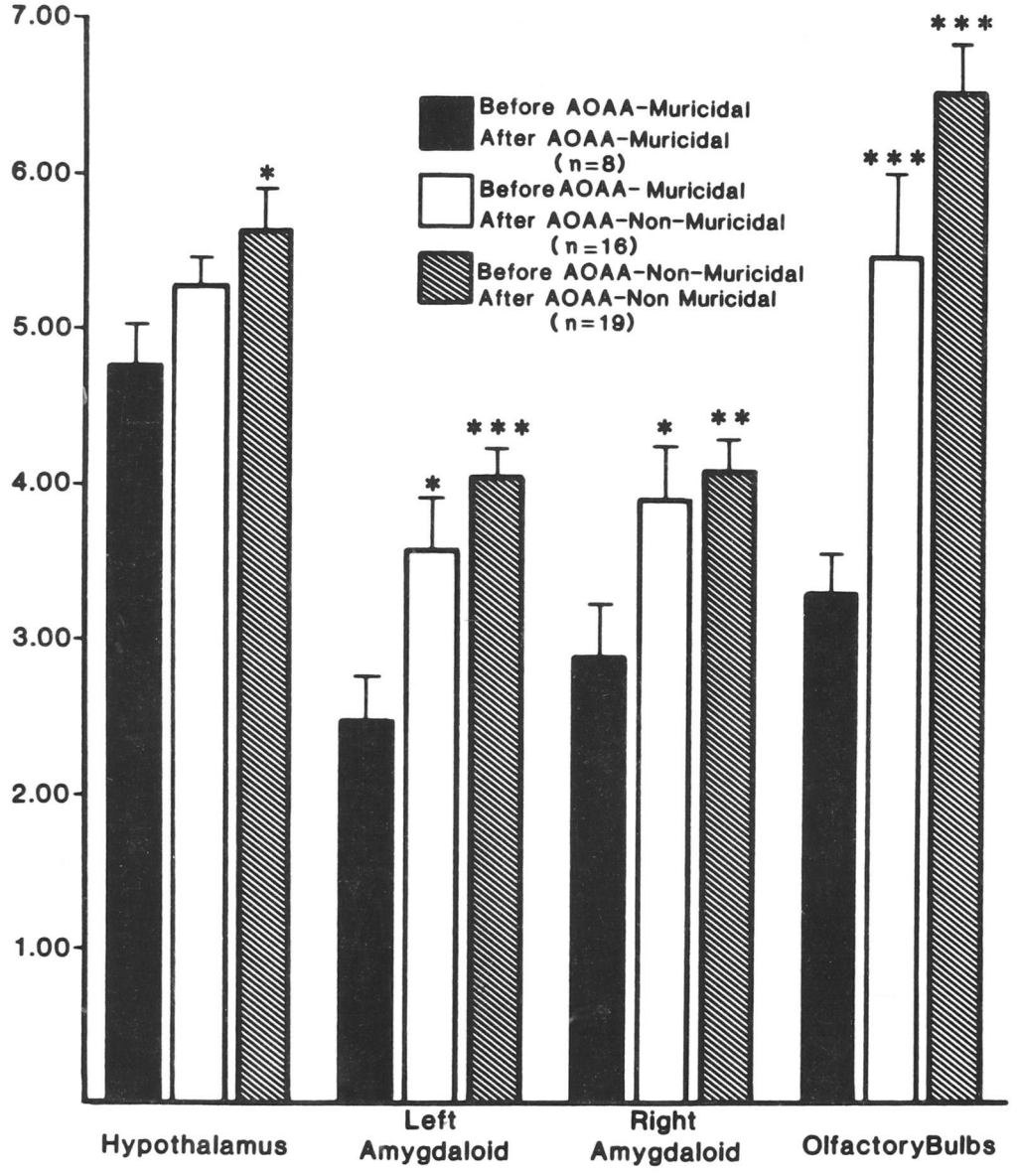

Figure 14 Behavioral changes reflecting altered GABA levels following AOAA treatment.
GABA values in μ mole/gram tissue (mean ± SEM)
Control group was Muricidal before and after AOAA
*p < 0.05, Student's t test
**p < 0.01, Student's t test
***p < 0.001, Student's t test

DISCUSSION

Resting GABA levels in the hypothalamus were found to be in agreement with those reported by Horton et al. (1979), Knieriem et al. (1977), Balcom et al. (1975), and Starr and Tanner (1975). Balcom et al. (1975) reported levels of GABA in the olfactory bulbs similar to those found in this study. However, these same investigators found higher GABA values in the amygdala than those found in this work. These discrepancies are probably due to differences in experimental protocol, specifically differences in social housing and rat strain.

Isolation was shown to significantly reduce levels of brain GABA in all areas studied. This effect has not been previously reported by investigators working with the muricidal rats. However, Earley and Leonard (1977) demonstrated lower levels of GABA in the amygdala and olfactory bulbs of isolated aggressive mice. DaVanzo and Sydow (1975) demonstrated non-significant reduction of whole brain GABA in isolated aggressive versus aggregated mice. Despite the fact that these studies using the mouse model did not have adequate behavioral controls to enable one to make conclusions on the effects of isolation, both these investigators and this thesis demonstrate a trend isolating low brain GABA with social isolation.

The mechanism by which the reduction of GABA takes place is still uncertain. Scott et al. (1951) and Mugford (1974) demonstrated that high testosterone is associated with the development of muricidal behavior. This would seem logical since isolation is definitely

stressful, the adrenocortical stress syndrome would predict an increase in 17-ketosteroids, catabolic end products of testosterone. Since isolation leads to increases in aggression, high testosterone would be associated with muricidal behavior. However, Early and Leonard (1975) published data associating low testosterone with low GABA and high levels of aggression in all areas studied except the amygdala. At present this dilemma remains unresolved.

Isolation was shown to be a dominant factor in the induction of muricidal behavior. Rats isolated for 7 days demonstrated a highly significant ($p<0.001$) increase in the incidence of muricide over their aggregated counterparts. This conclusion is in agreement with the work of Karli (1956) and Broderick (1979). However, it disagrees with the work of Korn and Moyer (1968) and Myer (1969) and sheds doubt on the statement of Gibbons et al. (1979) that the isolation protocol is merely a convenience.

Since isolation can lead to potentiation of the incidence of muricide, it can allow for more cost-effective experimentation in rat strains with low innate aggressiveness. It also adds convenience to experimental design and allows the rat to become acquainted with the behavioral testing site, a factor which Janssen et al. (1962) found to be essential for muricide induction.

In this study, it was shown that there was a 53% incidence of muricide in a random population of isolated Long Evans rats. These data are slightly higher than those reported by Broderick (1979) who found a 40% muricide ratio and Barr (1979) who found a 45% incidence

of muricide, both using the Long Evans rat. These differences are easily explained, however, due to variation in behavioral classification methodology.

As mentioned previously in the review of the literature, the methodology involved in muricidal classification varies greatly. The behavioral selection protocol is totally at the discretion of the experimenter. Even in the muricidal model with its precise behavioral end point, the behavioral populations can vary with differences in muricidal test duration. However, it is interesting to note that when particulate GABA levels were plotted against the latency of the muricidal response, the correlation coefficient showed no significance.

Muricidal rats were shown to have lower levels of GABA in the hypothalamus, right amygdala and olfactory bulbs with the hypothalamus being significantly lower at the 0.001 level of significance. This same effect on the olfactory bulbs has been previously shown by Mark et al. (1975) and Mandel et al. (1978) using the muricidal model. Earley and Leonard (1977) demonstrated lower GABA levels in the olfactory bulbs and bilateral amygdala of isolated aggressive mice when compared to aggregated controls. These data hint of a modulatory role for GABA on muricidal behavior. When considering the voluminous data demonstrating the modulatory effect of other inhibitory neurotransmitters, however, one cannot conclude that GABA modulates muricidal behavior. Yet, one can speculate that with further research in this area, it seems reasonable to predict that specific neurotransmitters will be shown to exert specific behavioral

modulation on specific anatomical sites.

The non-specific GABA transaminase inhibitor, AOAA, is known to have a specific curve for anti-convulsant activity and for GABA elevation (See FIGURES 3 and 4, p. 10 and 11). However, these two curves do not mirror one another. There is much speculation as to why this occurs. Glial GABA transport and possible isomers of GABA transaminase have been suggested as causes. The purposes of this study was not to answer this question, however, it did influence the experimental protocol.

When considering the time between injection and behavioral testing, several factors must be considered:

1. Recovery from possible neurotoxicity (approximately 45 min)
2. Recovery from possible hyperactivity due to injection (approximately 10 min)
3. The mean kill time for previously muricidal rats should be within a period of maximal GABA elevation.

If the theory of glial transport of GABA is valid, the test period should coincide with the time of maximal pharmacological activity to insure maximal available GABA (approximately 30 min). All of these factors were taken into consideration in choosing the timing sequence of the experimental protocol.

AOAA was shown to elevate particulate brain GABA in all areas studied with marked elevation in the olfactory bulbs. This is in agreement with the data of Starr and Tanner (1975) and DaVanzo and Sydow (unpublished).

AOAA was shown to be an effective anti-muricidal agent in both isolated and aggregated rats, with AOAA having a significantly greater effect in isolated rats. Even though this anti-aggressive effect has not been shown in the muricidal rat, it has been shown in the intraspecies model of DaVanzo and Sydow (unpublished).

APPENDIX I:

ANATOMICAL DISSECTION COORDINATES

The localization and subsequent dissection of the hypothalamus and bilateral amygdala were performed according to the method of Barr et al. (1979). For removal of the olfactory bulbs and further analysis of the anatomical position of the hypothalamus and amygdala, Pellegrinó's "A Stereotoxic Atlas of the Rat Brain" was consulted.

Three coronal cuts were made in the brain following its removal from the skull (See FIGURE 15). The most rostral cut demonstrated the point of resection for the olfactory bulbs (the bulbs were lifted away from the overlying frontal cortex before this cut was made). The central cut, made at the optic chiasma, constituted the rostral border of the central tissue section from which the hypothalamus and amygdala were removed for assay. The most caudal cut (See FIGURE 15) demonstrated the caudal aspect of the central tissue section. The caudal aspect of the mammillary bodies served as the landmark for this caudal dissection.

A transverse cut was then made caudal to rostral through the central tissue section utilizing the two rhinal fissures as landmarks (See FIGURE 16). Two oblique cuts were then made along the internal capsule to separate the hypothalamus from the bilateral amygdala.

For anatomical localization of individual CNS nuclei, PLATES 4 and 5 demonstrate the planes of view, rostral and caudal, respectively to the optic chiasma. PLATES 6 and 7 demonstrate the planes of view rostral and caudal, respectively to the caudal aspect of the mammillary bodies.

From these plates and figures, several points should be made about

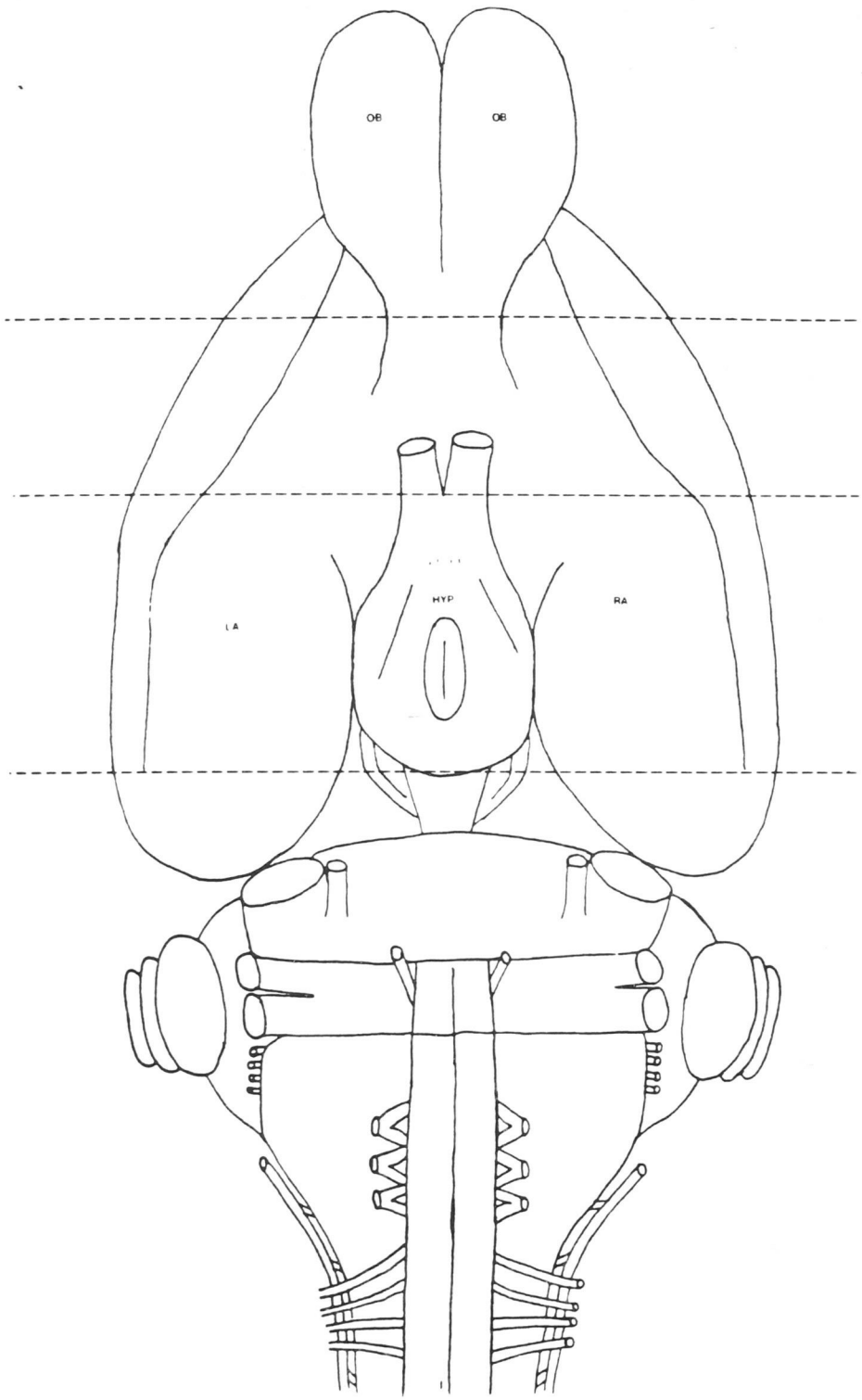

FIGURE 15. The ventral surface of a rat brain. (HYP-hypothalamus, LA-left amygdaloid, OB-olfactory bulbs, RA-right amygdaloid).

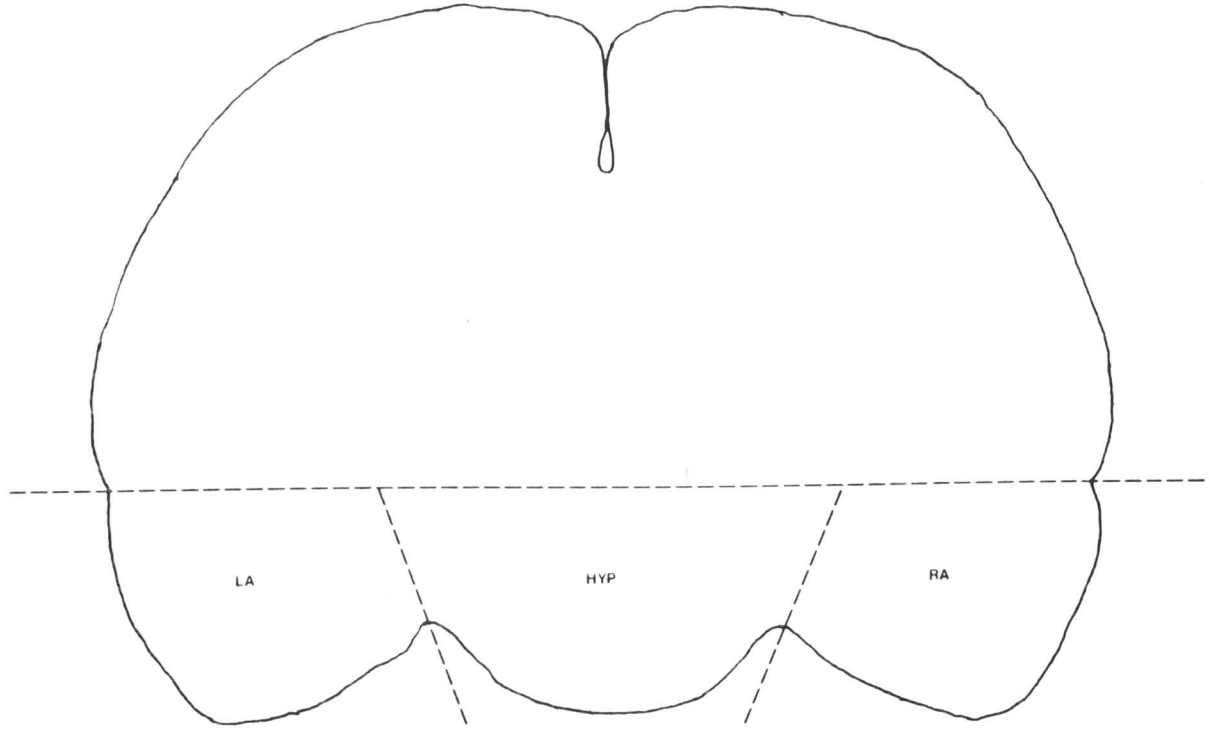

FIGURE 16. A rostral view of the central tissue section. (HYP-hypothalamus, LA-left amygdaloid, RA-right amygdaloid).

LIST OF ABBREVIATIONS FOR PLATES

AAA - Anterior amygdaloid area

ABL - Basal amygdaloid nucleus, lateral part

ACB - Lateral parolfactory area

ACO - Cortical amygdaloid nucleus

AL - Lateral amygdaloid nucleus

BCA - Bed nucleus of the anterior commissure

CA - Anterior commissure

CC - Corpus callosum

CE - External capsule

CI - Internal capsule

CL - Nucleus of Luys

CLA - Claustrum

CO - Optic chiasma

CP - Posterior commissure

CPU - Caudate nucleus

DBB - Diagonal band of Broca

ENT - Entorhinal cortex

FA - Amygdaloid fissure

FD - Dentategyrus

FH - Hippocampal fissure

FI - Fimbra of the hippocampus

FLD - Dorsal fasciculus of Schutz

FR - Rhinal fissure

FX - Fornix

GLD - Lateral geniculate body, dorsal part

GLV - Lateral geniculate body, ventral part

GM - Medial geniculate body

H - Habenula

HL - Lateral habenular nucleus

HM - Medial habenular nucleus

HP - Habenulo-interpedunuclar tract

HPC - Hippocampus

IP - Interpeduncular nucleus

LM - Medial lemniscus

LS - Lateral septal nucleus

LT - Lateral nucleus of the thalamus

LTP - Lateral nucleus of the thalamus, posterior part

MD - Dorsomedial nucleus of the thalamus

MFB - Median forebrain nucleus

ML - Lateral mammillary nucleus

MM - Medial mammillary nucleus

MP - Posterior mammillary nucleus

MPA - Medial parolfactory area

MPO - Medial preoptic area

MS - Medial septal nucleus

MT - Mammillothalamic tract

NPT - Posterior nucleus of the thalamus

OT - Optic tract

PC - Cerebral peduncle

PF - Parafascicular nucleus of the thalamus

PIR - Piriform cortex

PM - Mammillary peduncle

POA - Lateral preoptic area

PRT - Pretectal area

PV - Paraventricular nucleus of the thalamus

PVG - Periventricular grey substance

RE - Reuniens nucleus of the thalamus

S - Subiculum

SC - Suprachiasmatic nucleus

SM - Stria medullaris thalami

SN - Substantia nigra

ST - Stria terminalis

SUM - Supramammillary nucleus

TOL - Lateral olfactory area

TP - Tuberculopyriform tract

TT - Mammillotegmental tract

TUO - Olfactory tubercle

VE - Ventral nucleus of the thalamus

VL - Lateral ventricle

V3 - Third ventricle

ZI - Zona incerta

II - Optic nerve

III - Oculomotor nerve

PLATE 4

CROSS SECTION ROSTRAL TO THE OPTIC CHIASMA

(3.0 mm rostral to bregma)

This plane of view is just rostral to the optic chiasma, the rostral plane of dissection for the removal of the central tissue section (See FIGURE 15). This section is the caudal view of the rostral portion of the brain not assayed.

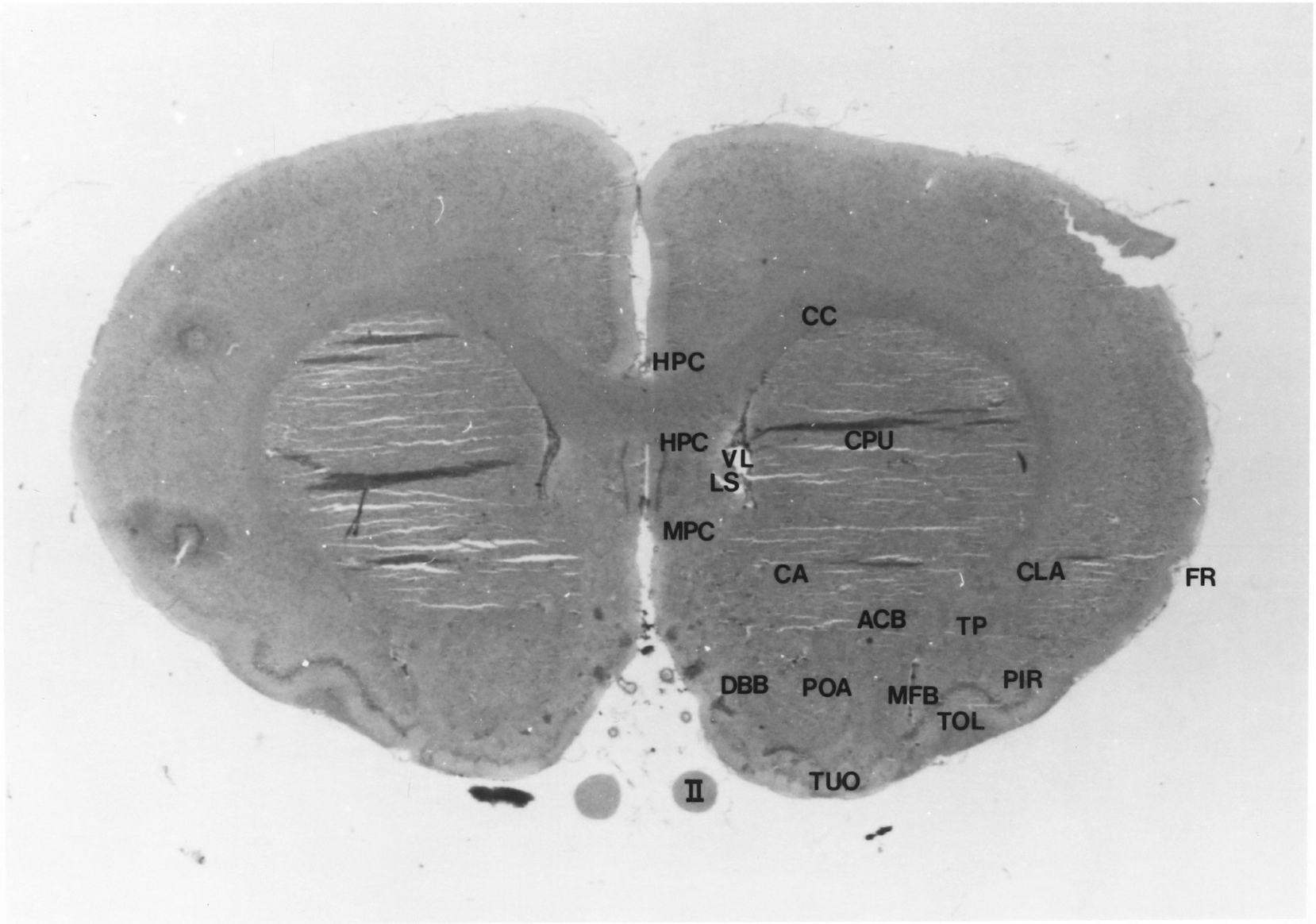
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PLATE 5

CROSS SECTION THROUGH THE OPTIC CHIASMA

(2.0 mm rostral to bregma)

This plane of view is approximately at the optic chiasma, the rostral plane of dissection for the removal of the central tissue section. This plane is a rostral view of the central tissue section.

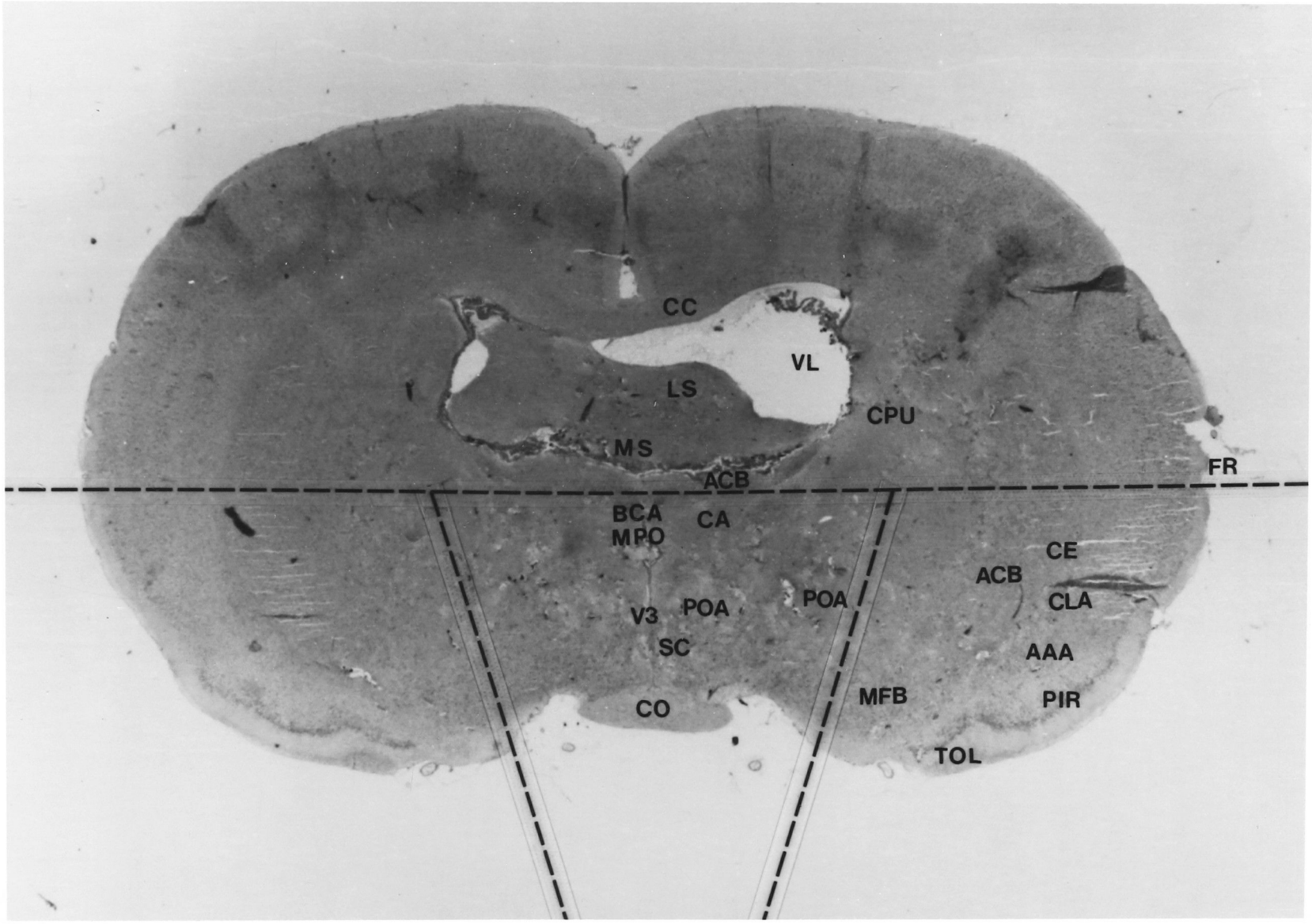
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PLATE 6

CROSS SECTION ROSTRAL TO THE MAMMILLARY FACICULUS

(1.8 mm caudal to bregma)

This plane of view is rostral to the mammillary faciculus, the caudal plane of dissection for removal of the central tissue section (see FIGURE 15). This plane is a caudal view of the central tissue section.

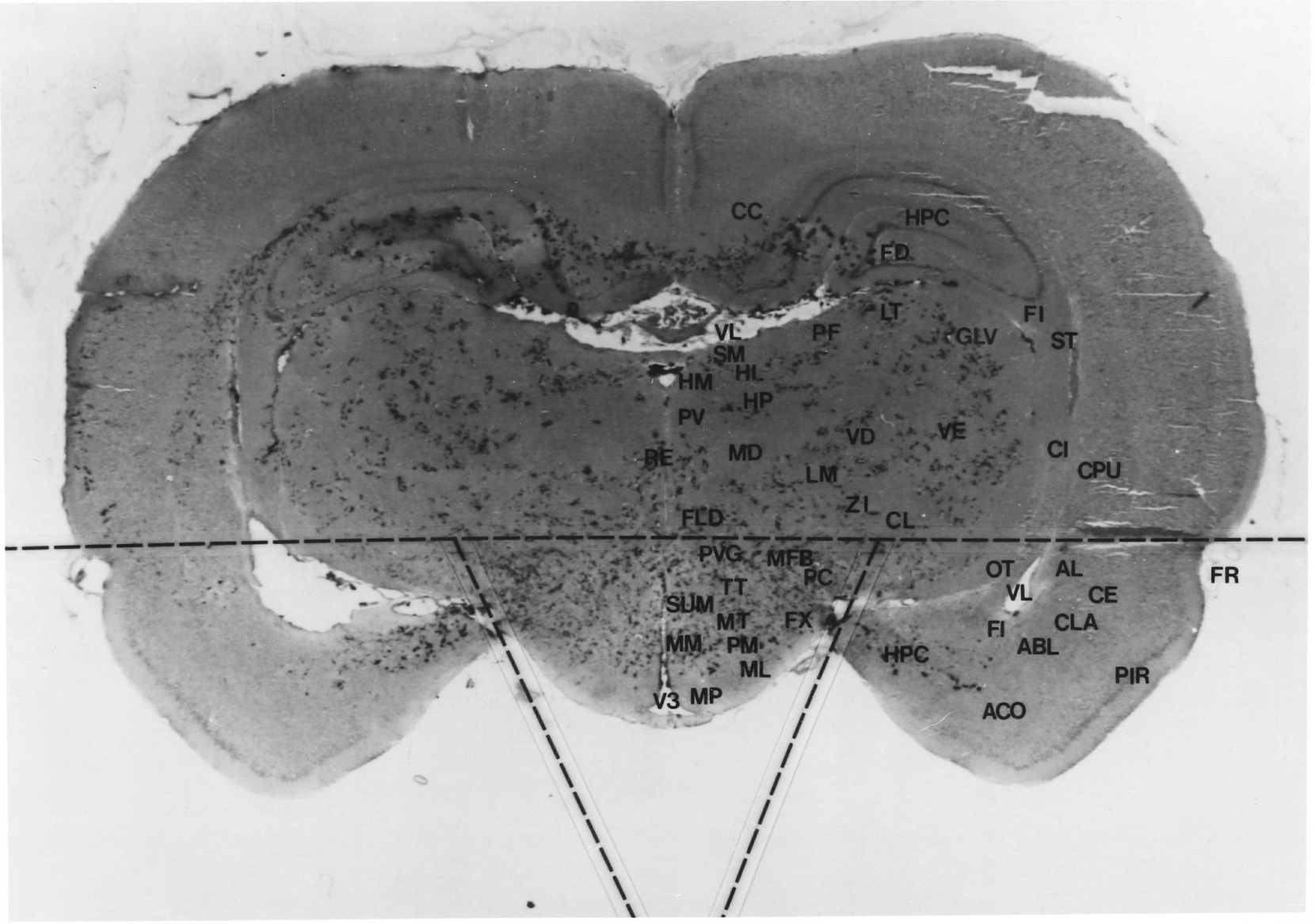
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PLATE 7

CROSS SECTION CAUDAL TO THE MAMMILLARY FACICULUS

(3.0 mm caudal to bregma)

This plane of view is just caudal to the mammillary faciculus, the caudal plane of dissection for the removal of the central tissue section. This section is a rostral view of the caudal portion of the brain not assayed.


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FA

the anatomical positions of various behaviorally important structures in relation to the dissection protocol.

1. The diagonal band of Broca ends 0.2 mm caudally to the optic chiasma.
2. The median forebrain bundle extends caudally to the optic chiasma for a distance of 0.8 mm.
3. The caudate nucleus completely transects the assayed tissue block. However, the caudate nucleus is dorsal to the transverse section made at the rhinal fissure.
4. Both the lateral and medial septal nuclei extend caudally beyond the optic chiasma; these structures are also dorsal to the transverse dissection at the rhinal fissure.
5. The entorhinal cortex extends 0.2 mm rostral into the tissue assayed for amygdaloid GABA.
6. The hippocampus extends 0.8 mm rostral to the caudal aspect of the mammillary bodies.

The entire amygdaloid complex (the baso-lateral, baso-medial, central, cortical, intercalated, lateral and medial nuclei; the transition amygdaloid zone and the anterior amygdaloid area except for its most rostral 0.4 mm portion) was dissected for assay. The entire pyriform cortex (except for the most rostral 0.6 mm portion) was included in the tissue assayed for amygdaloid GABA.

The entire hypothalamus was removed (the arcuate, dorso-medial, paraventricular, posterior supraoptic and ventromedial nuclei plus the anterior and lateral hypothalamic areas). Some thalamic nuclei were

included in the tissue assayed for hypothalamic GABA.

This dissection was performed to determine GABA levels in various brain areas, not to isolate CNS nuclei, per se. This appendix demonstrates the consistency with which these dissections were made.

APPENDIX II:

AMYGDALOID LATERALITY: CORRELATION WITH ISOLATION, MURICIDAL BEHAVIOR AND GABA.

Recently, it has been suggested that functional laterality exists within the amygdala (personal communication, Dr. McNeil). In order to test the hypothesis of behavioral laterality within the amygdala, both the left and right amygdala were run through the assay procedure separately. Many of the conclusions that follow range from simple observations of trends within the data to statistical analysis of derived laterality values. The major observations were the differences between the mean GABA levels for the left and right amygdala within a specific behavioral and social housing group.

Initially, it is interesting to note that in almost all behavioral and social housing groups, levels of GABA in the right amygdala exceed those in the left amygdala. It was observed that while the left amygdala was approximately the same for both the non-muricidal and muricidal groups (1.99 and 1.96 moles/g tissue respectively), the right amygdala demonstrated a 0.26 μmole higher GABA level in the non-muricidal group (See FIGURE 9, p. 40). However, the left amygdala experienced a greater drop in levels of GABA due to isolation than did the right amygdala (See TABLE 2, p. 38).

The effects of AOAA upon the amygdala are quite complex. Considering all drug treated rats, the left amygdala and right amygdala experienced the same percent GABA elevation (81.2% and 81.4% respectively) (See TABLE 5, p. 45). However, when these groups are separated according to behavior and pre-test housing experience, the similarity ceases. In the left amygdala, both the isolated and aggregated groups experienced the same percent GABA elevation (80.0% and 79.3%

respectively). Yet the right amygdala from aggregated rats experienced a 91.5% GABA elevation, while the right amygdala from isolated rats only experienced a 74.2% increase (See TABLE 4, p. 42).

Moreover, when the social housing groups are divided according to pre-AOAA treatment behavior, both isolated behavioral groups experienced a larger percent GABA elevation in the left amygdala than did their aggregated counterparts. In the right amygdala, both aggregated behavioral groups experienced the greater percent GABA elevation.

To further establish laterality within the amygdala, the values RELAG and RERAG were formulated. These values are the mean μmoles/gram tissue difference from the random population means for each behavioral and pre-test housing group. RELAG is the relative difference for the left amygdala as RERAG is for the right amygdala. The laterality difference expresses the difference in μmole/gram tissue between RELAG and RERAG for a particular behavioral and housing group.

When considering all non-AOAA treated animals, the laterality difference for non-muricidal rats is greater (Δ0.22 μmoles) than that for muricidal rats (Δ0.01 μmoles). This same effect is seen in FIGURE 9 (p. 40). Within the muricidal rats, the aggregated group experienced the greater difference (Δ0.22 μmoles) in amygdaloid GABA compared to Δ0.07 μmoles for the isolated group. However, within non-muricidal rats, the isolated group experienced the greater difference (Δ0.27 μmoles compared to Δ0.16 μmoles).

In previously muricidal rats, AOAA-treated rats experienced a laterality difference of Δ0.39 μmoles (for rats muricidal after

AOAA) and a Δ0.36 μmoles (for rats non-muricidal after AOAA) compared to a difference of Δ0.01 μmoles for non-drug treated. In the previously non-muricidal group, the non-drug treated group experienced a laterality difference of Δ0.22 μmoles whereas, the AOAA treated group experienced a difference of Δ0.03 μmoles. Essentially, this demonstrates that in muricidal rats, the AOAA treated group experienced the greater difference in laterality whereas, in the non-muricidal group, the non-drug treated animals experienced the greater difference. Also, when comparing AOAA-treated, previously muricidal rats, those remaining muricidal had almost the same laterality differences (Δ0.39 μmoles) as did the group that becames non-muricidal (Δ0.36 μmoles).

An equation by which one can calculate a derived percent GABA elevation was formulated. See FIGURE 17 for a diagram of this formula. This formula essentially gives a measure of GABA elevation with regards to behavioral changes also induced by AOAA-treatment.

From TABLE 11, it is shown that while the hypothalamus and olfactory bulbs demonstrated little difference between the aggregated and isolated groups, the amygdala showed a dramatic difference, with the right amygdala experiencing a greater difference in GABA elevation due to pre-test housing.

The possibility of behavioral laterality in the amygdala has never been studied in the muricidal rat. From this study, four major conclusions were found in regard to laterality:

FIGURE 17 FORMULA FOR DERIVED PERCENT GABA ELEVATION

$$\text{Derived Percent GABA Elevation} = \frac{\left|\bar{X}\ \text{GABA}\ \mu\text{mole/gram}\ \text{Before AOAA-MURICIDAL} - \bar{X}\ \text{GABA}\ \mu\text{mole/gram}\ \text{Before AOAA-MURICIDAL}\right|}{\left|\bar{X}\ \text{GABA}\ \mu\text{mole/gram}\ \text{After AOAA-NON-MURICIDAL} - \bar{X}\ \text{GABA}\ \mu\text{mole/gram}\ \text{After AOAA-MURICIDAL}\right|}$$

TABLE 11. THE EFFECT OF ISOLATION ON DERIVED PERCENT GABA ELEVATION

	Aggregated	Isolated
Hypothalamus	84.7%	73.8%
Left Amygdaloid	97.8%	38.0%
Right Amygdaloid	123.6%	36.0%
Olfactory Bulbs	79.2%	58.0%

1. In most behavioral and housing groups, the right amygdala contains a higher concentration of GABA than does the left amygdala.
2. Behavioral differences are reflected by greater differences in GABA levels within the right amygdala than in the left amygdala.
3. However, the left amygdala experiences the greater drop in GABA due to isolation.
4. The effect of AOAA on laterality is too complex to formulate any pure trends in regard to laterality.

APPENDIX III:

THE EFFECT OF AGE ON MURICIDAL BEHAVIOR

Myer (1971) observed that age is not a controlling factor in determining the time of muricide induction. However, this author found no mention in the literature that age can, in fact, influence a rat's probability of eliciting a muricidal response.

Throughout this study, an isolation period of one week was used. Other investigators (Karli, 1956, Broderick, 1979) have reported effects of longer isolation periods on muricidal behavior. These investigators observed that with extended isolation, there was a reduction in the increase of the incidence of muricidal behavior. Neither Karli nor Broderick, however, used sufficient experimental controls to account for the aging of the rat.

It was this investigator's opinion that this leveling effect may be due to some factor involving age. To test this hypothesis, a group of twelve rats, 13 weeks old, were isolated for one week and then tested for muricidal behavior.

The observation was made that the 13 week old group had a highly significant lower incidence of muricide than the 8 week old group (See TABLE 12 below). Subsequent GABA assays of the hypothalamus, left amygdaloid, right amygdaloid and the olfactory bulbs from 13 week old rats showed no significant differences from the 8 week old group.

TABLE 12. THE EFFECT OF AGE ON MURICIDAL BEHAVIOR

Group	Percent Muricide[a]
13 week old rats 440-470g (n=12)	25% (3/12)***
8 week old rats 225-270g (n=34)	53% (18/34)

***p<0.001, chi-square analysis

[a]muricidal testing followed one week of isolation

These results suggest that as a rat's age increases, aggressiveness decreases (a striking resemblance to human behavior) and that this effect may, in part, explain the leveling-off of the effects of isolation on muricidal behavior observed in extended isolation periods. Further work will have to be done, however, before this hypothesis is proved definitely.

SUMMARY

Isolation was shown to be a dominant factor in the induction of muricidal behavior. Isolated rats demonstrated lower GABA levels in the hypothalamus, left amygdala, right amygdala and olfactory bulbs. Muricidal rats showed reduced levels of particulate GABA in all areas except the left amygdala. The above statements lead to the conclusion that isolation, increased aggressiveness and reduced particulate brain GABA are interrelated.

AOAA was shown to be an effective anti-muricidal agent. This drug also elevated particulate GABA levels, the olfactory bulbs showing the most marked elevation. Isolated rats demonstrated greater percent GABA elevation and greater percent muricide reversal due to AOAA than did their aggregated counterparts.

The amygdala seemed to be associated with a slight degree of both social and behavioral laterality. The right amygdala showed changes in GABA levels correlated with behavioral differences, while the left amygdala did not mirror these changes. Interestingly, a more dramatic response of GABA levels to isolation was seen in the left amygdala than in the right amygdala. Age also reflected a behavioral parameter with older rats having a significantly lower incidence of muricide than younger rats.

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