EFFECT OF FIGHTING (DEFEAT) ON BRAIN LEVELS OF SEROTONIN, NOREPINEPHRINE AND MONOAMINE OXIDASE

by

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B. S., Purdue University, Lafayette, Indiana, 1963

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1967

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Introduction

The study of population densities, and the response of individuals to different densities has been the object of a considerable amount of research in the past decade, ranging from observations of organ weight change to systemic biochemical studies. A variety of changes have been reported to occur in animals after grouping. As the population density increases, the body weight decreases (Christian, 1959), and there is a decrease in the circulating eosinophils (Vandenbergh, 1960) and weight of the thymus gland (Bronson and Eleftheriou, 1963). Inflamation reactions and antibody formation are inhibited as a result of grouping (Christian, 1963; Vessey, 1964), and an inhibition is observed in the defense mechanism causing a diminished resistance to endoparasitism and infection (Davis and Read, 1958; Christian, 1963). Splenic enlargement occurred after grouping owing to splenic extramedullary hematopoiesis (Christian and LeMunyan, 1958; Rapp and Christian, 1963). It was also found that the weights of the testes, seminal vesicles and preputial glands in mice decreased linearly as the logarithm of the population increased (Christian, 1955). A high rate of epinephrine secretion from the adrenal medulla was observed as a result of grouping animals (Welch and Welch, 1964). An increase in adrenal weight was observed as the population density increased (Christian, 1959; Welch and Klopfer, 1961; Bronson and Eleftheriou, 1962, 1963), and in addition, the adrenals of mice from high density populations produced more corticosterone when incubated in vitro than those from control mice (Varon and Touchstone, 1964). Bronson and Eleftheriou (1962, 1963) reported that the adrenal ascorbic acid content decreased when mice were put in groups of four or more mice per cage. Keeley (1962) showed that mice born of mothers which had been subjected to crowded conditions during

pregnancy were less active than controls and slower to respond when confronted by unfamiliar stimuli.

One factor which may have an influence on some of these observed changes is that increased density causes an increase in the aggressive interactions between individuals of the population (Clarke, 1953; Barnett, 1958; Bronson and Eleftheriou, 1963; Christian, 1955; Davis and Christian, 1957; Southwick and Bland, 1959; Vandenbergh, 1960). It has been shown that many mammals such as mice, rats, dogs, rabbits or woodchucks establish social hierarchies when placed together in groups so that there are dominant animals, second ranking animals and so on down to the most subordinant animal (Urich, 1938; Scott, 1958; Christian, 1965) and that fighting among individuals may, in some cases, be necessary to form these hierarchies. It may be that aggressive and subordinate temperaments are mediated to a large extent by endocrine factors. Davis and Christian (1957) have shown that adrenocortical morphology and function are negatively related to social rank or dominance. The testicular hormones have been convincingly demonstrated to have a profound effect on the aggressive nature in chickens (Allee, Collias and Lutherman, 1939; Allee and Foreman, 1955; Guhl, 1942; Guhl, Collias and Allee, 1945; Guhl, 1964). Luteinizing hormone administration was demonstrated by Davis (1963) to result in dominance in starlings if the rank was not previously established, thus implicating a pituitary gonadotropic hormone in the aggressive-subordinate relationship among animals.

The work mentioned thus far by Christian and others concerned hormonal changes under conditions of population stress. Unfortunately, in these experiments it was not possible to study aggression by high ranking animals and defeat in subordinate animals. In an attempt to clarify the matter of aggression and defeat, Bronson and Eleftheriou (1964, 1965a, 1965b, 1965c) used the controlled method of fighting devised by Scott (1946) to measure some of the effects of fighting <u>per se</u>

on a defeated mouse. They demonstrated an increase in plasma corticosterone levels and an absolute increase in adrenal corticosterone levels as a result of defeat by fighting. There was no increase observed in adrenal corticosterone, however, if the increase in adrenal weight was taken into account (Bronson and Eleftheriou, 1964, 1965a, 1965b, 1965c). It was further reported that an increase in pituitary adrenocorticotropic hormone content results after repeated frustration by fighting (Bronson and Eleftheriou, 1965c) which again implicates the pituitary in aggressive interactions between animals. Barnett (1958) showed that there was a depletion in lipid content of the adrenal cortex after fighting in rats.

These results demonstrate that there is a pituitary-adrenal and a pituitary-gonadal relationship which is intimately associated with the aggressive interactions between animals and the frustration brought on in the subordinate animals by these interactions. Thus far, however, there has been no report of an experiment conducted to study the involvement of the brain, biochemically, in the frustration caused by repeated defeat of a subordinate animal exposed to a dominant animal of the same species.

Since the discovery of the biologically active amines, serotonin and norepinephrine, in specific areas of the brain (Amin, Crawford and Goddum, 1954; Twarog and Page, 1953), it has been suggested that they may be involved with brain function in these specific areas (Woolley and Shaw, 1954a, 1954b, 1954c; Gaddum, 1954). Serotonin has been shown to be a potent cerebral synaptic inhibitor in mammals and has been suggested to be an endogenous neurohumor (Marrazzi and Hart, 1955; Marrazzi, 1957; Marrazzi, 1958). It has been widely demonstrated that serotonin is involved in certain aspects of neural function both normally and after treatment with some pharmacologic agents (Brodie and Shore, 1957; Costa, 1960; Stark and

Boyd, 1964; Aprison and Hingtgen, 1965). Changes in brain serotonin have also been reported to have a direct effect on changes in behavior (Aprison, Wolf, Poulos and Falkerth, 1962; Aprison, 1965; Udenfriend, Weissbach and Bogdanski, 1957a, 1957b, 1957c; Aprison and Ferster, 1961a, 1961b; Hingtgen and Aprison, 1963; Brodie and Shore, 1957; Bogdanski, Weissbach and Udenfriend, 1958; Costa and Rinaldi, 1958; Shore, Pletscher, Tomich, Carlsson, Kuntzman and Brodie, 1957). For example, Aprison et al. (1962), Aprison and Ferster (1961) and Hingtgen and Aprison (1963) reported that pigeons trained to work on a multiple fixed-ratio fixed-interval schedule showed a marked change in their behavioral pattern which corresponded to a change in the serotonin concentration of the telencephalon and diencephalon. Bogdanski et al. (1958) reporting on the effects of administering 5-hydroxytryptophan, which increases the level of serotonin in all tissues including the brain stated "Dogs and cats given low or intermediate doses showed less spontaneous activity. At first, the animals were alert, but later they appeared to be sedated. Both species could be readily roused, and cats sometimes responded aggressively to handling. Larger doses elicited excitement and apparent disorientation. Dogs given a single dose of 40-60 mg/kg showed widely opened eyes, mydriasis, and a rather steady gaze which was not directed at any particular object. The animals walked hesitantly, and obstacles were not avoided or bypassed when encountered, but rather the animals whined or howled as they attempted to pass through them. Petting or lightly grasping the flanks caused howling and fixed escape reactions. Defensive maneuvers or aggressive reactions were never observed. The behavior of dogs suggested fear, while cats, at an equivalent phase, showed sham rage reactions with poorly directed movements. Both species failed to respond to visual or auditory stimuli. Spontaneous cries regularly occurred at this stage. Other behavioral

effects, such as licking automatism, occurred in individual animals. Rodents showed little change on gross observation following subexcitatory doses. Intermediate doses caused excitement, slight tremors and increased spontaneous motor activity."

After increasing the brain serotonin levels in rabbits, Costa and Rinaldi (1958) reported, "The most constant and frequent characteristics of this symptomatology were champing movements of the mouth, panting, tremors, piloerection, incoordinated leg movements and a condition of enduring behavioral alertness. Despite such clear signs of excitation the animals were much less sensitive to auditory and tactile stimuli".

Udenfriend et al. (1957c) showed that after increasing the levels of brain serotonin as high as 10 to 20 times, dogs reacted with generalized skeletal muscle tremors, loss of placing reactions, postural incoordination, lacremation, salivation, piloerection, increased heart rate, increased gastrointestinal activity, loss of response to visual stimuli, pupillary dilatation and loss of the light reflex.

Brodie and Shore (1957) reported that a persistant "excess" of free serotonin increased alertness, increased motor activity, increased body temperature, increased depth and rate of respiration, increased blood pressure, caused pupil dilatation, increased heart rate, caused piloerection and apparently made the animal temporarily blind.

Norepinephrine has also been shown to be directly involved in behavior (Brodie and Shore, 1957; Aprison and Hingtgen, 1965). In addition, norepinephrine has been reported to be connected with the behavioral changes observed when an animal is attacking or being attacked. This work was done by electrically stimulating the amygdala (Gunne and Reis, 1963), an area of the brain which has a partial control over anger, fear, rage and anxiety (Goddard, 1964; Gunne and Reis, 1963; Reis and

Gunne, 1965).

The suggestion has been made that the balance between the brain amines is more important to behavior than the absolute level of any one amine (McGeer, McGeer and Wada, 1963). McGeer et al. have further postulated a mechanism of action for the aromatic amines in which they act through a chain involving other neurotransmitters such as acetylcholine by modifying the environment so that acetylcholine or a similar agent will be more or less effective at the receptor site. It was stated by McCaman and Aprison (1964) after a study on the developing brain, that serotonin, norepinephrine and acetylcholine play a significant role in the functioning of the fully integrated nervous system of the adult and that it may be to some extent at least, the differences in relative concentrations of these substances between young animals and adults which explain the difference in maturity. In addition, both serotonin and norepinephrine have been shown to be directly involved in the learning ability of the mouse (Woolley, 1962; Woolley, 1963; Woolley and van der Hoeven, 1963). An increase in brain catecholamines and a decrease in brain serotonin was reported to improve the performance of a conditioned avoidence response while an increase in serotonin and a decrease in catecholamines caused a reduction in performance (McGeer et al., 1963). Furthermore, both norepinephrine and serotonin have been shown to be involved in self-stimulation behavior (Poschel and Ninteman, 1963; Stark and Boyd, 1964).

With this previous work in mind, the present study was undertaken to determine the effects of frustration (brought on by repeated defeat of an animal exposed to a trained fighter) on two of the brain amines, serotonin and norepinephrine, which are neurohumors acting in the limbic (emotional) system of the brain. In addition, an analysis of the enzyme monoamine oxidase, which oxidatively deaminates serotonin and norepinephrine, was carried out in order to study a phase of the

metabolism of these two amines and thereby show a possible mechanism of action for serotonin and norepinephrine in response to fighting (defeat) stress.

Materials and Methods

The animals used in this study were all Mus musculus, strain C57Bl/6J, developed at the Jackson Memorial Laboratory in Bar Harbor, Maine, and taken from a colony maintained at Kansas State University. This strain was chosen because of its highly aggressive nature (Scott, 1946; King, 1957).

It has been observed that when animals are kept in isolation for an extended period of time after weaning, they become aggressive to other animals of the same species placed in the home cage of the isolated animal (Scott, 1946). This defense of the home territory was used in the present study as a basis for training a colony of fighter mice which were later to be used as fighters, by means of which the other animals used in this experiment were exposed to aggression and defeat. After weaning at 21-25 days of age, the mice were isolated in opaque plastic cages, 5 x 7 x 11 in covered with a wire mesh lid until they were at least 100 days of age. These cages were never cleaned after the time of weaning, and the mice were never handled. Each isolated mouse then was exposed to a smaller mouse (bait) in the fighter's home cage. The "bait" mice were housed together in groups of six mice per cage. Eventually the potential fighter would attack the "bait" and at this point the "bait" was immediately removed. After a five minute rest period, the above procedure was repeated. This training method was carried out each day until the latent period before an attack was made decreased to about 5-10 seconds. The fighter was then ready for use (Scott, 1946).

The experimental mice used in this study were also mice of the C57Bl/6J strain and were weaned and isolated as described previously. After at least forty days of isolation, these "naive" mice (not trained to fight) were exposed to a trained fighter in the fighter's home cage for a varied number of times, killed and brains excised and frozen for later analysis.

The fighting was done in two different ways in order to determine the manner in which a defeated mouse adapts itself after exposure to fighting aggression encountered intermittently over a prolonged period versus that encountered repeatedly for a short period. The prolonged fighting procedure was to expose a "naive" mouse to a trained fighter for two 5-minute periods a day for 1, 2, 4, 8, 14 or 20 days. There was at least a six-hour period maintained between fights on the same day. Groups of twelve mice were used for each of the different fighting periods in the three prolonged fighting experiments, that is, serotonin, norepinephrine and monoamine oxidase studies except in the case of the 20-day norepinephrine determination in which only six mice were used.

The short term intensive fighting procedure was to expose the "naive" mice to trained fighters for one, four or eight 5-minute periods in one day. There was a five minute rest period allowed between these fights. Measurements of monoamine oxidase, serotonin and norepinephrine were made on individuals in groups of ten mice per period. In all cases, fighters were rotated so they never had to fight more than once per day.

The defeated animals from both the short and long term experiments were all killed twenty minutes after their last fight. Brains were quickly excised and treated in one of two ways. In the case of the brains which were to be assayed for serotonin or norepinephrine, the whole brain was weighed to the nearest 0.1 mg on a torsion balance and frozen in acetone-dry ice. For the monoamine oxidase assay, the hypothalamus (chosen for its active role in neuroendocrine interactions and stress response), amygdala (chosen for its involvement in aggression, fear, anger and anxiety)(Goddard, 1964; Gunne and Reis, 1963; Reis and Gunne, 1965), and the frontal cortex (chosen as a representative of a higher brain area) were excised and frozen in acetone-dry ice and then weighed as described above. The time required to kill the animals and remove the

brain or brain areas was 2-2½ minutes for whole brain and 4-5 minutes for the three areas mentioned.

It would have been desirable to measure serotonin and norepinephrine in the same areas as monoamine oxidase rather than whole brain, but unfortunately, the equipment available was not sensitive enough to determine norepinephrine in small quantities of brain tissue. It was, therefore, decided to measure serotonin in whole brain in order to obtain a correlation between the free levels of these two amines.

Determination of Serotonin

Assays for serotonin were done in triplicate by a modification of the methods described by Bogdanski, Pletscher, Brodie and Udenfriend (1956); McCaman, McCaman, Hunt and Smith (1965); and Vanable (1963) and is outlined in Figure 1. Fluorescence was read on a Turner Model 110 Filter Fluorometer with primary filter, Corning 7-51, and a secondary filter combination of a Corning 3-71 plus a Corning 4-72. Fluorescent values were converted to ug of serotonin by reading from a standard curve made with known amounts of serotonin taken through the entire procedure along with each assay. Figure 2 is the average standard curve constructed from the pooled data obtained from each determination and used to calculate the quantity of serotonin contained in each sample of brain homogenate. Once values were obtained from this curve, they were extrapolated to ug of serotonin/g of brain tissue.

Determination of Norepinephrine

Norepinephrine was estimated by a modification of the methods described by Shore and Olin (1958); Cohen and Goldenberg (1957a, 1957b); and Euler and Lishajko (1959, 1961). Fluorescence was determined on a Turner fluorometer with a primary filter combination of a Corning 3-75, a 405 mu interference filter and a Corning 7-51. Secondary filters were a combination of a Corning 3-71 and a Corning 4-70. Figure 3 is a flow sheet of the procedure used in this

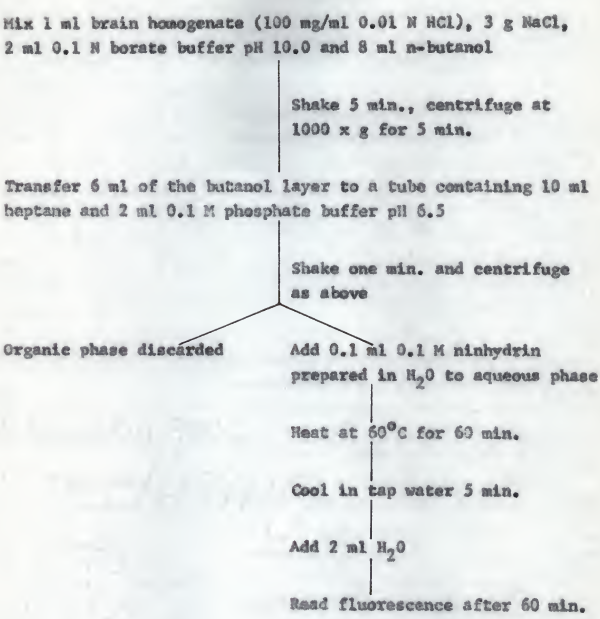
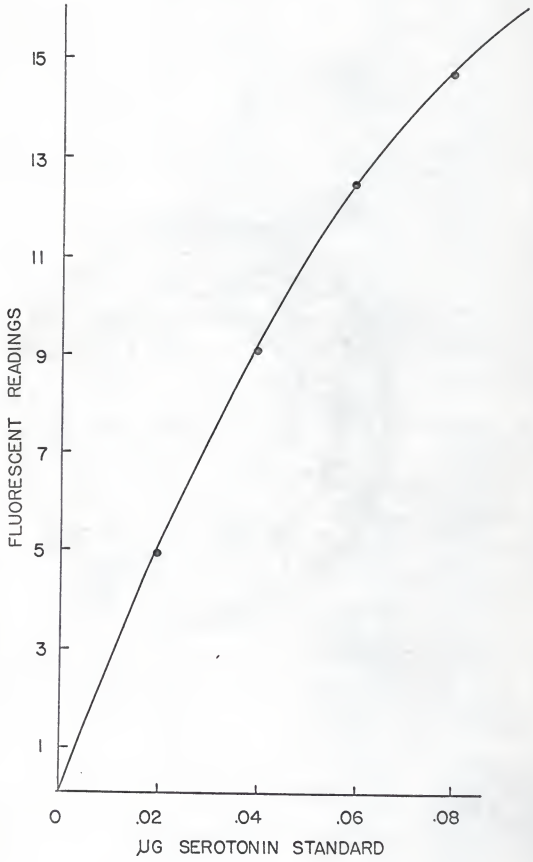


Fig. 1. Flowsheet of assay method for serotonin.

Fig. 2. Standard curve for serotonin used to convert fluorescent readings into ug of serotonin.


15
13
11
9
7
5
3
1
FLUORESCENT READINGS
0
.02
.04
.06
.08
µG SEROTONIN STANDARD

determination. The calculation of quantities of norepinephrine/g brain tissue was done in the same manner as that previously described for serotonin. Figure 4 is the standard curve used for the norepinephrine determinations.

Monoamine Oxidase Determination

Monoamine oxidase was determined radioactively using a modification of the methods published by Wurtman and Axelrod (1963) and by McCaman et al. (1965). Each sample was assayed in triplicate by the method shown in Figure 5. Radioactivity was counted in a Packard Tri Carb Model 3224 Scintilation Spectrometer with gain set at 10, window C at 50, window D at 600 and using a toluene scintilation fluid made with 4 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis-2 (5-phenyloxazoleyl)-benzene per liter of toluene. The substrate was 5-hydroxytryptamine-2-^{14}C purchased from the New England Nuclear Corporation. A 40 mM stock solution of this compound was made in 0.01 M HCl from which the 0.8 mM working standard was made. Radioactivity was converted to units of enzyme (one unit of monoamine oxidase was defined as that amount of enzyme contained in one g of tissue which converted one umole of 5-hydroxytryptamine to one mole of 5-hydroxyindole-3-acetic acid in one hour) by the following equation:

$$\text{umoles serotonin/gm tissue/hr} = \frac{\text{N mmoles substrate} \times 1000 \times \text{counts of sample/30 minute incubation} \times 2}{2/3 \times \text{counts of N mmoles substrate} \times \text{gm of sample}}$$

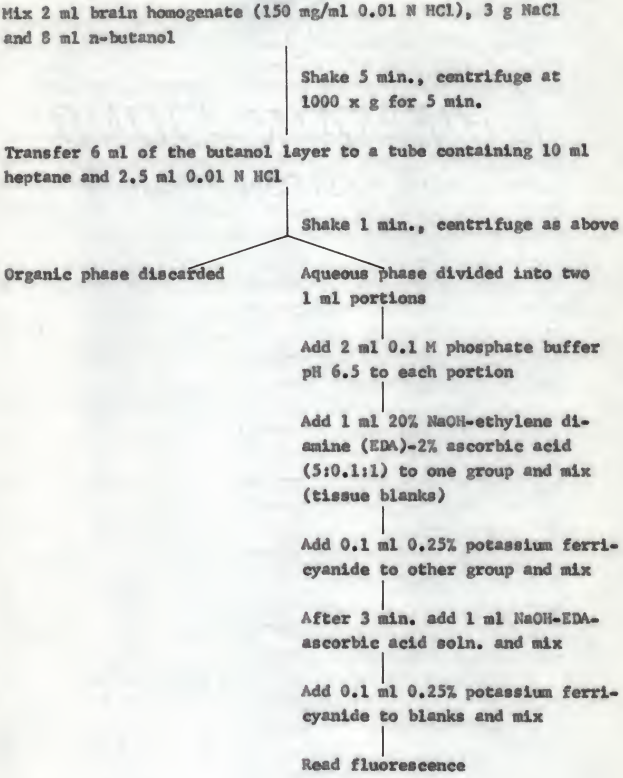
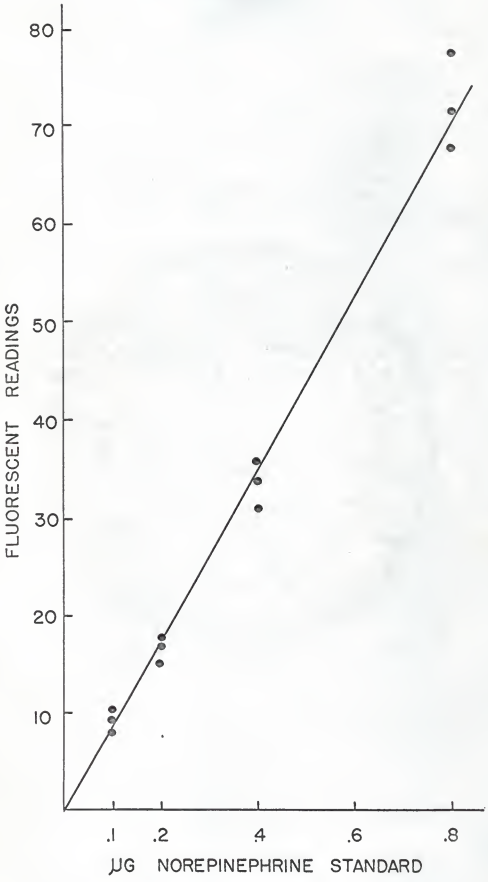


Fig. 3. Flowsheet of assay method for norepinephrine.

Fig. 4. Standard curve for norepinephrine used to convert fluorescent readings into ug of norepinephrine.


80
70
60
50
40
30
20
10
FLUORESCENT READINGS
.1
.2
.4
.6
.8
µG NOREPINEPHRINE STANDARD

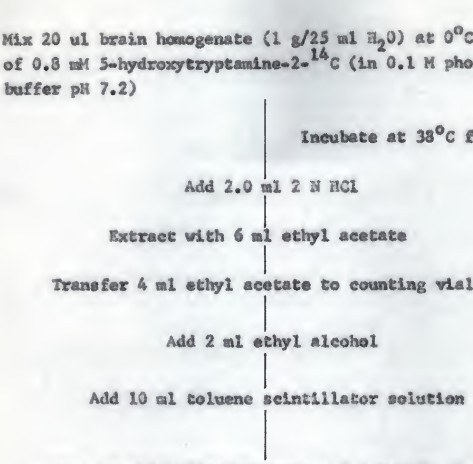


Fig. 5. Flowsheet of assay method for monoamine oxidase.

Plates I and II. Typical fighting encounters between trained fighter mice and untrained mice of the C57Bl/6J strain. The trained fighter is found either on top or to the right in each picture.

Results

Results indicated that both short and long-term exposure of a defeated mouse to a trained fighter caused a significant change in the serotonin concentration of whole brain and on the monoamine oxidase concentration of the hypothalamus, amygdala and frontal cortex. Norepinephrine, however, demonstrated no significant changes from normal as a result of either of the fighting procedures although a significant decrease was observed between the values obtained after one and two days of fighting and those obtained after four, eight, fourteen and twenty days of the long-term experiment. All values discussed are means obtained from the various phases of the experiment.

Short-term Fighting Experiment

Serotonin increased significantly ($p < 0.005$) after one fight from a normal value of 0.986 ug/g to 2.574 ug/g brain tissue. After four and eight exposures in one day, the values of 2.759 and 2.585 ug/g obtained, respectively, were not significantly different from the value obtained after one fight, however, these values were still significantly ($p < 0.005$) higher than normal. Figure 6 graphically represents the effect of short-term fighting on the whole brain content of free serotonin in defeated mice.

Norepinephrine remained essentially unchanged throughout the short-term study. The normal value was found to be 0.306 ug/g brain tissue. After one fight, a very slight decrease to 0.303 ug/g was observed followed by a slight increase after four and eight exposures to values of 0.307 and 0.315 ug/g, respectively. None of these fluctuations were found to be significantly different with respect to each other or with the normal value. Figure 7 shows the effect of short-term fighting on the norepinephrine content of the brain of defeated mice.

Monoamine oxidase exhibited different trends over the

Fig. 6. Effect of exposure to a trained fighter for one, four or eight 5-minute periods in one day on the brain serotonin content of defeated mice.

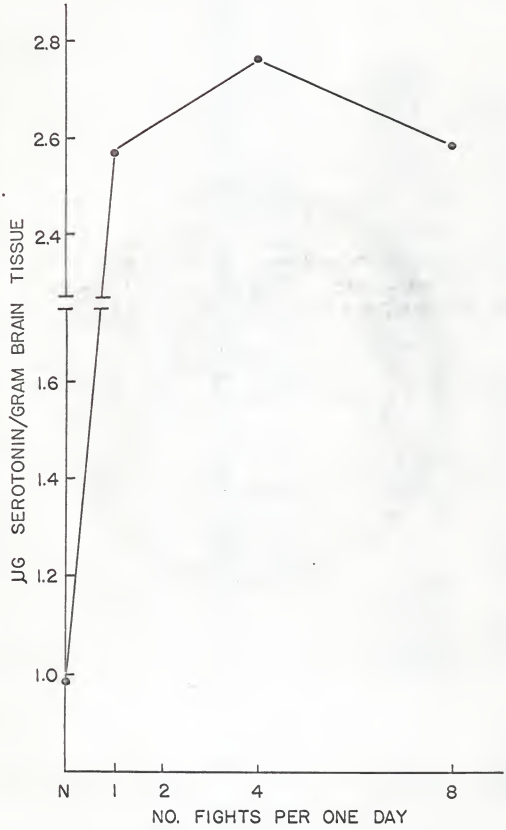
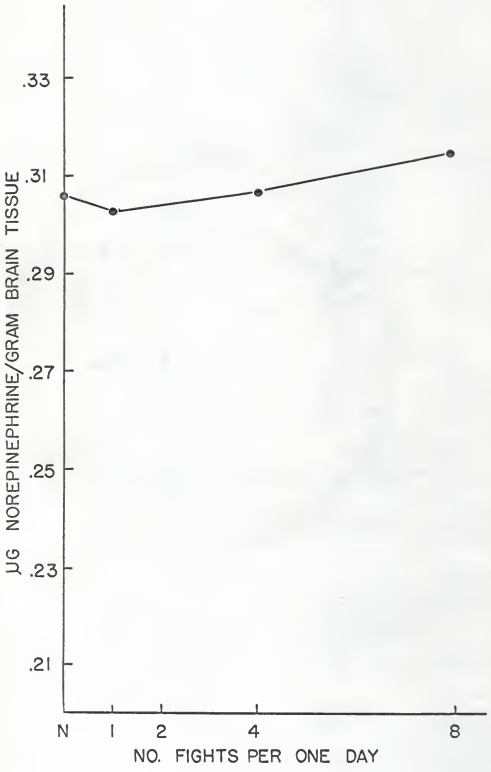

2.8
2.6
2.4
1.6
1.4
1.2
1.0
µG SEROTONIN/GRAM BRAIN TISSUE
N
1
2
4
8
NO. FIGHTS PER ONE DAY

Fig. 7. Effect of exposure to a trained fighter for one, four or eight 5-minute periods in one day on the brain norepinephrine content of defeated mice.


.33
.31
.29
.27
.25
.23
.21
µG NOREPINEPHRINE/GRAM BRAIN TISSUE
N
1
2
4
8
NO. FIGHTS PER ONE DAY

short-term experiment for each of the areas studied. The normal value for the hypothalamus was found to be 6.467 enzyme units which was higher, but not significantly, than the frontal cortex or the amygdala which had normal levels of 5.973 and 5.659 units, respectively. After one fight, a nonsignificant increase was observed in the hypothalamus to a value of 9.009 units while the amygdala showed a significant ($p < 0.005$) increase to a value of 7.513 enzyme units. The frontal cortex remained at a value of 5.993 units and was observed to have no change after one fight. Four fights brought the monoamine oxidase content of the hypothalamus to a value of 9.704 units which was significantly ($p < 0.05$) higher than normal but not significantly different from the one fight value. The amygdala returned to a normal level of 5.763 units after four fights which was a significant ($p < 0.005$) decrease from the one fight value. The frontal cortex was still found to be unchanged, having a value of 5.987 units. Eight exposures to a trained fighter caused a significant ($p < 0.05$) decrease below normal in the amygdala to a value of 4.672 units, and a decrease in the frontal cortex to 4.412 units which approached significance at the 0.05 level. The enzyme content of the hypothalamus decreased to 8.847 units which was no longer significantly higher than the normal level. Figure 8 shows the effect of short-term fighting on the monoamine oxidase content of the hypothalamus, amygdala, and frontal cortex of defeated mice. It should be noted that although the curve for the hypothalamus appears to demonstrate significant changes, the variation in the results obtained precluded any significance except between normal and four exposures per day. Table I shows the effect of short-term fighting on the brain content of serotonin, norepinephrine and the monoamine oxidase activity of the hypothalamus, amygdala and frontal cortex.

Fig. 8. Effect of exposure to a trained fighter for one, four or eight 5-minute periods in one day on the monoamine oxidase activity (uM serotonin/gram/hour) in the hypothalamus, amygdala and frontal cortex of defeated mice.

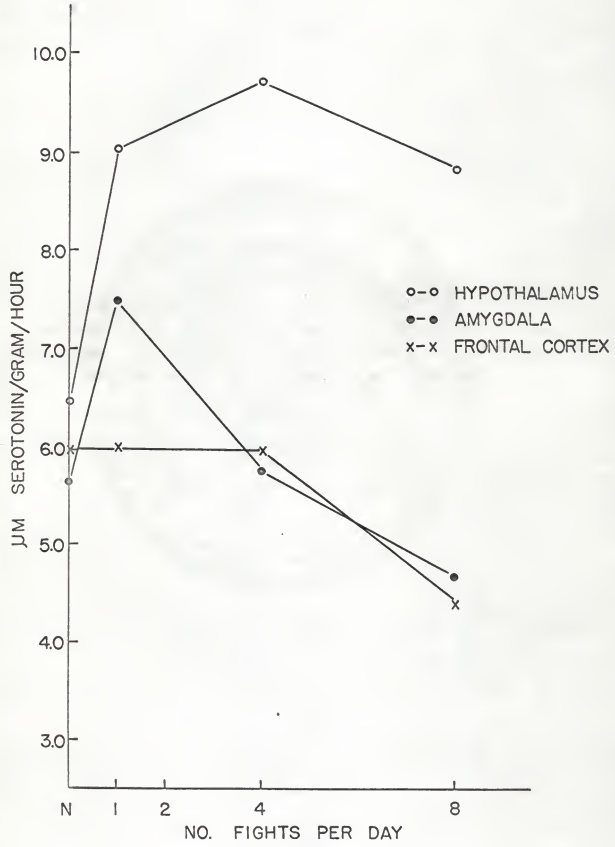

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
µM SEROTONIN/GRAM/HOUR
o–o HYPOTHALAMUS
•–• AMYGDALA
x–x FRONTAL CORTEX
N
1
2
4
8
NO. FIGHTS PER DAY

Table 1. Effect of exposure to trained fighters for a different number of 5-minute periods in one day on the brain serotonin and norepinephrine content and on the monoamine oxidase activity in the hypothalamus, amygdala and frontal cortex of defeated mice.

Fights/Day (No.)	Serotonin ug/g	Norepinephrine ug/g	Monoamine Oxidase uM Serotonin/g/hr Hypo*	Amyg*	F.C.*
0	0.986	0.306	6.467	5.659	5.973
1	2.574	0.303	9.009	7.513	5.993
4	2.759	0.307	9.704	5.763	5.987
8	2.585	0.315	8.847	4.672	4.412

* Hypo-Hypothalamus, Amyg-Amygdala, F.C.-Frontal Cortex

Long-term Fighting Experiment

Serotonin was found to decrease significantly ($p < 0.01$) from a normal level of 0.986 ug/g to 0.775 ug/g after one day of exposure to a trained fighter for two 5-minute periods a day. Two days of fighting left the value essentially at this level with a mean of 0.783 ug/g. However, after four days of fighting, the level decreased further to 0.722 ug/g which was significantly ($p < 0.005$) lower than normal but not from the value obtained from two days. At eight and fourteen days, the level of brain serotonin was found to be steadily increasing to values of 0.791 and 0.891 ug/g, respectively. These values were not significantly different from each other nor was the fourteen day value significantly different from the normal. The eight-day level, however, was significantly ($p < 0.01$) lower than normal. Twenty days of fighting brought on another decrease to a level of 0.796 which again was significantly ($p < 0.01$) lower than normal. Figure 9 shows the response of brain serotonin in a defeated mouse to a long-term fighting procedure.

Figure 10 is a graphic representation of the effect of long-term fighting on the brain norepinephrine content of the defeated mouse. A normal value of 0.306 ug/g was obtained from which none of the values deviated significantly over the long-term study. Fluctuations were observed, however, from high values of 0.337 and 0.333 ug/g obtained after one and two days of fighting, respectively, to levels of 0.281, 0.249, 0.284 and 0.243 ug/g obtained after four, eight, fourteen and twenty days, respectively. These values were all significantly lower, at the 0.05 level or less, than those obtained after one and two days of fighting.

Monoamine oxidase exhibited a significant ($p < 0.005$) increase in the hypothalamus over the normal value of 6.467 enzyme units to levels of 8.750 and 9.860 units after one and two days of fighting, respectively. The level after two days

Fig. 9. Effect of exposure to a trained fighter for two 5-minute periods a day for a varied number of days on the brain serotonin content of defeated mice.

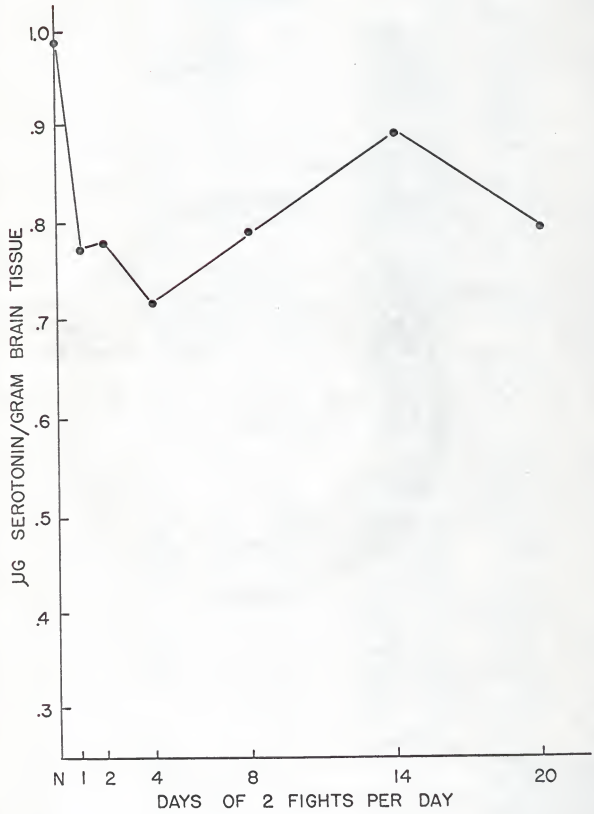
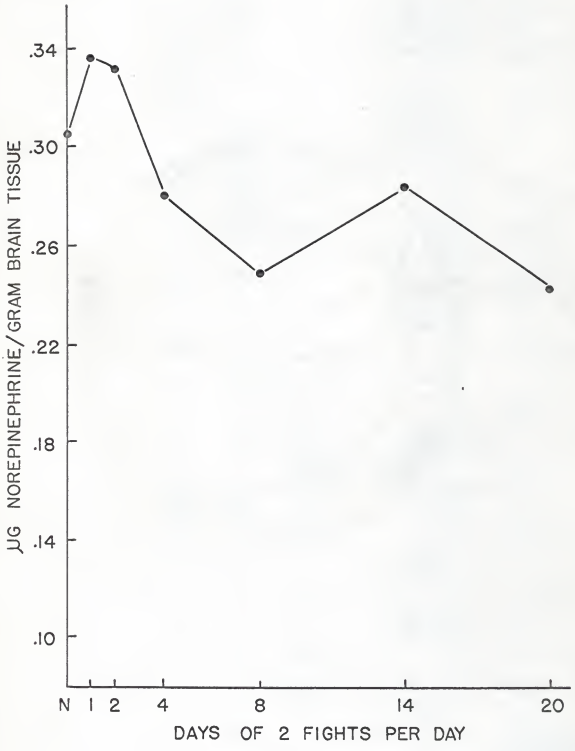

1.0
.9
.8
.7
.6
.5
.4
.3
µG SEROTONIN/GRAM BRAIN TISSUE
N 1 2 4 8 14 20
DAYS OF 2 FIGHTS PER DAY

Fig. 10. Effect of exposure to a trained fighter for two 5-minute periods a day for a varied number of days on the brain norepinephrine content of defeated mice.


.34
.30
.26
.22
.18
.14
.10
µG NOREPINEPHRINE/GRAM BRAIN TISSUE
N
1
2
4
8
14
20
DAYS OF 2 FIGHTS PER DAY

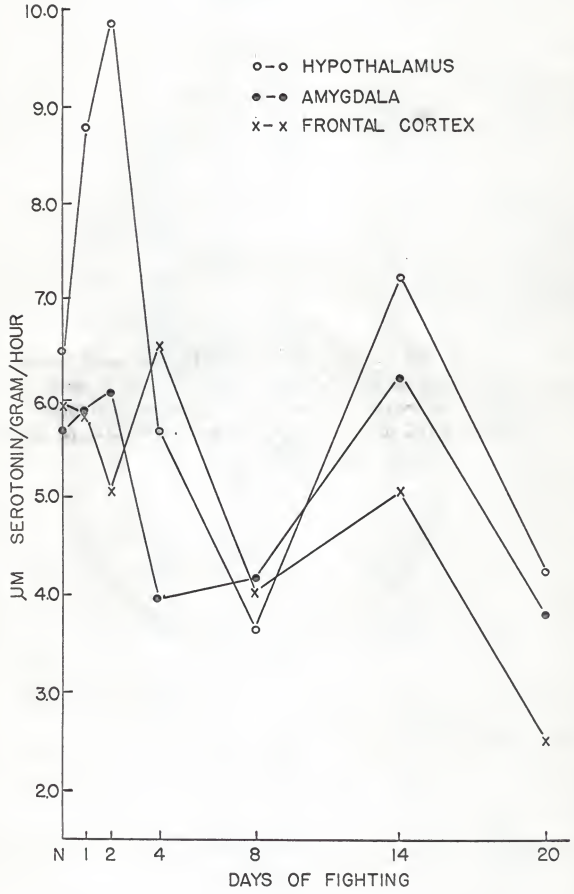
was also significantly ($p < 0.01$) higher than the one day response. Four days of fighting brought on a significant ($p < 0.005$) decrease from the two day value to 5.688 units which, however, was not significantly different from normal. There was a further significant ($p < 0.005$) decrease in the hypothalamus to 3.676 units after eight days of fighting from which the level increased significantly ($p < 0.025$) to 7.281 units after fourteen days. This was followed by another significant ($p < 0.05$) decrease between fourteen and twenty days of fighting to a value of 4.249 units.

The amygdala showed no significant change over the first two days of fighting although a slight increasing trend was observed to values of 5.928 and 6.070 units for one and two days, respectively. After four days of fighting, the amygdala exhibited a significant ($p < 0.005$) decrease from the two day value to 3.972 units which was also significantly ($p < 0.005$) lower than normal. Eight days of fighting brought on a non-significant increase to 4.168 units followed by a significant ($p < 0.005$) increase to a value of 6.270 units after fourteen days. The amygdala again decreased significantly ($p < 0.005$) to a level of 3.799 units after twenty days of fighting.

One and two days of fighting caused no significant change in the monoamine oxidase activity of the frontal cortex although a decreasing trend was observed to values of 5.915 and 5.092 units after one and two days, respectively. A significant ($p < 0.01$) increase to 6.533 units was observed after four days followed by a significant ($p < 0.005$) decrease to 4.058 units after eight days of fighting. A nonsignificant increasing trend to a value of 5.055 units was observed in the frontal cortex between eight and fourteen days. The frontal cortex showed the same response as the hypothalamus and amygdala between fourteen and twenty days of fighting with a significant ($p < 0.005$) decrease to 2.510 units.

The twenty-day values for the hypothalamus, amygdala and

Fig. 11. Effect of exposure to a trained fighter for two 5-minute periods a day for a varied number of days on the monoamine oxidase activity in the hypothalamus, amygdala and frontal cortex of defeated mice.


10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
μM SEROTONIN/GRAM/HOUR
o–o HYPOTHALAMUS
•–• AMYGDALA
x–x FRONTAL CORTEX
N
1
2
4
8
14
20
DAYS OF FIGHTING

frontal cortex were also significantly lower ($p < 0.05$) than the respective normal values. Figure 11 shows graphically the effect of long term fighting stress on the monoamine oxidase activity of the hypothalamus, amygdala and frontal cortex of defeated mice. Table II summarizes the effect of long-term fighting stress on the whole brain content of serotonin and norepinephrine and on the monoamine oxidase activity of the hypothalamus, amygdala and frontal cortex of defeated mice.

Table II. Effect of exposure to trained fighters for two 5-minute periods a day for a varied number of days on the brain serotonin and norepinephrine content and on the monoamine oxidase activity of the hypothalamus, amygdala and frontal cortex of defeated mice.

Days of Fighting (No.)	Serotonin ug/g	Norepi-nephrine ug/g	Monoamine Oxidase uM Serotonin/g/hr		
			Hypo*	Amyg*	F.C.*
0	0.986	0.306	6.467	5.659	5.973
1	0.775	0.337	8.750	5.928	5.915
2	0.783	0.333	9.860	6.070	5.092
4	0.722	0.281	5.688	3.972	6.533
8	0.791	0.249	3.676	4.168	4.058
14	0.891	0.284	7.281	6.270	5.055
20	0.796	0.243	4.249	3.799	2.501

* Hypo-Hypothalamus, Amyg-Amygdala, F.C.-Frontal Cortex

Discussion

It is difficult to correlate the results obtained from the monoamine oxidase, serotonin and norepinephrine determinations since they were not all done in the same areas of the brain. Therefore, these different phases of the experiment are discussed separately.

The increase in serotonin observed during the short-term study of fighting up to eight times in one day agrees with previous results. Barchas and Freedman (1963) showed that rats forced to swim to exhaustion in 15° water had a 15% increase in brain serotonin. They also reported that rats on a treadwheel for more than 3 hr showed a 10% increase in serotonin. These increases are rather small, however, compared to the 64% increase observed in the present study. If it is assumed that this increase in serotonin is a stress response, then the difference in the increases found may be due to differential degrees of stress. It is difficult to say which of the situations is more stressful. The period spent in the water or on the treadwheel may require more physical exertion due to the prolonged time involved but the fighting encounters may produce a more severe emotional trauma. Fighting stress has indeed been reported by Bronson and Eleftheriou (1964, 1965a, 1965b, 1965c) to be a severe stress in view of the response shown by the adrenal cortex to fighting. If this is the case, the animal being the most severely stressed may show the greater increase of free serotonin. There is also a species difference involved which could explain the differences observed.

After fighting on a prolonged basis, however, there was found to be a decrease in serotonin. This response has not previously been reported. However, other work involving stress and serotonin was done only on a short-term basis. Barchas and Freedman (1963) did, however, place rats on a treadwheel for up to 24 hr after which they observed a 10% increase

in serotonin. It is important to note that this was a continuous stress and not as severe as that when exposed to fighter mice. The long-term fighting procedure lasted for twenty days with the maximum decrease occurring after four days of fighting for two 5-minute periods/day.

It may be that there are two different mechanisms involved in serotonin changes during short and long-term fighting stress. It is known that serotonin is stored in a bound or inactive state and must be released to a free form to be active (Waleszek and Abood, 1957; Shore et al., 1957; Haggendal, 1963; Asheroft, Eccleston and Crawford, 1965). When an animal encounters a severe stress, it may be that serotonin is rapidly liberated from the bound into the active free form. This could be viewed as an initial response to a stress. A rapid increase in free serotonin is in fact observed after short-term stress both in the present work and in the work by Barchas and Freedman (1963).

It is not known what the function of serotonin is in the central nervous system, but if it is involved in the learning process as has been postulated (Woolley, 1962; Woolley, 1963; Woolley and van der Hoeven, 1963) then the results obtained after prolonged fighting stress can be explained. Woolley and his coworkers have shown that a decrease in brain serotonin to below normal causes a marked increase in the learning ability of adult mice, whereas an increase in brain serotonin inhibits the learning ability. This was done by experimentally altering the brain serotonin content by the use of drugs. From this it may be assumed that in the natural situation, serotonin also decreases when an animal is forced to learn. It seems that a defeated mouse must be going through a learning process while trying to cope with or evade the fighter mouse. This in fact was observed since the actual contact time during the 5-minute fighting period decreased steadily until about four days and remained essentially at this level throughout the remaining

sixteen days. This observation has also been made by Bronson and Eleftheriou (1965a, 1965b).

The cage in which the fighter mouse was housed and in which the fighting encounters took place was covered with a wire-mesh lid. Protruding into the cage from this lid was a food hopper also made of wire mesh. It was noted that after about three or four days of fighting, the defeated mouse had learned to cling to the food hopper and move around trying to keep the hopper between himself and the fighter mouse. In addition, from about four days throughout the remainder of the experiment, the defeated mouse would usually climb onto the food hopper immediately upon being placed into the fighter's cage even though the fighter did not attack. It, therefore, seems that under conditions of fighting stress, the defeated mouse is forced into a learning process in order to cope with the attacks made by the fighter. After about four days of fighting, the defeated mouse learns that by using the food hopper as an obstacle between himself and the fighter he receives the least amount of injury. The observed changes in brain serotonin content is in accord with this hypothesis since there is a decrease through four days of fighting followed by an increase back to an essentially normal level at fourteen days.

The decrease between fourteen and twenty days is difficult to explain. It could be that at this time the animal goes into a state of exhaustion from its attempt to cope with the attacks of the fighter. Another possibility is that the animal is forced into another learning situation. Since the attacks still persist, the mouse may again be trying to find a way to alleviate the situation.

Norepinephrine remained essentially unchanged throughout the short-term study. However, a significant decrease was observed between the values obtained after one and two days of fighting and those obtained after four, eight, fourteen and

twenty days in the prolonged fighting study. In previous work determining norepinephrine as a function of stress, the brain amine was found to decrease. This was with severe stress such as swimming rats to exhaustion in 15° water (Barchas and Freedman, 1963) or electroshock to rats (Levi and Maynert, 1962). After less severe stress, such as shaved animals exposed to an environment of 4°C for 3 to 5 hr, 72 hr of food and water deprivation or anoxia induced gradually in a nitrogen chamber (Barchas and Freedman, 1963), no change in brain norepinephrine was observed. These experiments were all conducted using rats as experimental animals.

Fighting stress is in fact a severe type of stress as mentioned previously. Therefore, in light of the previous work with norepinephrine and stress, the norepinephrine content should have decreased in the short-term study as well as the long-term. It is possible that a species difference could account for the discrepancy between the results from the short-term study and those reported previously. Woolley and van der Hoeven (1963) and Woolley (1962, 1963) have shown that a decrease in both norepinephrine and serotonin in the brain increases the learning ability of adult mice. If this is indeed the case, the same argument used for serotonin can also be used here since a decrease was observed during the prolonged fighting study.

Another alternative should be mentioned for explaining the difference in the results obtained in the short vs. long-term study for both serotonin and norepinephrine. It is possible that there are differential types of adaptation. One used to cope with a stressful situation initially and another for adapting to a prolonged stress.

The study of an enzyme with respect to purely behavioral manipulation has not previously been reported in the literature. The present study shows that fighting stress has a profound effect on the monoamine oxidase activity of the hypothalamus,

amygdala and frontal cortex of a defeated mouse. After one fight, monoamine oxidase activity is increased in the amygdala while no effect is observed in the frontal cortex and only an increasing trend is shown in the hypothalamus. After eight fights in one day the amygdala and frontal cortex both fell below normal and the hypothalamus was maintained above normal.

During prolonged fighting, the monoamine oxidase activity in the hypothalamus increased above normal after one and two days of fighting and dropped below normal after four and eight days. The amygdala was essentially unchanged until four days, at which time the monoamine oxidase activity dropped below normal where it stayed through eight days of fighting. The frontal cortex was essentially unchanged until eight days when it dropped below normal. At fourteen days all three areas showed increases to essentially normal values from which all three areas again had a fall in monoamine oxidase activity to below normal at twenty days.

There appear to be two different mechanisms involved which govern monoamine oxidase activity during short and long-term stress. It is interesting that the activity in the hypothalamus increases and remains above normal throughout the short-term fighting study whereas both the amygdala and frontal cortex decrease eventually to below normal. It may be that there is a shift in the activity of monoamine oxidase from these areas to other areas such as the hypothalamus where it may be needed to a greater extent. During the prolonged stress study, the activity in the frontal cortex seemed to have a general decreasing trend which also could be indicating a shift of the enzyme activity from there to other areas following a corresponding change in the amine levels. The hypothalamus was the only area which under long-term stress showed a significant increase above normal and that only during the first two days. The rise shown by all three areas at fourteen days is also of interest, especially since both serotonin and

norepinephrine also have a peak at this time.

Not very much can be said about these results without knowing how the amines themselves are changing in these specific areas as well as the synthetic enzyme systems involved. It should be mentioned that several workers (Van Woert and Catzias, 1966; Guha and Murti, 1965; Gorkin, Komisarova, Lerman and Veryovkina, 1964) have published some convincing evidence that there may be different monoamine oxidases for different amines or that there may be more than one active site involved. If this is true, it should be noted that in the present study, serotonin was used as the substrate in the enzyme analysis and, therefore, the monoamine oxidase actually measured was that for serotonin.

The hypotheses suggested in this thesis need further investigation, particularly, the elucidation of the entire metabolic pathways for the various brain amines in the same brain areas as a function of fighting stress. However, the present work shows there is a differential response in brain serotonin and norepinephrine to the same stressor. In addition, it was shown for the first time that purely behavioral manipulation can alter the activity of an enzyme in the brain.

Summary

A study of the effects of frustration (caused by repeated defeat after exposure of an untrained mouse to a trained fighter) on the brain content of serotonin and norepinephrine and on the monoamine oxidase activity of the hypothalamus, amygdala and frontal cortex was conducted. It was shown that there was a causal relationship between the behavioral manipulation and the brain biochemistry observed. There was found to be a difference in concentration of the brain amines and enzyme activity depending on the fighting procedure used.

After short-term fighting stress (one to eight 5-minute fights/day), serotonin was found to increase 64% while norepinephrine showed no change from normal. The monoamine oxidase activity of the hypothalamus increased significantly to above normal where it stayed throughout eight fights/day. The amygdala showed an initial significant increase after one fight followed by a significant decrease to below normal after four and eight fights/day. The frontal cortex did not change until the eighth fight when it was found to be significantly below normal.

Long-term fighting stress (two 5-minute fights/day for periods from one to twenty days) elicited different responses than short-term stress. Serotonin decreased significantly until four days after which a significant increase to fourteen days was observed. This was followed by another decrease at twenty days. Norepinephrine showed an initial increasing trend followed by a significant decrease after four and eight days. Minor fluctuations then occurred at fourteen and twenty days. Monoamine oxidase activity in the hypothalamus increased significantly until two days and then decreased significantly below normal until eight days. Another significant increase was observed at fourteen days followed by a significant decrease at twenty days. The amygdala showed a significant decrease at

four days followed by a significant increase at fourteen days A decrease was then observed at twenty days. The frontal cortex decreased significantly at eight days following minor fluctuations at one, two and four days. There was also a significant increase at fourteen days and a significant decresse at twenty days as in the hypothalamus and amygdala.

The reason for the different responses obtained from the two types of fighting procedures was not clear. It is postulated that the response to short-term aggression is strictly a stress response whereas long-term aggression induces a learning process in the defeated mouse or possibly that there are differential adaptive mechanisms for short and long-term stress.

More work is needed in all phases of the metabolic pathways of the various brain monoamines to clarify their role in the adaptive response to fighting stress.

ACKNOWLEDGMENTS

The author wishes to express appreciation to Dr. B. E. Eleftheriou, major professor, for his assistance and advice in experimental work and in preparation of this thesis.

Appreciation is also expressed to Dr. R. R. Consigli for the use of his scintillation spectrometer to count radioactive samples.

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EFFECT OF FIGHTING (DEFEAT) ON BRAIN LEVELS OF SEROTONIN, NOREPINEPHRINE AND MONOAMINE OXIDASE

by

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

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1967

Abstract

Experiments on population densities have indicated that certain alterations take place varying from changes in body weight to systemic biochemical differences as a result of increasing population density. It may be that fighting among individuals is to some degree responsible for some of these changes. A need was evident to investigate some factors involved in brain biochemistry as a function of behavioral manipulation. It was, therefore, decided to study the effects of short and long-term fighting stress on two brain amines, serotonin and norepinephrine, and on the enzyme monoamine oxidase, which oxidatively deaminates these two amines.

The procedure involved two different types of fighting. Naive mice (not trained to fight) were exposed to trained fighters for one, four or eight 5-minute periods in one day which constituted a short-term exposure to aggression. Long-term fighting aggression was accomplished by exposing naive mice to trained fighters for two 5-minute periods a day for one, two, four, eight, fourteen or twenty days.

The results showed that fighting stress had an effect on brain serotonin and norepinephrine content and on the monoamine oxidase activity in the hypothalamus, amygdala and frontal cortex. In addition, the effect was observed to be different for short and long-term fighting stress.

Serotonin was found to increase after one fight in the short-term study to a level at which it remained through eight fights. Norepinephrine, however, showed no change at all during the entire short-term experiment. The monoamine oxidase activity was observed to increase significantly in the hypothalamus and remain above normal from one to eight fights/day. The amygdala increased initially but then dropped below normal after eight fights. The frontal cortex did not change after one and four fights but decreased significantly after eight

fights/day.

The long-term stress study showed a significant decrease in brain serotonin content through four days of fighting two fights per day. The level then increased at eight and fourteen days followed by a decrease at twenty days. Norepinephrine was observed to have an increasing trend at one and two days of fighting but then decreased significantly at four and eight days. An increase was then found at fourteen days followed by another decrease at twenty days. The monoamine oxidase activity in the hypothalamus increased at one and two days of fighting. It then decreased significantly at four and eight days, returned to normal at fourteen days and again decreased significantly at twenty days. The amygdala showed no significant change until it decreased between two and four days. The level remained below normal at eight days but then returned to normal at fourteen days. A decrease was then observed between fourteen and twenty days. The frontal cortex demonstrated minor fluctuations until eight days when it decreased significantly below normal. As in the other two areas and in the serotonin and norepinephrine content, the frontal cortex increased at fourteen days and then decreased at twenty days of fighting.

It is difficult to find an explanation for these results especially since the function of serotonin and norepinephrine in the brain is not known. It may be, however, that the response observed in the short-term study was a response to an external stress. During the long-term fighting procedure, however, it may have been that a learning process was taking place while the defeated animal was being forced to adapt itself to the repeated fighting encounters. However, the possibility cannot be overlooked that differences obtained between the short and long phases of the present study are due to differential types of adaptation, one to cope with a stress initially and another for adaptation to a prolonged stress.