Pharmacology Biochemistry & Behavior, Vol. 20, pp. 793–797, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Biochemical Indices of Reactivity and Habituation in Rats With Hippocampal Lesions[1,2]

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Received 19 September 1983

KANT, G. J., J. L. MEYERHOFF AND L. E. JARRARD. *Biochemical indices of reactivity and habituation in rats with hippocampal lesions.* PHARMACOL BIOCHEM BEHAV **20**(5) 793–797, 1984.—The response of rats with hippocampal lesions to acute and repeated footshock stress was assessed by measurement of pituitary cyclic AMP, plasma corticosterone and plasma prolactin. Levels of pituitary cyclic AMP and plasma prolactin and corticosterone were similar in never-shocked sham controls, and never-shocked hippocampal and neocortical lesion groups. Acute first time shock markedly elevated all measured stress indices with no statistically significant differences observed among surgical groups. In rats subjected to repeated stress (one 15 min footshock session per day for 10 days) and sacrificed 24 hours after the last shock session, levels of pituitary cyclic AMP and plasma hormones were similar to levels in never-shocked shams with the exception of the hippocampal animals. The rats with hippocampal lesions had higher levels of pituitary cyclic AMP, plasma corticosterone and plasma prolactin compared to never-shocked animals. We suggest that these data reflect a hyperreactive response of the hippocampal animals to a situation previously associated with shock. Finally, rats in all surgical groups subjected to repeated stress and sacrificed immediately after the last shock session showed a diminished cyclic AMP response to the stressor as compared to first footshock session response, demonstrating a habituation to the stressor as measured by this index. No differences in habituation were observed among hippocampal, neocortical and sham groups. Plasma hormone responses did not habituate in any group. These data support the behavioral observations of hyperreactivity in hippocampal animals and indicate that hippocampal animals are able to habituate to repeated stressful stimuli.

Cyclic AMP Prolactin Corticosterone Pituitary Hippocampus Habituation

RODENTS with hippocampal lesions appear to be more reactive than intact animals to both internal and external stimuli [3, 8, 10, 30, 34]. Thus, hippocampal lesioned rats react more vigorously to the presentation of intense stimuli [6,25], are more active than controls in home cage and novel situations [9,10], and habituate more slowly than controls in an open field [7]. Behavioral measures and/or levels of plasma corticosterone were used in the above studies to assess "reactivity" or "habituation."

It is known that the hippocampus is involved in the regulation of corticosterone release via feedback of circulating corticosterone on pituitary and hippocampal glucocorticoid receptors [18, 24, 32]. Thus the interpretation of plasma corticosterone response to stressful stimuli in animals with hippocampal lesions must consider not only any issues of "reactivity" or "habituation" but also the direct effects of the lesion on regulation of ACTH and corticosterone release. Therefore, it would be useful to measure other indices of stress in addition to corticosterone.

We have been investigating biochemical responses in rats to stressful environmental stimuli and have shown that various stressors including footshock elevate levels of pituitary cyclic AMP and plasma prolactin as well as plasma corticosterone [2, 13, 14, 27]. The cyclic AMP and plasma prolactin responses to stressors are proportional to the severity of the stressor and habituate to repeated exposure of the same stressor [15,16]. Plasma corticosterone, in our studies, was maximally elevated by relatively mild stressors and did not habituate to repeated moderate stressors.

We decided to evaluate the role of the hippocampus in the response to a stressful stimulus, footshock. Both reactivity to the initial footshock session and habituation to repeated sessions were tested by measuring pituitary cyclic AMP, plasma prolactin and plasma corticosterone responses in

[1]The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense, (para 4–3, AR 360-5).

[2]In conducting the research described in this report, the investigator(s) adhere to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.

sham animals and rats with hippocampal or neocortical lesions.

Since it has been recently demonstrated that hippocampal lesions can also affect dopamine metabolism [35], we measured levels of dopamine and norepinephrine in terminal areas of catecholamine ascending pathways as well.

METHOD

Animals

Sprague-Dawley male rats (300–350 g) were housed individually in a temperature (23°) and light controlled (lights on from 0600 to 1800) room. (In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.) Other rats were also housed in the room. Food and water were freely available. The surgical procedures used in lesioning the hippocampus were similar to those employed in other studies reported by one of us [9,10]. For the surgical procedures, animals were anesthesized with pentobarbital, ketamine and atropine. Bilateral holes were drilled above the hippocampus in the skull, overlying cortex was removed and the hippocampus was aspirated medially and ventrally. Histological examination of the lesioned animals was not performed in the present study since the animals were sacrificed by microwave irradiation and since some brain regions were dissected for catecholamine analysis (see below). In similar studies using this lesion procedure, approximately 80 to 85% of the hippocampus was found to be removed, with the sparing of the ventral tips [9,10].

In the neocortical animals, only overlying cortex was removed and sham animals were prepared with only burr holes. The holes were filled with acrylic anchored with nylon screws prior to wound closure to permit sacrifice of the animals by microwave irradiation. This procedure was followed since unfilled burr holes would permit pressure-induced distortion of the brain during microwave irradiation with loss of brain tissue, and metal screws would interfere with the microwave field. Animals were allowed to recuperate from surgery for 1 week prior to initiation of daily handling or stress.

Stress

Each surgical group (sham, neocortical, and hippocampal) was divided into 2 treatment groups of 12 animals each. One treatment group was simply handled each day for 10 days. Handling included habituating the rats to traversing an open-ended plastic cylinder similar to the microwave sacrifice applicator. The other treatment group was subjected to one 15 min session of footshock each day for ten days. Footshock (1.2 mA) was delivered to the floor bars of an operant box on a variable time schedule on the average of once per 30 seconds. Shock duration was 5 sec.

Experimental Procedures

On the 11th day, each treatment group (handled only or repeatedly stressed) was again divided in two. One subgroup was sacrificed immediately upon removal from the home cage and the other group was subjected to a 15 min footshock session and then immediately sacrificed following stressor termination. Thus each surgical group (sham, neocortical and hippocampal) provided 4 experimental groups: never-shocked, once-shocked and sacrificed immediately following the only shock session, 10 day repeatedly stressed but not stressed on day of sacrifice, and 10 day repeatedly stressed and sacrificed immediately following an 11th stress session. The experiment was conducted between 0830 and 1230 on two consecutive days and animals were sacrificed alternatively from the different surgical groups to minimize any circadian effects. Animals were sacrificed 18 days post-surgery.

Sacrifice and Assay Procedures

Animals were sacrificed by a 5 sec exposure to high power microwave irradiation, a technique which has been shown to eliminate post mortem changes in cyclic AMP [12,23]. The microwave power generator was a modified Varian PPS-2.5 with an output of 2.5 kW at a frequency of 2450 Megaherz [26]. Rats were placed into a plastic applicator tube and a plunger was inserted behind the rat to prevent backward movement [22]. Sacrifice was accomplished in less than one minute after removal from the home cage or shock chamber.

After microwave sacrifice, the rats were decapitated and trunk blood was collected in heparinized beakers. The blood was centrifuged and the plasma frozen at −20° for later analysis of prolactin and corticosterone. The heads were cooled briefly on dry ice and the pituitaries were dissected, weighed and sonicated in one ml of 50 mM sodium acetate buffer, pH 6.2. After centrifugation the supernatants were stored at −70° until assayed for cyclic AMP.

In the never-shocked groups only, additional regions (corpus striatum, n. accumbens, o. tubercle, septal region, amygdala, pyriform cortex, frontal cortex, and entorhinal cortex) were dissected, weighed and sonicated in sodium acetate buffer as above. Dissection was performed essentially as described previously [1]. Supernatants were stored at −70° until assayed for norepinephrine or dopamine.

Material for the prolactin assays was provided by the National Institute of Health through the Rat Pituitary Hormone Distribution Program. Rat prolactin was radioiodinated as previously described [21]. Plasma was assayed for corticosterone using an antibody produced in our laboratory in rabbits [29]. Within assay variation was <5% and between assay variation was <12%. Recovery of added corticosterone was >92%.

Cyclic AMP was determined by radioimmunoassay using an antibody developed and characterized in our laboratory [21,36]. A highly specific antiserum was used at a final dilution of 1:400,000. The antiserum exhibited cross-reactivities for ATP and cyclic GMP of less than 0.00007 and 0.14% respectively. The assay data were analyzed by computer [31]. Within assay variation was 7% and between assay variation was 18%. Phosphodiesterase treatment of tissue extracts reduced cyclic AMP to undetectable levels.

Norepinephrine and dopamine were determined by radioenzymatic assay as described previously [21].

Statistics

Two way analysis of variance was performed for the data displayed in Table 1 and 2. No significant effects were seen for the catecholamine data shown in Table 2. Significant F values were found for the pituitary cyclic AMP, plasma prolactin and plasma corticosterone data shown in Table 1.

TABLE 1

EFFECT OF STRESS ON PITUITARY CYCLIC AMP, PLASMA PROLACTIN AND CORTICOSTERONE

Days 1–10	No shock	No Shock	Shock	Shock
Day 11	No shock	Shock	No shock	Shock
		Pituitary Cyclic AMP (pmoles/mg wet weight)		
Sham	1.51 ± 0.28	13.45 ± 3.26*	1.56 ± 0.19	3.82 ± 0.91*‡
Neocortical	1.34 ± 0.12	11.96 ± 1.53*	1.45 ± 0.14	2.89 ± 0.72*‡
Hippocampal	1.42 ± 0.13	20.08 ± 4.93*	1.75 ± 0.32	5.30 ± 1.21*‡
		Plasma Prolactin (ng/ml plasma)		
Sham	10.6 ± 2.0	212 ± 23*	12.6 ± 1.9	166 ± 24*
Neocortical	18.2 ± 8.3	172 ± 28*	15.4 ± 1.5	261 ± 63*
Hippocampal	14.8 ± 3.0	236 ± 30*	33.0 ± 6.7†	225 ± 28*
		Plasma Corticosterone (μg/100 ml plasma)		
Sham	4.5 ± 1.4	27.2 ± 1.6*	7.4 ± 3.7	24.4 ± 3.9*
Neocortical	3.2 ± 1.2	26.3 ± 2.2*	6.7 ± 2.6	34.0 ± 2.5*
Hippocampal	7.1 ± 2.7	26.0 ± 2.7*	17.0 ± 3.3†	36.5 ± 3.5*

Values represent the mean ± SEM. N=6. *Significantly different than no shock/noshock group, $p<0.05$, Student's t-test. †Significantly different than sham or neocortical shock/noshock, $p<0.05$, Student's t-test. ‡Significantly different than no shock/shock, $p<0.05$, Student's t-test.

Selected group comparisons were then made using Student's t-test.

RESULTS

As shown in Table 1, levels of pituitary cyclic AMP, plasma prolactin and plasma corticosterone in never-shocked animals were similar in sham, neocortical and hippocampal groups. Acute first-time stress markedly elevated all indices, with no statistically significant differences observed between the groups. Hippocampal animals did, however, have higher pituitary cyclic AMP and plasma prolactin levels than the other groups. Twenty four hours after their last shock, repeatedly stressed rats exhibited similar levels of cyclic AMP and hormones to never-shocked animals with the exception of the hippocampal group. The hippocampal animals had higher levels of pituitary cyclic AMP, plasma prolactin and corticosterone as compared to either similarly treated sham and neocortical rats or to hippocampal never-shocked rats. All groups of rats habituated to repeated stress as shown by comparing levels of pituitary cyclic AMP in the rats sacrificed immediately following the 11th stressor session to levels following the first shock session. Plasma hormone responses did not habituate under these conditions.

As shown in Table 2, levels of DA in the striatum, n. accumbens, and o. tubercle tended to be higher in the hippocampal animals than controls. However none of the changes were statistically significant.

TABLE 2

EFFECT OF NEOCORTICAL AND HIPPOCAMPAL LESIONS ON LEVELS OF DOPAMINE AND NOREPINEPHRINE

Region	Sham	Neocortical	Hippocampal
	Dopamine (μg/g wet weight)		
Striatum	18.0 ± 3.4	21.5 ± 2.1	27.1 ± 5.3
N. Accumbens	4.2 ± 0.5	4.7 ± 1.0	5.8 ± 1.1
O. Tubercle	5.1 ± 0.5	4.4 ± 0.7	6.2 ± 0.8
Amygdala	0.75 ± 0.22	0.72 ± 0.13	0.79 ± 0.18
Septal Region	0.61 ± 0.14	1.42 ± 0.42	0.83 ± 0.33
	Norepinephrine (μg/g wet weight)		
Frontal Ctx	0.19 ± 0.03	0.28 ± 0.05	0.26 ± 0.05
Pyriform Ctx	0.10 ± 0.02	0.13 ± 0.03	0.10 ± 0.03
Entorhinal Ctx	0.11 ± 0.02	0.18 ± 0.05	0.12 ± 0.04
Amygdala	0.25 ± 0.03	0.27 ± 0.07	0.22 ± 0.05
Septal Region	0.25 ± 0.04	0.32 ± 0.06	0.38 ± 0.08

Values represent the mean ± SEM. N=6. All rats were taken from the never-shocked group.

DISCUSSION

In the present study, we have compared reactivity and habituation to a stressful stimulus in rats with hippocampal lesions and controls using biochemical indices of stress response.

Acute stress increases plasma corticosterone and plasma prolactin in the rat [19, 21, 27, 33]. We have also found that acute stress elevates levels of pituitary cyclic AMP *in vivo* and have suggested that this cyclic AMP response is involved in the control of release of some pituitary hormones in response to stress [2, 13, 14, 27]. Neither prolactin or corticosterone, however, seem to be the hormones directly regulated by stress-induced pituitary cyclic AMP increases

as shown by recent work from our laboratory [17,28].

Previous studies of hippocampal lesioned animals have generally measured only plasma corticosterone levels with somewhat contradictory results [4, 11, 20]. We find that plasma corticosterone is a sensitive marker of mild stress or arousal but that corticosterone peaks at lower stress intensities than plasma prolactin or pituitary cyclic AMP [16]. Probably because plasma corticosterone is so sensitive a marker, it is difficult to demonstrate habituation using corticosterone as an index except for the most gentle stimuli [5,11]. We have previously found that plasma prolactin and pituitary cyclic AMP responses to some stressors (forced running, immobilization, footshock) habituate with repeated exposure [15].

Resting levels of plasma corticosterone were similar in never-shocked sham animals, neocortical lesioned rats, and hippocampal lesioned rats in agreement with previous reports in the literature that used various types of stressors and measurement techniques [4, 11, 20, 30]. Plasma corticosterone response to stress in rats with hippocampal lesions has been reported as being either similar to intact controls or elevated as compared to control stress response [4, 11, 20, 30]. In the present study, both response patterns were seen depending upon the intensity of the stressor. Acute footshock, a relatively severe stressor, markedly elevated plasma corticosterone in all surgical groups with no significant differences observed among hippocampal, neocortical and sham groups. This finding is similar in pattern and magnitude to that reported for ether stress in hippocampal lesioned rats [20]. In the present research, the animals previously subjected to 10 days of footshock and then sacrificed 24 hours after the last shock can be viewed as a mild stress group, since handling and transport to the experimental area had been previously associated with footshock. In this treatment group, differences in plasma corticosterone were seen among surgical groups with hippocampal rats demonstrating a hyperreactive corticosterone response. These findings are similar to those reported in rats with hippocampal lesions during social interaction in a territorial situation and in rats with dentate gyrus lesions exposed to novelty stress [4,11]. Plasma prolactin data confirm the hyperreactive stress response in the hippocampal group handled 24 hours after the last shock. An alternative explanation for the increased levels of prolactin, corticosterone and pituitary cyclic AMP seen in the hippocampal animals handled 24 hr after the last shock is that the hippocampal group returned to basal levels more slowly than the other groups following shock. This possibility might particularly pertain to the corticosterone response since negative feedback of corticosteroids at hippocampal receptors has been lost.

Plasma corticosterone and plasma prolactin did not habituate in any surgical group, although we have previously observed habituation of plasma prolactin response under similar experimental conditions [15]. In the previous study we examined the effects of daily 15 min sessions of footshock, immobilization, forced running and cold exposure. Overall analysis of the data showed that pituitary cyclic AMP and plasma prolactin habituated to repeated stressors while corticosterone did not. However, in that study the attenuation of the prolactin response in the daily footshock group was only 25% of the large stress-induced rise in prolactin. In the present study, we chose footshock for the stressor (despite the only moderate prolactin habituation observed in the previous study) because of the marked habituation of the pituitary cyclic AMP response and because this stressor is more easily exactly replicated by other laboratories. In an effort to possibly increase the degree of prolactin habituation, we decreased the shock intensity from 1.6 mA to 1.2 mA in the present study. Nevertheless, prolactin did not habituate in the present study. However, pituitary cyclic AMP responses to footshock were markedly attenuated in the eleventh session as compared to those following first footshock. Pituitary cyclic AMP responses habituated in sham, neocortical and hippocampal rats; no differences in habituation were seen among sham, neocortical and hippocampal animals. These data should not be interpreted as indicating which biochemical indices of stress habituate to stress, in general, since habituation to repeated stress as measured by biochemical indices depends upon the characteristics of the stress, particularly stress severity. Different indices show different habituation rates. One way of looking at habituation with regard to biochemical response is that repeated exposure to a stressor decreases the perceived severity or intensity of the stressor. If the decrease in perceived intensity drops the stressor severity significantly below the level that evokes maximal response for that biochemical index, then biochemical "habituation" or attenuation of the response is seen. The differences in threshold and maximum response plateaus among prolactin, corticosterone, and pituitary cyclic AMP were one reason for selecting several indices of stress response. Apparently in the present study, the stressor severity was sufficiently high to preclude observable habituation of the plasma corticosterone and prolactin responses.

Springer and Isaacson [35] recently reported that 7 days after hippocampal damage there was an increase in DA levels, a decrease in DA utilization and a decrease in NE levels in the n. accumbens; both of these measures returned to control levels within 28 days. It was suggested that removal of the hippocampal input to basal ganglia results in a loss and then subsequent recovery of DA utilization. In the present study, animals were sacrificed 18 days after hippocampal lesion by which time catecholamine levels were statistically similar in lesioned and control animals. However, levels of DA were somewhat elevated in corpus striatum, n. accumbens, and olfactory tubercle of the hippocampal animals. If removing the hippocampal input does have an effect similar to that proposed by Springer and Isaacson, our data indicates that the recovery of DA and NE systems occurs by day 18.

In summary, after measuring three biochemical indices of stress response in hippocampal lesioned rats, we conclude that hippocampal lesioned rats have hyperreactive hormonal responses to mild stressful stimuli. The hippocampal lesions did not prevent habituation of the pituitary cyclic AMP response to repeated stress. Thus, compared to control animals, the hippocampal animals exhibited increased "reactivity" and similar habituation.

ACKNOWLEDGEMENTS

We wish to thank Edward Mougey, Lee Pennington, David Collins, Clint Wormley, and Willie Gamble for performing the cyclic AMP and hormone assays. We also appreciate the excellent technical assistance provided by Clyde Kenion, Golden Driver, SP5 Leigh Landman-Roberts, and SP4 Terry Eggleston and the secretarial support provided by Pat Conners.

REFERENCES

1. Balcom, G. J., R. H. Lenox and J. L. Meyerhoff. Regional GABA levels in rat brain determined after microwave fixation. *J Neurochem* **24:** 609–613, 1975.
2. Bunnell, B. N., G. J. Kant, R. H. Lenox, L. L. Pennington, D. R. Collins, E. H. Mougey and J. L. Meyerhoff. Pituitary cyclic AMP in rats is increased by psychological stress. *Soc Neurosci Abstr* **7:** 282, 1981.
3. Coover, G. D. and S. Levine. Auditory startle response of hippocampectomized rats. *Physiol Behav* **9:** 75–77, 1972.
4. Ely, D. L., E. G. Greene and J. P. Henry. Effects of hippocampal lesion on cardiovascular, adrenocortical and behavioral response patterns in mice. *Physiol Behav* **18:** 1075–1083, 1977.
5. Hennessy, M. B. and S. Levine. Effects of various habituation procedures on pituitary-adrenal responsiveness in the mouse. *Physiol Behav* **18:** 799–802, 1977.
6. Ireland, L. C. and R. L. Isaacson. Reactivity in the hippocampectomized gerbil. *Psychonom Sci* **12:** 163–164, 1968.
7. Isaacson, R. L. The hippocampus. In: *The Limbic System*. New York: Plenum Press, 1974, pp. 161–218.
8. Jarrard, L. E. The hippocampus and motivation. *Psychol Bull* **79:** 1–12, 1973.
9. Jarrard, L. E. and B. N. Bunnell. Open-field behavior of hippocampal-lesioned rats and hamsters. *J Comp Physiol Psychol* **66:** 500–502, 1968.
10. Jarrard, L. E. Behavior of hippocampal lesioned rats in home cage and novel situations. *Physiol Behav* **3:** 65–70, 1968.
11. Johnson, L. L. and B. P. Moberg. Adrenocortical response to novelty stress in rats with dentate gyrus lesions. *Neuroendocrinology* **30:** 187–192, 1980.
12. Jones, D. J. and W. B. Stavinoha. Microwave inactivation as a tool for studying the neuropharmacology of cyclic nucleotides. In: *Neuropharmacology of Cyclic Nucleotides*, edited by G. C. Palmer. Baltimore: Urban and Schwarzenberg, 1979, pp. 253–281.
13. Kant, G. J., G. R. Sessions, R. H. Lenox and J. L. Meyerhoff. The effects of hormonal and circadian cycles, stress and activity on levels of cyclic AMP and cyclic GMP in pituitary, hypothalamus, pineal, and cerebellum of female rats. *Life Sci* **29:** 2491–2499, 1981.
14. Kant, G. J., J. L. Meyerhoff, B. N. Bunnell and R. H. Lenox. Cyclic AMP and cyclic GMP responses to stress in brain and pituitary: Stress elevates pituitary cyclic AMP. *Pharmacol Biochem Behav* **17:** 1067–1072, 1982.
15. Kant, G. J., B. N. Bunnell, E. H. Mougey, L. L. Pennington and J. L. Meyerhoff. Effects of repeated stress on pituitary cyclic AMP, and plasma prolactin, corticosterone and growth hormone in male rats. *Pharmacol Biochem Behav* **18:** 967–972, 1983.
16. Kant, G. J., E. H. Mougey, L. L. Pennington and J. L. Meyerhoff. Graded footshock stress elevates pituitary cyclic AMP, and plasma β-endorphin, β-LPH, corticosterone, and prolactin. *Life Sci* **33:** 2657–2663, 1983.
17. Kant, G. J., R. H. Lenox, B. N. Bunnell, E. H. Mougey, L. L. Pennington and J. L. Meyerhoff. Comparison of stress response in male and female rats: pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. *Psychoneuroendocrinology*, in press.
18. Knigge, K. M. and M. Hays. Evidence of inhibitive role of hippocampus in neural regulation of ACTH release. *Proc Soc Exp Biol Med* **114:** 67–69, 1963.
19. Krulich, L., E. Hefco, P. Illner and C. B. Read. The effects of acute stress on the secretion of LH, FSH, prolactin, and GH in the normal male rat, with comments on their statistical evaluation. *Neuroendocrinology* **16:** 293–311, 1974.
20. Lanier, L. P., C. Van Hartesveldt, B. J. Weis and R. L. Isaacson. Effects of differential hippocampal damage upon rhythmic and stress-induced corticosterone secretion in the rat. *Neuroendocrinology* **18:** 154–160, 1975.
21. Lenox, R. H., G. J. Kant, G. R. Sessions, L. L. Pennington, E. H. Mougey and J. L. Meyerhoff. Specific hormonal and neurochemical responses to different stressors. *Neuroendocrinology* **30:** 300–308, 1980.
22. Lenox, R. H., O. P. Gandhi, J. L. Meyerhoff and H. M. Grove. A microwave applicator for *in vivo* rapid inactivation of enzymes in the central nervous system. *IEEE Trans Microwave Theory Tech* **24:** 58–61, 1976.
23. Lenox, R. H., J. L. Meyerhoff, O. P. Gandi and H. L. Wray. Regional levels of cyclic AMP in rat brain: pitfalls of microwave inactivation. *J Cyclic Nucleotide Res* **3:** 367–379, 1977.
24. McEwen, B. S. and D. W. Pfaff. Chemical and physiological approaches to neuroendocrine mechanisms: attempts at integration. In: *Frontiers in Neuroendocrinology*, W. F. Ganong and L. Martini. Toronto: Oxford University Press, 1973, pp. 267–335.
25. McNew, J. J. and R. Thompson. Role of the limbic system in active and passive avoidance conditioning in the rat. *J Comp Physiol Psychol* **61:** 173–180, 1966.
26. Meyerhoff, J. L., R. H. Lenox, P. V. Brown and O. P. Gandhi. The inactivation of rodent brain enzymes *in vivo* using high intensity microwave irradiation. *Proc IEEE* **68:** 155–159, 1980.
27. Meyerhoff, J. L., G. J. Kant, G. R. Sessions, E. H. Mougey, L. L. Pennington and R. H. Lenox. Brain and pituitary cyclic nucleotide response to stress. In: *Perspectives in Behavioral Medicine*, vol 2, edited by R. B. Williams. New York: Academic Press, in press.
28. Meyerhoff, J. L., G. J. Kant, C. J. Nielsen, E. H. Mougey and L. L. Pennington. Adrenalectomy abolishes stress-induced increase in pituitary cyclic AMP. *Soc. Neurosci Abstr* **9:** 1123, 1983.
29. Mougey, E. H. A radioimmunoassay for tetrahydrocortisol. *Anal Biochem* **91:** 566–582, 1978.
30. Murphy, H. M., C. H. Wideman and T. S. Brown. Plasma corticosterone levels and ulcer formation in rats with hippocampal lesions. *Neuroendocrinology* **28:** 123–130, 1979.
31. Rodbard, D., R. H. Lenox, H. L. Wray and D. Ramseth. Statistical characterization of the random error in the radioimmunoassay dose-response variable. *Clin Chem* **22:** 350–358, 1976.
32. Rotsztejn, W. H., M. Normand, J. Lalonde and C. Fortier. Relationship between ACTH release and corticosterone binding by the receptor sites of the adenohypophysis and dorsal hippocampus following infusion of corticosterone at a constant rate in the adrenalectomized rat. *Endocrinology* **97:** 223, 1975.
33. Seggie, J. A. and G. M. Brown. Stress response patterns of plasma corticosterone and growth hormone in the rat following handling or exposure to novel environment. *Can J Physiol Pharmacol* **53:** 629–637, 1975.
34. Smith, R. F. Mediation of footshock sensitivity by serotonergic projection to hippocampus. *Pharmacol Biochem Behav* **10:** 381–388, 1979.
35. Springer, J. E. and R. L. Isaacson. Catecholamine alterations in basal ganglia after hippocampal lesions. *Brain Res* **252:** 185–188, 1982.
36. Steiner, A. L., A. W. Parker and D. M. Kipnis. Radioimmunoassay for cyclic nucleotides. *J Biol Chem* **247:** 1106–1113, 1972.