EFFECTS OF EXPOSURE TO AGGRESSION, DEFEAT, AND HORMONE TREATMENT ON REGIONAL BRAIN RNA BASE RATIOS IN THE MOUSE

by

MARY ELLEN LENZ

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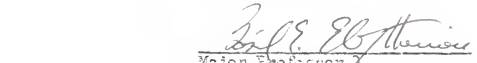

Major Professor

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INTRODUCTION

Aggression is defined as an unprovoked attack, involving engagement in aggressive behavior, which includes only offensive behavior. The term agonistic behavior includes both offensive and defensive behaviors and may include both physical conflict (fighting) and nonphysical conflict such as gestures, postures and other communicative signals. Aggressiveness is a relatively permanent state of potential for committing acts of aggression (Welch, 1968).

Aggression in laboratory animals can be elicited by various methods such as surgical techniques, painful stimuli, drug treatment and prolonged isolation-frustration. Frontal lobectomy (Karli, 1955), ablation of the olfactory bulbs (Vergnes and Karli, 1963), bilateral lesions of the lateral olfactory bandelets and the destruction of the prepyriform cortex (Karli and Vergnes, 1963) are experimental surgical techniques which induce aggressive behavior in rats. In addition, lesions of the septal nuclei produce septal irritability accompanied by aggression (Brady and Nauta, 1953). Electric foot shock also can induce aggression when applied to mice or rats (O'Kelly and Steckle, 1938; Miller, 1948; Ulrich, Hutchinson, and Azrin, 1965). Treatment with hallucenogenic drugs (Brown, 1960; Everett, 1961), convulsant and amphetamine-like drugs (Reinhard, Plekes, and Scudi, 1960) results in the appearance of aggressive behavior. Prolonged periods of isolation produce aggressive behavior in male mice (Garattini, Giacolone, and Valzelli, 1967; Yeu, Stanger and Millman, 1959). Isolation-frustration produces an aggressive behavior which is most "natural", in the sense that it includes elements of species characteristic behavioral patterns.

The study reported herein is one of a series of experiments on the endocrine and neurochemical effects of exposure to agonistic behavior in the male C57BL/6J mouse. In all cases the training method of Scott (1946) was utilized to establish a colony of male fighting mice. This work on agonistic behavior originated from a study of population density and its effect on the endocrine system (Christian, 1957). Several separate studies indicate that agonistic behavior might be as important in causing adrenal responses as density (Barnett, 1958; Bronson and Eleftheriou, 1963; Clark, 1953). The effects of aggression and defeat on adrenal weights (Bronson and Eleftheriou, 1965a), plasma bound and unbound corticosterone (Bronson and Eleftheriou, 1965b), pituitary ACTH (Bronson and Eleftheriou, 1965a) and plasma and pituitary levels of LH (Eleftheriou and Church, 1967) and TSH (Eleftheriou, Church, Norman, Pattison, and Zolovick, 1968) reveal a trend toward systemic endocrine adaptation which develops earlier in aggressive mice than in defeated mice.

Since aggressive or defeat behavior patterns are mediated through the central nervous system a series of experiments was initiated into the study of factors in the brain which might affect agonistic behavior. It is well known that changes in the concentration of serotonin in the brain affect behavior (Aprison and Ferster, 1961a; Aprison and Ferster, 1961b; Aprison, Wolf, Poulos and Folkerth, 1962; Bogdanski, Weisbach, and Udenfreind, 1958; Brodie and Shore, 1957; Costa and Rinaldi, 1958; Hingtgen and Aprison, 1963; Shore, Pletscher, Tomich, Carlsson, Kuntzman and Brodie, 1957; Udenfriend, Weisbach and Bogdanski, 1957a; Udenfriend, Weisbach, and Bogdanski, 1957b; Udenfriend, Weisbach and Bogdanski, 1957c). Norepinephrine is altered also in various types of behavior (Aprison and Hingtgen, 1965), and is related to

behavioral alterations seen when an animal is attacked or is being attacked (Gunne and Reis, 1963). In addition, both serotonin and norepinephrine are involved in the learning ability of mice (Woolley, 1962; Woolley, 1963; Woolley and van der Hoeven, 1963). Initial studies on brain monamine oxidase (Boehlke and Eleftheriou, 1967) and brain 5-hydroxytryptophan decarboxylase (Eleftheriou and Church, 1968a) revealed inconsistent changes in enzyme activity in the hypothalamus, amygdala, and frontal cortex of mice exposed to aggression and defeat. When serotonin and norepinephrine levels in these areas were measured, a persistent effect of exposure to aggression was evident up to sixteen day fights in defeated mice indicating a lack of neurochemical adaptation when systemic adrenal-pituitary adaptation has occurred (Eleftheriou and Church, 1968b).

With increasing information on the roles of ribonucleic acid (RNA) in the cell, the effects of aggression and defeat on regional brain RNA in the mouse were studied to gain an insight into the mediation of aggressive and defeat behavior patterns. Brain RNA has been a subject of study only in recent years. Brain RNA is located in both microsomal and nuclear fractions (Brody and Bain, 1952; Aldridge and Johnson, 1959). A DNA-dependent RNA polymerase has been identified in nuclei from calf and rat brain (Barondes, 1964), and its activity is higher in the brain than in the liver for all species studied (Bondy and Waelsch, 1965). Hiatt (1962) identified an RNA fraction in rat liver nuclei which, on the basis of its size, rate of labeling, and base composition, resembled bacterial messenger RNA. The existence of rapidly labelled RNA in the brain has been demonstrated in the 4-18 S region of a sucrose density gradient (Barondes and Jarvik, 1964; Kimberlin and Hunter, 1965). In addition, a rapidly labelled RNA was eluted from a

methylated albumin-kieselghur column (Bertermann, Mahler, Moore, Dutton, and Thompson, 1965). A rapidly labelled RNA with the following properties was obtained from rat brain: its base composition is close to that of rat DNA; it sediments in sucrose density gradients in a broad range, a part of it sedimenting faster than 28 S ribosomal RNA; it is eluted from a methylated albumin-kieselghur column at higher salt concentration than ribosomal RNA, and its synthesis is inhibited by actinomycin-D (Jacob, Stevenin, Jund, Judes, and Mandel, 1966). These results support the theory that synthesis of high molecular weight RNA of the messenger type also occurs in the rat brain.

The level of stimulation of neuronal cells in the eye alters the RNA content of the cells. Bratgaard (1952) reported that light deprivation led to a rapid decrease in RNA concentration in the ganglion cells of the rabbit retina and light stimulation resulted in an increased synthesis of RNA proportional to the total light stimulation received. These observations were confirmed and the conclusion reached that adequate light stimulation is a major variable controlling the development of normal ribonucleoprotein levels in retinal cells (Rasch, Swift, Riesen, and Chow, 1961). Stimuli which alter the levels of brain RNA have been noted. The compounds, hexenal, caffein, and camphor, increase and circulatory hypoxia of the brain decreases the nucleic acid content of the brain (Baranov and Pevzner, 1963). The concentration and total amount per cell of cytoplasmic RNA were markedly increased in rat Purkinje cells, as compared with controls, following prolonged physical exertion (Attardi, 1957). Convulsions produced by metrazol resulted in depletion of pentose-nucleic acid content in nuclear fractions of the rat brain (Talwar, Sadasivudu, and Chitre, 1961). Electroshock treatment of the cat produced a pronounced decrease in the RNA content in various brain areas (Mihailovic, Jankovic, Petrovic, and Isakovic, 1958).

In addition to demonstrations that RNA synthesis is affected by neural stimulation, a number of studies have attempted to link RNA with the capacity of the central nervous system to store information which corresponds to learning and memory. Increased RNA synthesis in the brains of goldfish during short term learning experiments has been demonstrated (Glassman, Schlesinger, and Wilson, 1966). RNA from mice trained to avoid a shock showed greater isotope incorporation than RNA from controls (Wilson, Boggan, Zemp, and Glassman, 1966). These investigators also showed by density gradient sedimentation patterns that there was increased RNA synthesis of a messenger-like RNA in mice brains during learning. Analysis of regional areas of mouse brain for incorporation of ^{3}H-uridine into RNA following fifteen minutes of avoidance conditioning showed increased labelling only in localized areas (Zemp, Wilson, and Glassman, 1967). These workers concluded that brain tissue responds to certain kinds of stimulation in the same way as do other tissues, i.e., an early chemical response to stimulation is the synthesis of RNA.

The purine-pyrimidine ratio was analyzed in nuclear and cytoplasmic RNA from vestibular Deiters' neurons in rats subjected to a learning experiment during which there was established a pattern of sensory and motor abilities (Hydén and Egyházi, 1962). The adenine/uracil ratio of the nuclear RNA increased significantly and there was an increased amount of RNA per nerve cell. These results suggested that the base ratios of RNA changed during learning. The RNA changes during learning were interpreted as a genomic activation of regions in the brain to produce nuclear RNA with highly specific base ratios. In a subsequent experiment Hydén and Egyházi (1964) studied changes in RNA content and base composition in cortical neurons of rats in a learning experiment involving transfer of handedness. The results showed an

increase of RNA occurred in the neurons of the part of the cortex engaged in the transfer of handedness. The purine/pyrimidine ratio was increased significantly in the RNA of the learning neurons compared to the controls. The conclusions from this study were that an acute learning situation with no precedence in the animal's life acts as a genomic stimulation resulting in a production of RNA with highly specific base ratios in the neurons immediately involved, and that the decrease of the ratio G+C/A+U suggests that the new RNA formed is of the messenger RNA type. Hydén and Lange (1965) summarized their work on RNA response in neurons early and late during two learning experiments. Early in a learning situation, which has not been encountered before, they postulated that the genome of the neuron is activated, and a small amount of RNA rich in adenine and uracil is synthesized. When the new behavior has become fixed, the formation of the adenine-uracil rich RNA declines, and RNA rich in guanine and cytosine is formed. At this time, the genic activation already has induced the synthesis of a specific protein by the adenine-uracil rich RNA needed for facilitation. Thus, an acute learning situation may select parts of the genome which become activated. The primary gene product is RNA rich in adenine and uracil. This is followed by a ribosomal type RNA which takes over the long term synthesis of protein necessary to sustain neural function of the new behavior.

Additional support for the role of RNA in neural stimulation, memory and learning is found in studies with ribonuclease (RNA-ase). Certain neural membrane transport systems may be dependent on RNA since the surface positive component of dendritic potentials are enhanced after topical application of RNA-ase to the cortex (Shtark, 1965). Tail sections of transected, conditioned planaria regenerated in exogenous ribonuclease performed randomly suggesting

that ribonuclease interferes with the transfer of information (Corning and John, 1961). Intracerebral injection of RNA-ase was shown to block retention of a conditioned defensive reflex in white mice (Krylov, Danylova, and Tongur, 1965).

Utilizing aggression and defeat as generalized nervous stimuli, regional brain levels of ribonuclease and total ribonucleic acid (RNA) were studied (Hamlet, 1969). Significant differences in hypothalamic and amygdaloid RNA-ase activity were found between day one and day sixteen of exposure to aggression in defeated mice. Male fighter mice during their early training period exhibited similar changes in RNA-ase which became reversed after training was completed. Total regional brain RNA content supported the findings obtained with RNA-ase. A persistent and significant decline in total RNA was obtained in the hypothalamus and amygdala of repeatedly defeated mice as well as in fighters during the early part of their training experience. Additional information regarding the nature of the changes in total regional nucleic acid content in mice exposed to aggression and defeat was desirable. The purpose of the present study was to determine the RNA base percent and base ratios in certain brain areas (hypothalamus, amygdala, frontal cortex, and cerebellum) of C57BL/6J male mice exposed to aggression and defeat. This was done in an effort to supplement previous studies dealing with aggression, defeat, and the mode of neural RNA adaptation.

MATERIALS AND METHODS

Five hundred male Mus musculus, strain C57BL/6J, were used in the study. All experimental mice were isolated following weaning at twenty-one to twenty-eight days of age, and were maintained under standard conditions of sixteen hours light, eight hours dark, 70° F, with food and water provided ad libitum. Isolation was achieved by dividing 7" x 11½" opaque cages into halves with metal cage dividers. One animal was placed in each compartment with its own bedding, food, and water supply. Isolation was maintained for a minimum of forty days before experimental treatment was initiated.

The five hundred mice were divided into ten experimental groups of fifty mice each. An entire experimental group of fifty animals each was killed on the same day by cervical dislocation. The brain areas (hypothalamus, amygdala, frontal cortex, and cerebellum) were dissected immediately from the brain and frozen in acetone-dry ice. These frozen brain areas from each group were placed in polyethylene tubes, and transferred to a liquid nitrogen storage tank for subsequent analyses. All mice, except one group which will be discussed later, were between the ages of 60-83 days.

Experimental Groups:

(1) Late fighters (LF): The late fighters were fifty male mice trained to fight according to the method of Scott (1946). Prior to training, each mouse and its soiled bedding was transferred and housed in a 7" x 11½" opaque cage. These cages were not cleaned, although fresh cedar shavings were added where needed. Each mouse was exposed twice daily (morning and late afternoon) for five-minute periods to a "trainer" mouse. Trainer mice were male and of the C57BL/6J strain

which were held by the tail and dangled in the cage of the mouse being trained according to the procedure of Scott (1946). The late fighters were trained in this manner for twenty-four days. As the late fighters' attacks became more aggressive, they were marked at the base of the tail by nail polish and the trainer mice were dropped into the late fighters' cages. The mice were carefully observed so that the late fighter was not repulsed in any attack. At the end of the twenty-four day training session, the most aggressive males were selected for use in subsequent fighting encounters. Criteria for selection were rate and vigor of attack. The late fighters were killed and the brain areas removed within five minutes after their last fight. Because of isolation, length of training period, and fighting encounters, the late fighters were between 114-121 days of age when killed.

(2) Early Fighters (EF): The early fighters were fifty male mice trained to fight exactly as the late fighters were trained, except they were killed within five minutes of the last training period on the fifth day of training.

(3) Naive mice and exposure to aggressors: After forty days of isolation, mice not trained to fight (naive) were exposed to the trained fighters for 0 (unfought control), 1, 4, 8, and 16 days. Fifty male mice were exposed at each period for a total of 250 mice. In all cases the naive mice were transferred to the cage of the late fighters for the five minute period. The mice were killed and brain regions removed within five minutes after the second fight on the last day. The control group of mice that were transferred to a clean cage without exposure

to a trained fighter was not included because it has been found that no significant hormonal or neurohormonal changes occur in these mice (Eleftheriou and Church, 1968b).

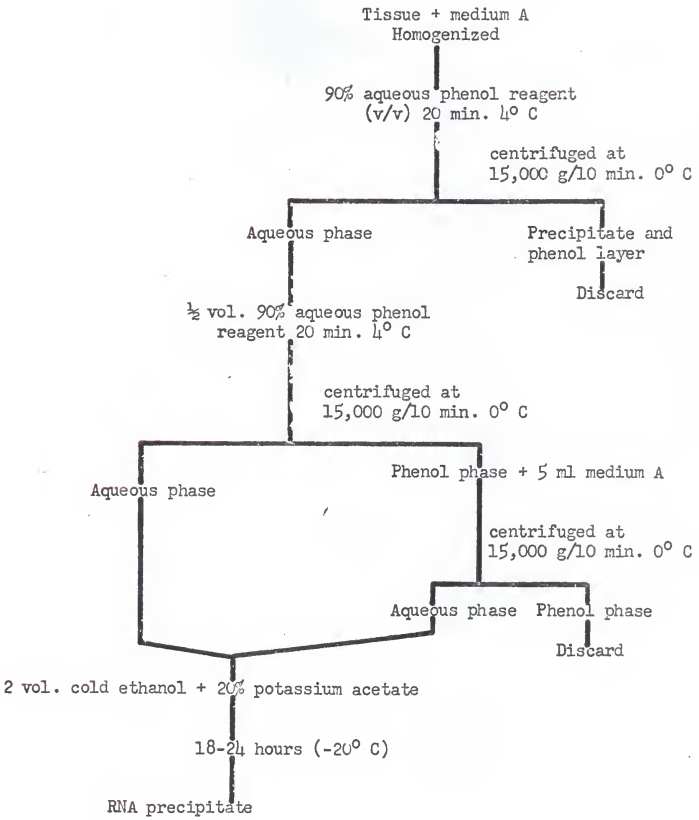
(4) Hormone Treatments: Serotonin, dopamine, and corticosterone were injected subcutaneously into a group of fifty animals each. The dosage level of each hormone was 200 ug/.1 ml/day for five days. The animals were killed on the sixth day following the initiation of injections. Serotonin and dopamine were injected in saline and corticosterone was injected in sesame seed oil.

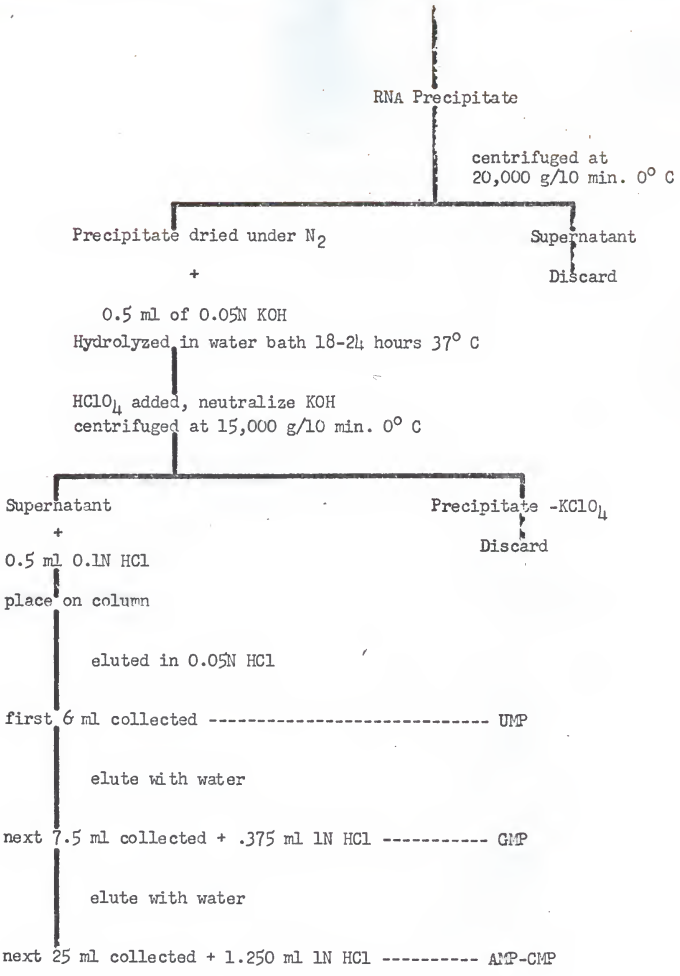
Extraction Procedure:

Total RNA was determined by the procedure of Kirby (1956). A modification of this technique was used to obtain RNA which was free of DNA, protein, and ribonucleases from both nuclei and cytoplasm (Popa, Cruceanu, and Lacatus, 1967).

At the time of analysis, a tube containing identical brain areas from one group was removed from the liquid nitrogen. All steps were carried out at 0°-4° C whenever possible. The brain areas were rapidly subdivided into five groups of 10 areas/group in order to obtain sufficient tissue for each analysis, and suspended in a glass homogenizer (Potter-Elvehjem type) containing 10 ml of ice-cold medium A (0.03M tris-HCl, 0.25M sucrose, 0.001M $MgCl_2$, and 0.006M mercaptoethanol, at pH 7.6). Prior to the addition of brain tissue, medium A was made 0.5% with respect to the detergents sodium dodecyl sulphonate (Crestfield, Smith and Allen, 1955), and sodium napthalene disulphonate (Jacob, Stevenin, Jund, Judes, and Mandel, 1966) to aid in the disruption of cells. Washed bentonite was added to medium A (1.25 mg/ml) to aid in the removal of nucleases (Petermann and Pavlovec, 1963). The suspension was homogenized using a teflon pestle and a power stirring apparatus.

Fig. 1. Flow diagram for the extraction of RNA from mammalian tissues (brain).




RNA Precipitate
centrifuged at
20,000 g/10 min. 0° C
Precipitate dried under N_2
Supernatant
Discard
+
0.5 ml of 0.05N KOH
Hydrolyzed in water bath 18-24 hours 37° C
$HClO_4$ added, neutralize KOH
centrifuged at 15,000 g/10 min. 0° C
Supernatant
Precipitate -$KClO_4$
Discard
+
0.5 ml 0.1N HCl
place on column
eluted in 0.05N HCl
first 6 ml collected ---------- UMP
elute with water
next 7.5 ml collected + .375 ml 1N HCl ---------- GMP
elute with water
next 25 ml collected + 1.250 ml 1N HCl ---------- AMP-CMP

The homogenate was diluted with an equal volume of 90% aqueous phenol reagent (90% phenol, 0.1% 8-hydroxyquinoline, and 10% m-cresol) and stirred for twenty minutes (Hiatt, 1962). The aqueous and phenol layers were separated by centrifugation at 15,000 g for ten minutes. The phenol extraction was repeated a second time by adding ½ volume of the phenol reagent to the aqueous phase and stirring for twenty minutes. The mixture was centrifuged at 15,000 g for ten minutes, the aqueous layer was removed and saved, while the last phenol layer was back washed by adding 5 ml of medium A. This mixture was centrifuged at 15,000 g for ten minutes and the aqueous phase pooled with the original aqueous extract. Two volumes of ice-cold ethanol, and a sufficient quantity of 20% potassium acetate were added to the aqueous extract to make the final solution 2% with respect to potassium acetate. The solution was shaken gently and stored at -20° C for a minimum of 18-24 hours. The RNA precipitate was collected by centrifugation at 20,000 g for 10 minutes. The supernatant was discarded and the RNA pellet dried under a nitrogen spray.

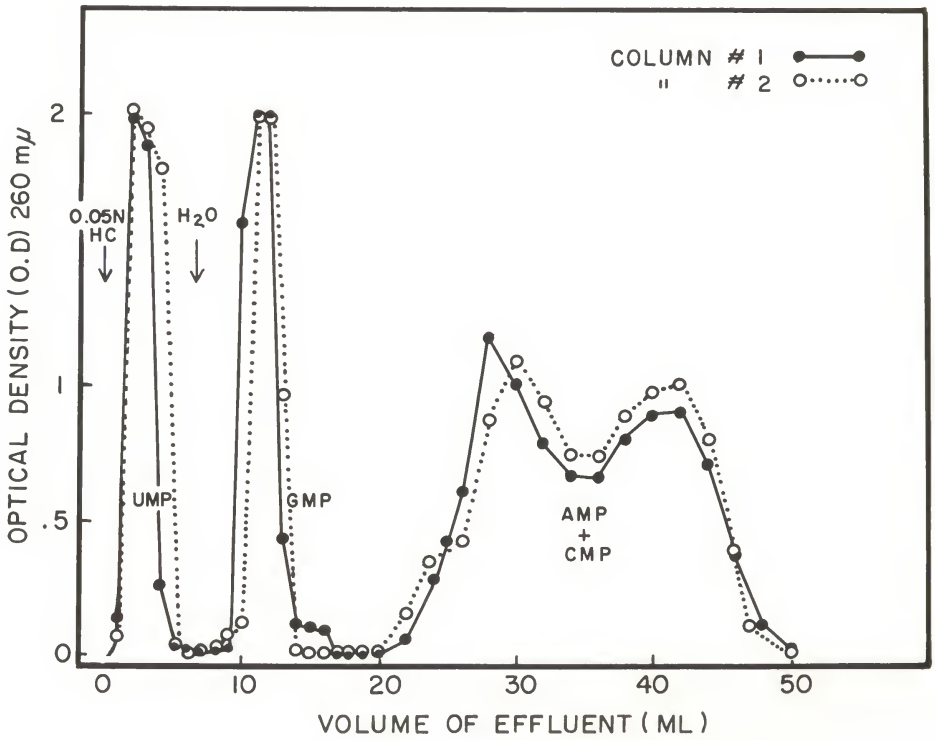
Base Ratio Analysis:

A technique developed by Katz and Comb (1963) using a small cation exchange column was used to isolate and determine the RNA bases. The RNA obtained from the extraction procedure was hydrolyzed by placing the pellet in a small polyethylene centrifuge tube, adding 0.5 ml of 0.5N KOH and incubating the tube in a hot water bath for 18-24 hours at 37° C. The solution was neutralized by adding 3-4 drops of 6N $HClO_4$, and the $KClO_4$ precipitate was removed by centrifugation at 15,000 g for 10 minutes. An equal volume of 0.1N HCl was added to the supernatant, and the solution was layered carefully on the top of a Dowex 50 H^+ column, 0.9 x 5 cm (200 to 400 mesh, 4 times cross linked). The column was prepared by washing and

decanting the fines of the resin in doubly distilled water (pH 7.6) three times. The resin then was washed with about 200 ml of 3N HCl, followed by water washing until neutral. The resin was packed into a 0.9 x 32.0 cm column to a height of 5 cm and 20 ml of 0.05N HCl passed through the column. The columns were standardized by placing 0.5 ml of a standard 2', 3'-ribonucleotide mixture plus 0.5 ml of 0.1N HCl on the column, allowing it to drain to the top of the resin and collecting the fraction. The column was washed down with 1.0 ml of 0.05N HCl which was allowed to drain to the top of the resin and collected. Five milliliters of 0.05N HCl were added and allowed to drain to the top of the resin and five 1 ml fractions collected. Buffered doubly distilled water (pH 7.6) was passed through the column and one milliliter fractions were collected for a total of forty-five 1 ml fractions. The collected fractions were read on a Beckman DU-spectrophotometer at a wave length of 260 mu. Results of standardization are shown in Fig. 2.

Each one milliliter sample of hydrolyzed RNA was layered carefully on a column which had been washed with 20 ml of 0.05N HCl prior to each run, and allowed to drain to the top of the resin. The column was washed down with 1.0 ml of 0.05N HCl which was allowed to drain to the top of the resin. Five milliliters of 0.05N HCl were added to the column and the first 6 ml of the HCl effluent collected and the rest discarded. These conditions wash quantitatively uridine monophosphate (UMP) through the column while the other nucleotides remain on the column. Doubly distilled water (pH 7.6) was added to the column carefully to prevent disturbing resin particles and allowed to pass through the column at a flow rate of not more than 1 ml/minute. The first 7.5 ml of water effluent, which quantitatively elutes guanosine monophosphate (GMP), were collected and brought to 0.05N HCl concentration by the addition

Fig. 2. Results of column chromatography standardization on hydrolyzed RNA. Each nucleotide or nucleotide combination was eluted from the Dowex columns in specific fractions. These were collected and their optical density values at 260 mu determined.


COLUMN # 1
" # 2
OPTICAL DENSITY (O.D) 260 mμ
2
1
.5
0
0.05N HC
H2O
UMP
GMP
AMP + CMP
0
10
20
30
40
50
VOLUME OF EFFLUENT (ML)

of 0.375 ml of 1.0N HCl. The next 25 ml of water passing through the column, which elutes cytidine monophosphate (CMP) and adenosine monophosphate (AMP) together, were collected and brought to 0.05N HCl concentration by the addition of 1.275 ml of 1N HCl.

Determination of Relative Mole Percent of Nucleotides (uM/100 uM Nucleotide):

The three fractions containing UMP, GMP, and AMP-CMP, were read on a Beckman DU-2 spectrophotometer at their respective maximal absorbancy wave lengths (UMP-260 mu; GMP-257 mu; AMP-CMP, 257 and 279 mu). The $A_{279}:A_{257}$ ratio in 0.05N HCl for AMP is 0.238 and, for CMP, 2.32 (Katz and Comb, 1963). The following equations (Loring, 1955) were used to calculate the absorbancy due to AMP and CMP in the mixture:

$$x = \frac{2.32\ (A_{257}) - A_{279}}{2.08}$$

$$y = A_{279} - 0.238\ x$$

where x is the absorbancy at 257 mu due to AMP alone, y is the absorbancy at 279 mu due to CMP alone, and A is absorbancy (optical density). The relative mole percent for each nucleotide was found by dividing the optical density of each nucleotide by its extinction coefficient, multiplying by the dilution factor (fraction volume), adding the total, and finding the percent.

Example:

$$x = \frac{2.32\ (A_{257}) - A_{279}}{2.08} = \frac{2.32\ (.115) - .074}{2.08} = .093$$

$$y = A_{279} - .238\ x = .074 - .238\ (.093) = .052$$

Base	O.D.	Extinction Coefficient	Dilution (fraction)	Product	Micromoles/ uM of RNA
UMP	.100	9,600	6.000	$.625 \times 10^{-4}$	10.71
GMP	.385	11,800	7.875	2.569 "	44.02
AMP	.115	14,900	26.250	1.633 "	27.98
CMP	.074	13,000	26.250	1.001 "	17.30
				5.837×10^{-4}	

Statistical Analyses:

Standard deviations were calculated for all nucleotide base percents. In addition, a two-way analysis of variance was performed (Winer, 1962).

RESULTS

Qualitative Analysis:

Brain RNA from C57BL/6J mice obtained using the cold phenol extraction procedure of Kirby (1956) exhibited an absorption curve typical of nucleic acids (Fig. 3). Previous studies on mouse brain RNA reported a maximal absorbance at 258 mu, minimal absorbance at 234 mu, and the 280 mu/260 mu ratio to be 0.47 (Popa, Cruceanu, and Lacatus, 1967). For RNA extracted from the hypothalamus, maximal absorbance was at 258 mu, minimal absorbance at 230 mu, and the 280 mu/260 mu ratio was equal to 0.49. The maximum absorbance for amygdaloid RNA was found to be at 258 mu, minimal absorbance at 234 mu, and the 280 mu/260 mu ratio was equal to 0.62, which was within the limits reported for mouse brain RNA. Previous work in this laboratory tested the purity of the RNA obtained by this procedure by fractionation on a methylated albumin kieselghur column, treatment with RNA-ase, and ^{32}P uptake (Fig. 4) (Pattison, 1969). Confirmation of isolation of various nucleotides also can be seen in Fig. 2.

Quantitative Analysis:

The two way analysis of variance (treatment x brain areas) for each nucleotide (uridine monophosphate (UMP), guanosine monophosphate (GMP), adenosine monophosphate (AMP), and cytidine monophosphate (CMP) showed that all experimental treatments had a significant effect ($P < .005$) on each base (Table 1). For the nucleotides AMP and GMP, all observed differences can be attributed solely to treatments since the block (variation among the four brain areas) and interaction terms are nonsignificant. Uridine monophosphate was the only nucleotide which exhibited a significant ($P < .005$)

Fig. 3. Comparison of UV absorption curves of a standard RNA preparation and RNA extracted from C57BL/6J mouse brain (hypothalamus and amygdala).

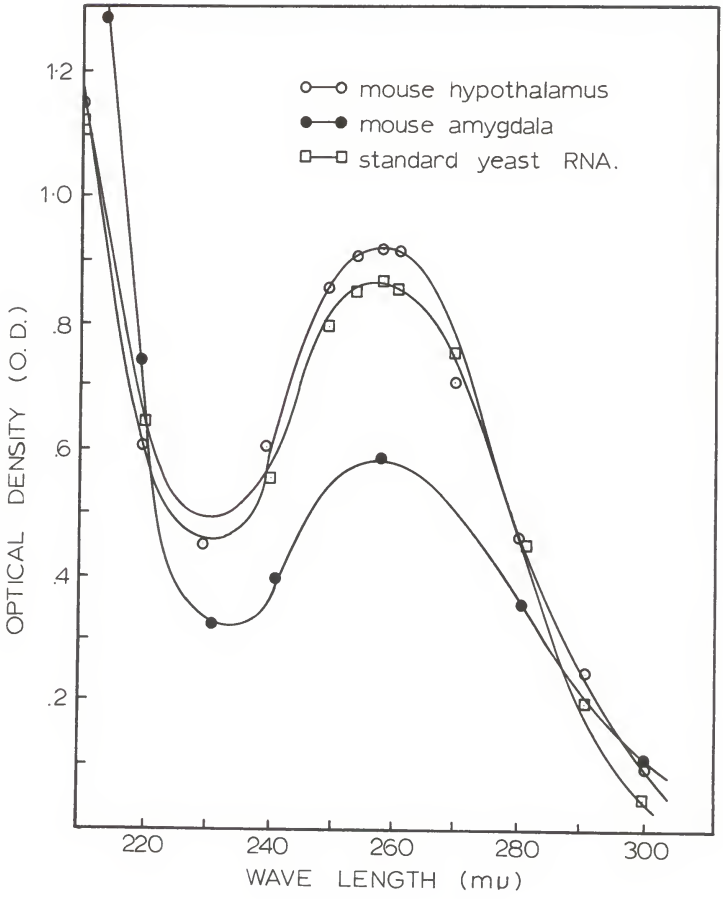

mouse hypothalamus
mouse amygdala
standard yeast RNA.
OPTICAL DENSITY (O. D.)
1·2
1·0
.8
.6
.4
.2
220
240
260
280
300
WAVE LENGTH (mμ)

Fig. 4. Results of MAK column chromatography. Extracted ^{32}P labelled RNA was separated by the column and 5 ml fractions collected. Fractions were divided into two equal portions with one set receiving RNA-ase, the other receiving no RNA-ase. Optical density for each fraction was read at 260 mu and plotted.

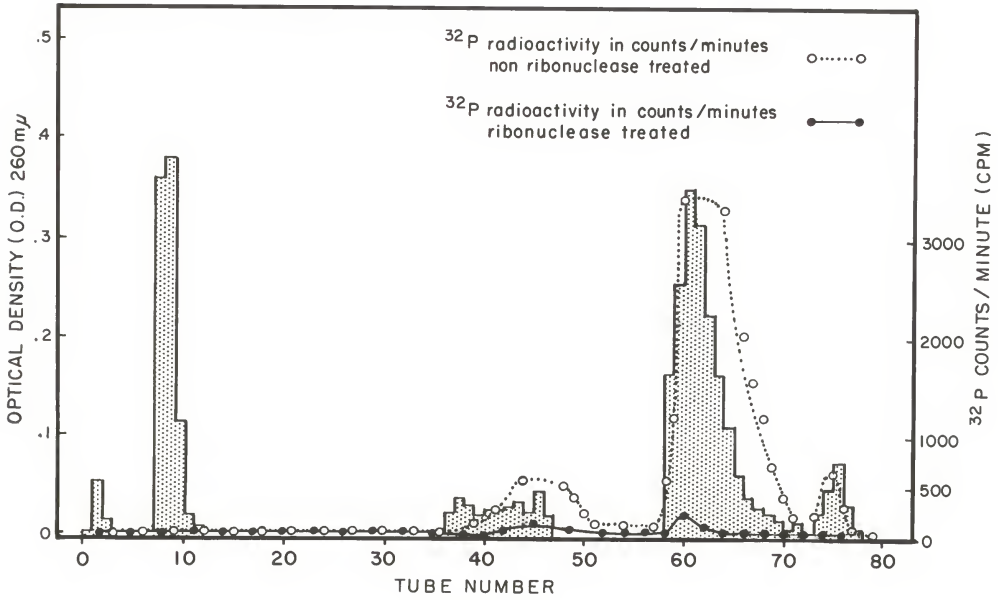

^{32}P radioactivity in counts/minutes non ribonuclease treated
^{32}P radioactivity in counts/minutes ribonuclease treated
OPTICAL DENSITY (O.D.) 260mμ
^{32}P COUNTS/MINUTE (CPM)
TUBE NUMBER
.5
.4
.3
.2
.1
0
3000
2000
1000
500
0
0
10
20
30
40
50
60
70
80

Table 1. Statistical treatment showing the results of the two way analysis of variance procedure.

Nucleotides	Source of variation	Mean square	Degrees of freedom	F ratio	P
AMP	Treatment Combinations	165.35	9	6.09	<.005
	Block (between main groups)	9.01	3	0.33	ns
	Interaction	39.23	27	1.44	ns
	Sampling error	27.17	160		
CMP	Treatment Combinations	59.60	9	6.68	<.005
	Block (between main groups)	12.58	3	1.41	ns
	Interaction	35.76	27	4.01	<.005
	Sampling error	8.92	160		
GMP	Treatment Combinations	402.16	9	5.49	<.005
	Block (between main groups)	180.00	3	2.46	ns
	Interaction	107.05	27	1.46	ns
	Sampling error	73.31	160		
UMP	Treatment Combinations	48.25	9	4.65	<.005
	Block (between main groups)	181.03	3	17.44	<.005
	Interaction	41.75	27	4.02	<.005
	Sampling error	10.38	160		

variation among brain areas. Interpretation of the data for UMP was made more complex by a significant ($P < .005$) interaction term. For CMP, the treatment and interaction terms were significant ($P < .005$) while the block term was nonsignificant.

The AMP base percent was maximal (30.85 ± 6.00) in the hypothalamus on day one, and minimal (18.97 ± 1.42) in the unfought controls. In the amygdala, AMP levels were highest (29.53 ± 1.29) following corticosterone treatment (200 ug) and lowest (17.86 ± 3.28) in the controls. The frontal cortex exhibited the greatest AMP level (28.44 ± 1.16) on day one, and the lowest AMP percent (20.50 ± 2.79) in the controls. The highest base percent of AMP (34.47 ± 4.72) in the cerebellum was obtained on day one, while the lowest percent (19.95 ± 1.55) was on day sixteen. In the hypothalamus, amygdala and cerebellum, there was a trend for an initial increase in AMP base percents on day one with a gradual decline through day sixteen (Tables 2, 3, and 5). In all four brain areas there was an increase in AMP levels in the late fighters when compared to the early fighters (Tables 2, 3, 4, and 5). The hormone injections increased AMP levels over the control levels in the hypothalamus, amygdala, and frontal cortex (Tables 2, 3, and 4), while only corticosterone had this effect in the cerebellum (Table 5).

Hypothalamic cytidine monophosphate (CMP) percents exhibited trends in defeated mice similar to those seen for hypothalamic AMP. The maximal CMP value (22.98 ± 3.54) was obtained on day one, and the minimal CMP level (14.05 ± 2.30) in the late fighters was not significantly different from the control value (14.56 ± 1.69). An initial increase in hypothalamic CMP levels on day one was followed by a decline through day sixteen (Table 2). There were no significant differences between hypothalamic CMP levels in the

Table 2. Effects of exposure to aggression and defeat, aggressive training, and hormonal injection on regional brain RNA base percents in C57BL/6J mice.

Treatment	HYPOTHALAMUS AMP	CMP	GMP	UMP
Unfought control	18.97 ± 1.42	14.56 ± 1.69	53.99 ± 2.88	12.48 ± 3.87
Day 1 (2 fights/day)	30.85 ± 6.00	22.98 ± 3.54	33.65 ±10.64	12.51 ± 3.40
Day 4 (2 fights/day)	27.65 ± 1.44	18.09 ± 0.88	41.11 ± 1.44	13.15 ± 1.16
Day 8 (2 fights/day)	24.03 ± 3.58	14.21 ± 1.71	49.59 ± 5.24	12.18 ± 0.87
Day 16 (2 fights/day)	22.53 ± 2.52	16.64 ± 1.71	50.18 ± 2.16	10.64 ± 0.99
Early fighters	20.26 ± 2.72	15.64 ± 1.44	52.88 ± 4.04	11.23 ± 1.39
Late fighters	25.98 ± 2.60	14.05 ± 2.30	47.49 ± 3.42	12.48 ± 1.54
Dopamine (200 ug)	26.77 ± 1.51	15.10 ± 1.28	46.22 ± 2.16	11.91 ± 1.60
Corticosterone (200 ug)	28.02 ± 2.20	15.34 ± 0.81	42.99 ± 1.87	13.64 ± 1.68
Serotonin (200 ug)	29.39 ± 1.61	16.73 ± 0.51	40.49 ± 2.59	13.39 ± 1.34

Table 3. Effects of exposure to aggression and defeat, aggressive training, and hormonal injection on regional brain RNA base percents in C57BL/6J mice.

Treatment	AMYGDALA			
	AMP	CMP	GMP	UMP
Unfought control	17.86 ± 3.28	14.37 ± 1.36	57.21 ± 5.00	10.57 ± 1.70
Day 1 (2 fights/day)	27.45 ± 7.70	18.77 ± 6.71	41.32 ± 5.25	12.46 ± 2.68
Day 4 (2 fights/day)	25.83 ± 2.20	15.74 ± 0.71	42.57 ± 2.20	15.86 ± 2.42
Day 8 (2 fights/day)	24.36 ± 2.42	19.46 ± 4.86	45.68 ± 1.77	10.50 ± 1.88
Day 16 (2 fights/day)	21.95 ± 5.08	16.00 ± 3.24	52.70 ± 8.93	9.85 ± 1.88
Early fighters	21.73 ± 4.83	13.94 ± 1.78	49.54 ± 4.52	14.78 ± 2.51
Late fighters	28.86 ± 5.17	15.89 ± 2.35	41.80 ± 3.31	13.45 ± 4.07
Dopamine (200 ug)	25.23 ± 1.14	17.36 ± 1.28	42.67 ± 2.11	14.74 ± 2.50
Corticosterone (200 ug)	29.53 ± 1.29	16.46 ± 2.03	41.56 ± 2.12	12.45 ± 0.69
Serotonin (200 ug)	26.97 ± 2.47	18.50 ± 2.73	42.27 ± 4.34	12.27 ± 1.55

Table 4. Effects of exposure to aggression and defeat, aggressive training, and hormonal injection on regional brain RNA base percents in C57BL/6J mice.

Treatment	FRONTAL CORTEX			
	AMP	CMP	GMP	UMP
Unfought control	20.50 ± 2.79	14.61 ± 1.79	54.57 ± 5.22	10.32 ± 1.73
Day 1 (2 fights/day)	28.44 ± 1.16	18.78 ± 1.96	38.97 ± 5.05	13.81 ± 4.13
Day 4 (2 fights/day)	22.78 ± 4.04	15.83 ± 3.80	36.64 ± 3.80	24.75 ± 8.79
Day 8 (2 fights/day)	24.48 ± 1.92	18.54 ± 1.67	42.48 ± 1.61	14.51 ± 1.64
Day 16 (2 fights/day)	27.08 ± 3.78	16.11 ± 1.36	44.39 ± 2.68	12.42 ± 2.25
Early fighters	22.21 ± 2.28	18.90 ± 3.27	37.82 ± 9.64	21.06 ± 7.30
Late fighters	26.36 ± 1.44	16.85 ± 0.48	45.88 ± 0.71	10.91 ± 1.75
Dopamine (200 ug)	28.12 ± 9.43	16.00 ± 1.10	40.43 ± 7.13	15.46 ± 1.39
Corticosterone (200 ug)	26.66 ± 2.15	18.23 ± 1.26	44.25 ± 1.83	10.85 ± 1.44
Serotonin (200 ug)	26.92 ± 1.60	17.13 ± 1.45	44.21 ± 2.14	11.74 ± 1.27

Table 5. Effects of exposure to aggression and defeat, aggressive training, and hormonal injection on regional brain RNA base percents in C57BL/6J mice.

	CEREBELLUM			
Treatment	AMP	CMP	GMP	UMP
Unfought control	30.37 ± 3.86	9.70 ± 1.98	38.74 ± 3.16	21.19 ± 5.51
Day 1 (2 fights/day)	34.47 ± 4.72	13.09 ± 5.51	36.17 ± 6.16	16.28 ± 2.47
Day 4 (2 fights/day)	24.93 ± 4.22	19.22 ± 3.41	44.50 ± 4.88	12.35 ± 3.25
Day 8 (2 fights/day)	23.27 ± 4.20	15.30 ± 2.60	45.05 ± 3.62	16.38 ± 0.60
Day 16 (2 fights/day)	19.95 ± 1.55	18.89 ± 1.46	47.62 ± 3.76	13.53 ± 1.35
Early fighters	22.46 ± 1.82	20.93 ± 2.77	37.63 ± 6.41	18.98 ± 2.74
Late fighters	24.32 ± 6.88	10.74 ± 3.58	48.74 ± 5.33	16.21 ± 1.00
Dopamine (200 ug)	22.92 ± 4.41	17.20 ± 3.07	42.49 ± 2.92	17.38 ± 5.46
Corticosterone (200 ug)	31.23 ± 1.31	11.36 ± 0.04	43.04 ± 1.30	14.37 ± 2.11
Serotonin (200 ug)	25.87 ± 1.31	23.09 ± 8.43	32.93 ± 6.07	18.11 ± 2.26

Table 6. Effects of exposure to aggression and defeat, aggressive training, and hormonal injection on regional brain G+C/A+U ratios in C57BL/6J mice.

Treatments	Hypothalamus	Amygdala	Frontal Cortex	Cerebellum
Unfought control	2.22	3.05	2.31	0.95
Day 1 (2 fights/day)	1.37	1.58	1.39	0.98
Day 4 (2 fights/day)	1.46	1.41	1.13	1.69
Day 8 (2 fights/day)	1.79	1.91	1.57	1.55
Day 16 (2 fights/day)	2.03	2.26	1.54	2.00
Early fighters	2.22	1.76	1.36	1.44
Late fighters	1.61	1.39	1.69	1.55
Dopamine (200 ug)	1.59	1.51	1.36	1.50
Corticosterone (200 ug)	1.41	1.39	1.67	1.20
Serotonin (200 ug)	1.35	1.57	1.59	1.28

early and late fighters, or among the groups injected with the three hormones. Cytidine monophosphate (CMP) percents in the amygdala showed no trends in the defeated mice (Table 3). Maximal amygdaloid CMP percent (19.46 ± 4.86) was obtained on day eight of fighting exposure, whereas 13.94 ± 1.78 represented the minimal CMP level found in the early fighters. All three hormone treatments resulted in slightly elevated CMP levels in the amygdala. The frontal cortical CMP levels were similar to those seen in the amygdala (Table 4). Cerebellar CMP levels exhibited the greatest overall changes with the various treatments. The lowest cerebellar CMP level of 9.70 ± 1.78 was exhibited by the unfought controls while the highest CMP level (23.09 ± 8.43) was found following serotonin treatment. In the defeated animals, there was a trend for an increase in cerebellar CMP levels as days of repeated defeat were prolonged.

Guanosine monophosphate consistently had the highest overall base percent in all brain areas studied and for all treatments (Tables 2, 3, 4, and 5). Maximal hypothalamic GMP percent (53.99 ± 2.88) was found in the controls, and the minimal GMP level of 33.65 ± 10.64 was seen on day one. In the hypothalami of defeated animals, GMP was affected in a manner opposite to that for both AMP and CMP after all treatments (Table 2). A similar situation was found between GMP and AMP when base values in the hypothalamus from the early and late fighters were compared. All three hormones resulted in decreased hypothalamic GMP levels when compared to the control value.

The inverse relationship between the two purines, AMP and GMP, also was seen in the amygdala and cerebellum (Tables 3 and 4) of the defeated animals. As in the hypothalamus, the highest amygdaloid GMP percent (57.21 ± 5.00) was found in the unfought controls, and the lowest GMP level (41.32 ± 5.25) was

observed in day one. There was a significant decline of GMP levels in the late fighters as compared to the early fighters in the amygdala (Table 3). All hormone treatments resulted in decreased amygdaloid GMP levels when compared to the control value. Treatments affected frontal cortical GMP levels in a manner similar to that found in the hypothalamus and amygdala (Table 4). The highest value for GMP in the cerebellum (48.74 ± 5.33) was found in the late fighters, and the lowest value (32.93 ± 6.07) was present in the serotonin treatment. In the defeated mice cerebellar GMP levels exhibited a gradual increase over the control value through day sixteen (Table 5). A significant increase in GMP levels between the early and late fighters was observed in the cerebellum, a trend opposite to that found in the hypothalamus and amygdala. GMP levels were elevated by dopamine and corticosterone, and lowered by serotonin in the cerebellum.

In the hypothalamus, uridine monophosphate did not exhibit any trends nor show any significant differences among the various treatments (Table 2). Uridine monophosphate levels in the amygdala showed minor differences in repeatedly defeated animals (Table 3). Amygdaloid UMP levels in early and late fighters, as well as with all hormone treatment, exhibited slight increases over the control value. In the frontal cortex, the low control UMP value of 10.32 ± 1.73 rose rapidly to the highest UMP level of 24.77 ± 8.79 on day four. Uridine monophosphate content of the frontal cortex also was increased in the early fighters and by dopamine treatment. In contrast to the other three brain areas, cerebellar UMP declined significantly with prolonged exposure to defeat (Table 5). Uridine monophosphate content of the cerebellum also was lowered by corticosterone treatment, as well as in the late fighters.

In the hypothalamus and amygdala, the G+C/A+U ratio (Table 6) declined at day one and increased gradually to a value near that of the control on day sixteen. The waning and waxing of the G+C/A+U ratio reflected primarily the changes in the two nucleotides, AMP and GMP. In defeated animals, the frontal cortex exhibited an initial decrease in the G+C/A+U ratio at day one followed by inconsistent and minor fluctuations through day sixteen. The G+C/A+U ratio in the cerebellum increased over the control value from day one to day sixteen. In the amygdala, hypothalamus, and frontal cortex, the G+C/A+U ratio was lower in both the early and late fighters when compared to the control value (Table 6), but a further decline in this ratio in the amygdala and hypothalamus and an increase in this ratio in the frontal cortex and cerebellum was noted as training competence increased. In the hypothalamus, amygdala, and frontal cortex, the three hormones produced a decline in the G+C/A+U ratio to varying degrees, whereas in the cerebellum the same treatment elevated this ratio.

In summary, the general trends in these data indicated that the nucleotides, AMP and GMP, exhibited the largest proportional change with most treatments while CMP exhibited lesser changes, and UMP had the least overall change. In general, AMP and GMP were affected in the opposite manner by the same treatments. The hypothalamus and amygdala exhibited corresponding affects of exposure to aggression and defeat on brain RNA base percentages and ratios. Of the four brain areas studied, the hypothalamus, amygdala and cerebellum displayed the most persistent and sustained affects of aggression and defeat on brain RNA base composition.

DISCUSSION

The study of agonistic behavior has as its ultimate goal the understanding of aggressive and defeat behavior, and is, therefore, of great pertinence to our society. Aggression, and to a lesser extent, defeat, have been studied psychologically through behavioral tests, physiologically, and more recently, biochemically. This report is the second of a series dealing with the macromolecular changes, specifically in RNA, of the central nervous system of mice exposed to repeated aggression and defeat.

Exposure to aggression and defeat, and early training of fighters all have been shown to be stressful when adrenal responses were used as an index (Bronson and Eleftheriou, 1963; Bronson and Eleftheriou, 1965a; Bronson and Eleftheriou, 1965b). Stimulation of the central nervous system by drugs (Baranov and Pevzner, 1963; Talawar, et al., 1961) and physiological exertion (Attardi, 1957) alters the RNA content of the brain, and overstimulation of the nervous system by electric shock (Mihailovic, et al., 1958) or drug therapy (Talwar, et al., 1961) results in fatigue and a decline in RNA levels in the brain. In the current studies on brain RNA, exposure to aggression and defeat is accepted as a generalized stressful nervous stimulus resulting in neural activation.

Work on regional brain ribonuclease (RNA-ase) and total regional RNA content (Hamlet, 1969) indicates that a decline in total RNA and a corresponding increase in RNA-ase is found in the hypothalamus and amygdala of defeated mice as well as early fighters. These observations on the response of brain RNA to the stress of exposure to aggression and defeat correspond to the response of RNA found in neural overstimulation.

Results of the present study indicate that in the hypothalamus, amygdala, and frontal cortex there is a significant decline in the G+C/A+U ratio upon exposure to aggression and defeat with the largest proportional changes occurring in AMP and GMP, while CMP and UMP showed only minor effects. Based on works of Hydén and Egyházi (1962, 1964), it may be assumed that the decrease in the G+C/A+U ratio indicates increased synthesis of a messenger type RNA. The relationship between the G+C/A+U ratio, and synthesis of a messenger type RNA was obtained by an interesting series of observations. The base composition of nucleolar RNA seems to agree with that of cytoplasmic RNA (Edström, Grampp, and Schor, 1961; Edström, 1960), and it was surmised that nucleolar RNA would mask chromosomal RNA if the latter were a copy of RNA. Microchemical studies revealed that chromosomal RNA did not have a base composition similar to that of DNA, nor did it have base symmetry (Edström and Beerman, 1962). Since nucleolar RNA in nerve cells comprised 25 percent of the total nuclear RNA (Hydén and Egyházi, 1962), nucleolar RNA could not mask the base ratio composition of the rest of the nuclear RNA, the chromosomal and nucleoplasmic RNA. Therefore, the production of nuclear RNA with an increased A/U ratio in a learning experiment in rats (Hydén and Egyházi, 1962) was concluded to be chromosomal with the characteristic of "messenger" RNA in bacterial systems. When an increase in the A+G/C+U and a decrease in the G+C/A+U ratio was found in intact cortical neurons during a learning experiment, and the observations made that the increase in RNA in each cortical nerve cell was moderate, and the ratio of nuclear to cytoplasmic was high, this meant that the newly synthesized RNA had highly specific base ratios. The decrease in the G+C/A+U ratio suggested that the newly synthesized RNA was of the messenger type (Hydén and Egházi, 1964). The steady increase

in the G+C/A+U ratio in the cerebellum upon exposure to aggression and defeat is assumed to indicate a synthesis of RNA primarily of the ribosomal type. This assumption is based on the observations by Hydén and Lange (1965), that a decrease in the G+C/A+U indicates base ratio composition of the ribosomal type. Although the ultimate answer as to which types of RNA are changing with exposure to aggression and defeat depends on the isolation of the various types of RNA not made in this study, the changes in the RNA base ratios can be used as a reliable estimate of the changes in the synthesis of various types of RNA.

It has been proposed that an acute learning situation with no precedence in the animal's life can act as a genomic stimulation resulting in the production of chromosomal RNA with highly specific base ratios in neurons (Hydén and Egyházi, 1964). Exposure to aggression and defeat, which results in significant changes in RNA base composition, may act in a similar manner, thus resulting in the production of RNA which is rich in AMP. This appears to be true initially in the hypothalamus, amygdala, and frontal cortex, all of which exhibit elevated AMP, lowered GMP, slightly increased CMP, and static UMP levels on day one of exposure to aggression and defeat. By day sixteen of exposure to aggression and defeat, hypothalamic and amygdaloid base percents and G+C/A+U ratios approach those found in the unfought controls. This return to "normal" RNA base composition in the amygdala and hypothalamus raises the question of neurochemical adaptation. On day sixteen of exposure to aggression and defeat, systemic adrenal-pituitary adaptation has occurred (Bronson and Eleftheriou, 1965a; Bronson and Eleftheriou, 1965b; Eleftheriou and Church, 1967; Eleftheriou, et al., 1968). Total hypothalamic RNA exhibits a return to normal control levels on day sixteen, while amygdaloid RNA levels

remain at reduced levels (Hamlet, 1969). It is possible that the adjustment in hypothalamic and amygdaloid base composition on day sixteen of exposure to aggression and defeat indicates an adaptative ability in these two areas. On day sixteen, the frontal cortical G+C/A+U ratio still is significantly lowered, indicating a lack of adaptation in this area, while the cerebellum exhibits the optimum increase in the G+C/A+U ratio on day sixteen.

An overall analysis of RNA base relationships in early and late fighters reveals that there are few significant differences between the two groups. This is relatively surprising considering the finding that total RNA levels are higher in late than in early fighters in all four brain regions and that the RNA-ase levels are lower in the hypothalamus and amygdala in the late fighters (Hamlet, 1969). The G+C/A+U ratio in the hypothalamus and amygdala shows a slight decrease in the late fighters as compared to the early fighters. However, in the frontal cortex, the G+C/A+U ratio is significantly lower in the early fighters. The significance of the changes in RNA base percents and ratios between early fighters and late fighters is not clear. Interpretation of the results obtained from the late fighters is complicated by the fact that the late fighters were significantly older than the other mice used in this study. Since the "early fighter" group was arbitrarily chosen for sacrifice on day 5, it well may be that due to psychological adaptation, this group was not chosen sufficiently early. Thus, additional studies to measure base RNA ratios before day five of training may be required.

The hormones, dopamine, corticosterone, and serotonin, were studied with the hope of gaining an understanding of how these hormones are associated with aggressive and defeat behavior patterns in changing RNA composition. The hormone injections appear to produce changes in the RNA base percents and

ratios similar to those seen in exposure to aggression and defeat. These results, however, are very incomplete and thus, any comment should be withheld until additional data are available.

A note of caution must be interjected at this point in the interpretation of these results. The work of Hydén and Egyházi (1962, 1964) on RNA base compositions was conducted in learning experiments in which there was noted an increase in total RNA. Previous work on exposure to aggression and defeat indicates that there is a significant decline in total regional RNA and a corresponding increase in ribonuclease activity (Hamlet, 1969). Therefore, the decline in the G+C/A+U ratio might be due to a more rapid turnover and utilization of RNA rich in guanosine and cytidine caused by defeat stress, or a complex interaction of breakdown and synthesis of various kinds of RNA. However, the reversal of the G+C/A+U ratio in the cerebellum, which also exhibits a decrease in total RNA, indicates that there is a differential effect. Without the specific additional information in the types of RNA changed, turnover rates, and nuclear RNA polymerase activity, any conclusions drawn from the data would be mere conjecture.

The experimental design for study of effects of exposure to aggression and defeat involves elements of conditioning, learning and memory. A colony of fighter mice were trained to be "winners" in all encounters. The defeated mice, on the other hand, appeared to be inert, and did not learn to defend themselves or escape from an aggressive attack. A growing volume of data indicating that RNA has a role in learning and memory is accumulating (Albert, 1966; Hydén and Egyházi, 1962; Hydén and Egyházi, 1963; Hydén and Egyházi, 1964; John, 1967). The general concept is that learning acts as a genomic stimulator altering the metabolism of DNA and RNA whose functions

would be to serve as repositories of memory and to dictate construction of molecules, such as proteins, which actually affect functional changes in the cell. Supporting evidence for this concept comes from the fact that there is a loss of recent memory in mice following inhibition of cerebral protein synthesis (Flexner, Flexner, Roberts, de la Haba, 1964). An electrokinetic mechanism for the "read-out" of memory has been proposed (Elul, 1966). In learning, changes in DNA and/or RNA alter protein structures of neuronal membranes, which in turn alters synaptic activity.

At this time, the role of regional brain RNA in aggressive and defeat behavior patterns is not clear. It has been found that exposure to aggression and defeat increases ribonuclease activity with a corresponding reduction in regional brain RNA (Hamlet, 1969). The results of the present study indicate that under these same conditions, there is an increased synthesis of a messenger type of RNA. Since this is one of the first attempts at correlating the results of a study on macromolecules with aggressive and defeat behavior, no specific assumptions can be drawn from these data. One can speculate that initial exposure to aggression and defeat somehow triggers an increase in brain RNA-ase which accounts for the decreased total regional RNA, but leaves the significance of the decrease in the G+C/A+U ratio an unanswered question. If exposure to the stress of aggression and defeat results in increasing demands for neurotransmitter in the brain, as learning has been proposed to do (Briggs and Kitto, 1962), a situation analogous to enzyme induction may occur such that lowering levels of neurotransmitters or other precursors inhibit repressor sites on DNA and cause the synthesis of RNA, which will in turn direct the synthesis of the neurotransmitter. The first and second possibilities are not mutually exclusive, but are given to indicate the

complex interaction of effects which may occur upon exposure to aggression and defeat. The significance of the present study on regional brain RNA base ratios lies in the fact that it indicates that RNA is changing with exposure to aggression and defeat. At this time, the mechanism of mediation of the RNA base changes found in this study cannot be clarified. However, the finding that changes do occur in neural RNA upon exposure to aggression and defeat is significant.

SUMMARY

The results of the present study can be summarized as follows:

(1) All experimental treatments had a significant effect on all nucleotides (AMP, CMP, GMP, and UMP).

(2) AMP and GMP exhibited the largest proportional change with most treatments while CMP exhibited lesser changes, and UMP had the least overall change. In general, AMP and GMP were affected in the opposite manner by the same treatments.

(3) The hypothalamus, amygdala, and cerebellum displayed the most persistent and sustained effects of aggression and defeat on brain RNA base composition. The hypothalamus and amygdala exhibited almost identical effects of exposure to aggression and defeat on brain RNA base percentages and ratios.

(4) In defeated animals, there was a significant decrease in the G+C/A+U ratio in the hypothalamus, amygdala, and frontal cortex, while there was an increase in the G+C/A+U ratio in the cerebellum upon initial exposure to aggression.

(5) A surprising finding was that there were no consistent differences in base composition between the early and late fighters.

(6) Dopamine, corticosterone, and serotonin appeared to produce changes in the RNA base percents and ratios similar to those seen in exposure to aggression and defeat.

(7) At this time, the significance and mechanism of mediation of the RNA base changes found in this study cannot be clarified without further study of types of RNA changed, nuclear RNA polymerase, and RNA turnover rates upon exposure to aggression and defeat.

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EFFECTS OF EXPOSURE TO AGGRESSION, DEFEAT, AND HORMONE TREATMENT ON REGIONAL BRAIN RNA BASE RATIOS IN THE MOUSE

by

MARY ELLEN LENZ

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ABSTRACT

A total of five hundred Mus musculus, strain C57BL/6J, were isolated, upon weaning, for a minimum of forty days, then divided into ten experimental groups of fifty mice each: late fighters, a colony of trained fighting mice; early fighters; unfought controls; exposure to trained fighters for one, four, eight, and sixteen days; and hormone treatments of dopamine (200 ug), corticosterone (200 ug), and serotonin (200 ug). Hormone injections were given subcutaneously once daily for a total of five days and animals were killed on the sixth day. Animals were killed by cervical dislocation, the brain regions dissected, and frozen immediately in liquid nitrogen for subsequent analyses. Ten brain areas were pooled for each analysis, and the RNA was extracted, hydrolyzed, and a base ratio analysis performed.

The two way analysis of variance for each nucleotide (adenosine monophosphate [AMP], cytodine monophosphate [CMP], guanosine monophosphate [GMP], and uridine monophosphate [UMP]) was significantly different ($P < .005$) for each treatment. Adenosine monophosphate (AMP) and GMP exhibited the largest proportional change with most treatments while CMP exhibited lesser changes, and UMP levels showed the least variation. In repeatedly defeated animals, the hypothalamic and amygdaloid levels of AMP showed a sharp initial increase at day one (30.85 ± 6.00 and 27.45 ± 7.7, respectively) with a gradual decline approaching control levels (18.97 ± 1.42 and 17.86 ± 3.28, respectively), while GMP levels exhibited an inverse relationship. Frontal cortical AMP and GMP levels exhibited the same initial effects of exposure to aggression and defeat as seen in the hypothalamus and amygdala but did not show a return to control levels at day sixteen. The same treatment in the

cerebellum resulted in decreasing levels of AMP and increasing levels of GMP through day sixteen. No consistent differences in base percents were observed between the early and late fighters. The base percents in the three hormone treatments (dopamine, corticosterone, and serotonin) differed significantly ($P < .005$) from the control levels.

With the exception of the cerebellum, the brain areas tested exhibited a significant decline in the G+C/A+U ratio upon initial exposure to defeat. Based on previous work, it was indicated that the decrease in the G+C/A+U ratio might denote increased synthesis of a messenger type RNA, rich in AMP. The steady increase in the G+C/A+U ratio in the cerebellum upon exposure to aggression and defeat may indicate a synthesis of a ribosomal type RNA, rich in guanosine and cytidine. However, since this is one of the first attempts to correlate this type of macromolecular data with exposure to aggression and defeat, the suggested syntheses of various types of RNA changes in base ratio are mere conjectures. Since it is known that there is decreased total RNA levels in these brain areas upon exposure to aggression and defeat, the changes in the base ratios may be due to a complex interaction of RNA breakdown and synthesis. Additional information regarding types of RNA specifically changed, turnover rates and nuclear RNA polymerase are needed. At this time, the mechanism of mediation of the RNA base changes found in this study cannot be clarified. However, the finding that changes do occur in neural RNA upon exposure to aggression and defeat is significant.