Pharmacology Biochemistry & Behavior, Vol. 3, pp. 775–780.

Changes in Brain Monoamine Metabolism and Carbon Dioxide Induced Amnesia in the Rat

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(Received 16 October 1974)

LEONARD, B. E. AND H. RIGTER. *Changes in brain monoamine metabolism and carbon dioxide induced amnesia in the rat.* PHARMAC. BIOCHEM. BEHAV. 3(5) 775–780, 1975. – The effect of treatment with carbon dioxide (CO_2) on the performance of rats 24 hr after receiving a foot shock in a passive avoidance task was studied. Foot shock induced avoidance. Carbon dioxide produced retrograde amnesia for the foot shock induced avoidance response. Changes in brain monoamine metabolism were studied in groups of rats which had been treated with CO_2, foot shock or foot shock + CO_2. The rats were killed 24 hr after treatment. Changes mainly occurred in the brain stem and hippocampus. In the rats which had received foot shock alone, brain stem and hippocampal serotonin concentrations were raised. This rise was not observed when the foot shock was followed by CO_2 treatment. Furthermore, it was found that there was an increased release of noradrenaline in those rats subjected to foot shock alone but a decreased release of this amine in the group which received foot shock followed by CO_2. It is suggested that the amnesic effect of CO_2 parallels changes in brain serotonin and noradrenaline metabolism.

Amnesia Passive avoidance Carbon dioxide Hippocampus Noradrenaline Serotonin

THE most common paradigm for amnesia studies in rats involves (1) a training situation in which the animal acquires an aversive experience (f.e., a foot shock), (2) followed by an amnesic treatment and (3) a retrieval test 24 hr later. Little is known about the role brain monoamines may play in amnesia. A number of investigators studied the effects of a frequently used amnesic agent, electroconvulsive shock, on brain monoamine levels [8, 13, 14, 20]. However, these studies did not intend to correlate the changes in brain amine concentrations with amnesia. Thus, either the training experience was lacking [8, 13, 14] or the time interval was different from the one used in amnesia studies [19,20].

We report on the very first step in an investigation aimed at assessing possible associations between brain monoamine metabolsim and amnesia. Carbon dioxide (CO_2), an effective amnesic agent [21, 22, 27], was used to produce amnesia for a one-trial step-through passive avoidance response. In Experiment 1, we demonstrate that amnesia is present 24 hr after amnesic treatment. In Experiment 2, we followed the same paradigm but instead of subjecting the animals to a retrieval test, the rats were killed 24 hr after amnesic treatment and brain monoamine levels were determined. In further studies [23,31], we attempt to clarify the relationships between the changes in brain amine concentrations and amnesia.

EXPERIMENT 1

ASSESSMENT OF CARBON DIOXIDE AS AN AMNESIC AGENT

Method

Animals and Apparatus. Forty male rats of an inbred Wistar strain weighing 230–250 g were used. The animals were housed 10 per cage with free access to food and water. The animals were trained in a step-through passive avoidance apparatus of the type described by Ader, *et al.* [1]. This consisted of a 40 X 40 X 40 cm Lucite chamber which had a grid floor and black walls. The front wall was positioned at the edge of a table and connected to a 6 cm wide and 25 cm long runway which projected over the floor. The runway was illuminated by means of a 40 W lamp held 40 cm above it; the chamber was in darkness. When placed on the runway, a rat could enter the darkened chamber through a 6 X 6 cm opening which could be closed by means of a hand operated guillotine door. A scrambled foot shock (0.5 mA for 3 sec) could be delivered through the metal grid floor of the chamber from a 500 V AC source.

Procedure. The rats were randomly divided into 4 groups of 10 each. They were given 3 pretraining trials on Day 1 of the experiment. A pretraining trial consisted of placing the rat at the end of the runway facing the entrance of the chamber; the time taken for the animal to enter the cham-

ber was recorded and defined as the step-through latency. Upon entering the chamber, the door was closed and the animal allowed to remain there for 10 sec. It was then returned to its home cage. The interval between the 3 pretraining trials was approximately 2 hr. On Day 2, the rats were subjected to a single acquisition trial. This was similar to the pretraining trials with the exception that a foot shock was given to 2 groups of animals 10 sec after they had entered the chamber (FS groups). The other 2 groups of rats did not receive foot shock (NoFS groups). Immediately on terminating the acquisition trial, the rats were removed from the chamber and subjected to either amnesic treatment (CO_2 groups) or sham amnesic treatment ($NoCO_2$ groups). The amnesic treatment consisted in placing an animal in a closed container through which CO_2 was passed until oxygen measurements yielded zero readings [22]. The rats were removed from the box as soon as respiratory arrest occurred and were revived by artificial respiration; the time necessary for respiratory arrest to occur was 30–35 sec. Rats receiving sham amnesic treatment were placed in an air filled box for 35 sec. All animals were returned to their home cages at the end of the amnesic or sham amnesic treatment. Thus at the end of the acquisition trial the 4 groups of rats had been subjected to the following treatments: NoFS-$NoCO_2$ – no foot shock, no CO_2 treatment; NoFS-CO_2 – no foot shock, but CO_2 given; FS-CO_2 – foot shock followed by CO_2 treatment; FS-$NoCO_2$ – foot shock but no CO_2.

Twenty-four hours after the acquisition trial, the rats were subjected to a single retrieval trial. The rats were placed at the end of the runway and the time taken for them to enter the darkened chamber was recorded. When a rat failed to enter within 300 sec, it was removed from the runway and a latency score of 300 sec was assigned. The retrieval scores were divided into 3 classes: (1) latencies of 0–10 sec; (2) latencies of 10–299 sec; (3) latencies of 300 sec. Rats entering the chamber within 10 sec were considered as not showing passive avoidance; previous experiments had shown that 10 sec represented the maximal latency for rats which had not received a foot shock at the time of the acquisition trial. Rats entering the chamber within 10–299 sec displayed incomplete passive avoidance while those that failed to enter within 300 sec were regarded to show a complete passive avoidance response. In the analysis of the results, the 3 classes received a weighting of 0, 1 and 2, respectively. The retrieval scores were analysed statistically using the Yates test [33]. For the analysis of the other data the two-tailed Mann Whitney U test was employed, for comparisons between groups, and the two-tailed sign test, for comparisons within groups.

Results

All groups of rats showed a reduction in their step-through latencies during the course of the pretraining trials. This was apparent from the significant differences between the latencies at the time of the first pretraining trial and those at the time of the acquisition trial ($p = 0.001$ for each group, sign test). The latencies at the time of the acquisition trial did not differ between the four groups (Table 1). In the 2 NoFS groups, there was no significant difference between the latencies at the retrieval and those at the acquisition trial ($p>0.10$ for both groups). The latencies of the NoFS groups at the test trial did not differ (Table 1). This suggests that the CO_2 treatment did not affect the step-through response.

The latencies of the four groups of animals at the time of the retrieval trial are shown in Fig. 1. Administration of CO_2 produced amnesia: the FS-CO_2 group had significantly shorter latencies than the FS-$NoCO_2$ group ($z = 4.13$; $p<0.001$, Yates test). Thus 8 out of 10 rats in the FS-CO_2 group had a latency similar to those of the NoFS-$NoCO_2$ group, while 9 out of 10 of the FS-$NoCO_2$ group showed complete passive avoidance and did not enter the chamber. The latencies of FS-CO_2 rats did not differ from those of NoFS-$NoCO_2$ or NoFS-CO_2 rats (in both cases: $z = 1.49$). On the other hand, the latencies of the FS-$NoCO_2$ group were longer than those of the NoFS groups ($z = 4.37$; $p<0.001$ in both cases).

TABLE 1

EFFECT OF PRETRAINING ON STEP-THROUGH LATENCIES OF GROUPS OF RATS

Group	Trials: Pretraining 1*	Pretraining 2*	Pretraining 3*	Acquisition*	Retrieval*
NoFS-$NoCO_2$	9.3 ± 2.0	3.8 ± 1.0	2.2 ± 0.6	1.4 ± 0.1	1.5 ± 0.2
NoFS-CO_2	8.1 ± 1.7	3.9 ± 0.9	2.0 ± 0.4	1.1 ± 0.3	1.6 ± 0.2
FS-CO_2	9.2 ± 1.8	5.4 ± 1.2	1.9 ± 0.4	1.5 ± 0.1	†
FS-$NoCO_2$	8.5 ± 1.5	5.2 ± 1.2	1.3 ± 0.3	1.1 ± 0.1	†

Step-through latencies (mean number of sec ± standard error of the mean) during pretraining trials and the acquisition trial.

*Differences between groups within column are not significant (two-tailed Mann-Whitney U test).

†Latencies at the test trial are only given for groups which did not receive footshock at the acquisition trial. FS: footshock; NoFS: no footshock; CO_2: CO_2-treatment; and $NoCO_2$: no CO_2-treatment.

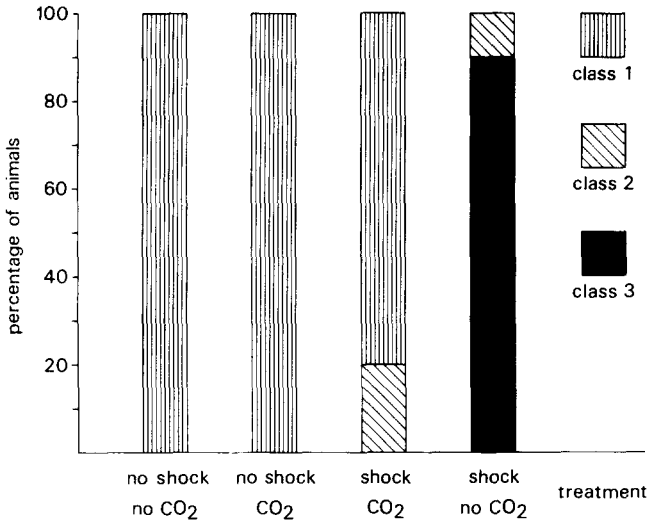

FIG. 1. Carbon dioxide induced amnesia for a passive avoidance response. The latency scores are divided into 3 classes: Class 1 indicates no avoidance; Class 2 incomplete avoidance; and Class 3 complete avoidance.

EXPERIMENT 2
CHANGES IN AMINE METABOLISM

Method

Four groups of 20 rats were used. The experiment was run in 5 randomized blocks. Each block contained 16 rats, 4 of each group. They were subjected to the pretraining and acquisition trials, as described above, and killed by decapitation 24 hr after the acquisition trial; they were not subjected to a retrieval trial before being killed. The brains were placed on ice and dissected within 3 min into the cortex, mid-brain, brain stem and hippocampus. The olfactory bulbs, cerebellum and pineal gland were removed. The brain stem was taken as the area posterior to the superior colliculi, i.e., colliculi, tegmentum, pons and medulla. The mid-brain comprised the striatum, hypothalamus, amygdala and septum. The hippocampal samples contained the area dentata and the subiculum in addition to the hippocampus proper dorsal to the rhinal sulcus. The hippocampus was studied separately, as a disturbance of its function has been implicated in the causation of amnesia in man and animal [18,34]. After the dissection, the brain areas were frozen on solid carbon dioxide. Brain areas from 4 rats were pooled for the biochemical determinations. The pooled brain areas were homogenized in 12 ml of 0.01 N HCl containing 1 ml of 10 percent sodium edate. After centrifugation at 800 G for 20 min, aliquots of the clear supernatant were removed for the fluorimetric determination of noradrenaline and dopamine [3,4], tyrosine [32], normetanephrine by the method of Anton and Sayre [5] as modified by Leonard and Tonge [15], homovanillic acid [2], tryptophan [12] and gamma-amino-n-butyric acid (GABA) [30]. The pellet and the remainder of the supernatant fraction were extracted with butanol; serotonin was determined by the method of Snyder, *et al.* [26] and 5-hydroxyindoleacetic acid (5-HIAA) by the method of Giacolone and Valzelli as modified by Tonge and Leonard [29]. All fluorescence measurements were made using a Hitachi-Perkin Elmer spectrophotofluorimeter Model 2A. The statistical significance of the results was assessed using a Student's t-test.

Results

The results are given in Figs. 2, 3 and 4. Neither CO_2, nor foot shock or the combination of both treatments induced significant changes in amine metabolism in the cortex. In the mid-brain, foot shock caused an increase in tyrosine in the FS-CO_2 as well as the FS-NoCO_2, group. None of the other parameters was affected.

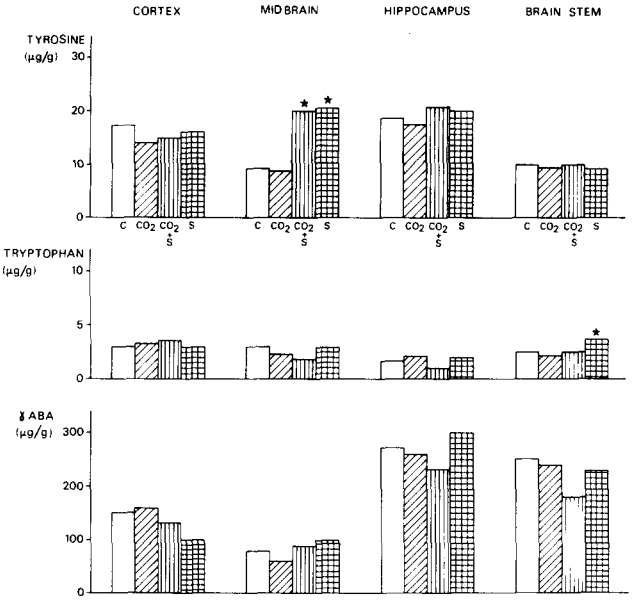

FIG. 2. Effect of treatment with foot shock and/or CO_2 at the time of the acquisition trial on the concentration of tyrosine, tryptophan and gamma-aminobutyric acid (GABA) in different brain areas at the time of the retrieval trial. C: control group receiving neither foot shock nor CO_2; CO_2: group subjected to CO_2 treatment alone; S: group subjected to foot shock alone; S+CO_2: group subjected to foot shock followed by CO_2 treatment.* The significance of the difference between the control group and the experimental groups shown by $p<0.05$.

All treatments decreased the concentration of dopamine in the brain stem. Moreover, the foot shock led to increased concentrations of tryptophan and serotonin in this area. This increase was not observed when foot shock was followed by CO_2.

The most marked changes in amine metabolism occurred in the hippocampus. A slight increase in hippocampal dopamine and homovanillic acid concentrations was found in both the FS-NoCO_2 and the FS-CO_2 groups. This could be indicative of an increased dopamine turnover as a consequence of the foot shock. Dopamine and homovanillic acid metabolism were unaffected in the NoFS-CO_2 group. There was a significant decrease of noradrenaline in the FS-NoCO_2 group and a significant increase in the FS-CO_2 group. These changes in noradrenaline were reflected in a slight decrease and increase respectively in the concentrations of normetanephrine. Hippocampal serotonin was

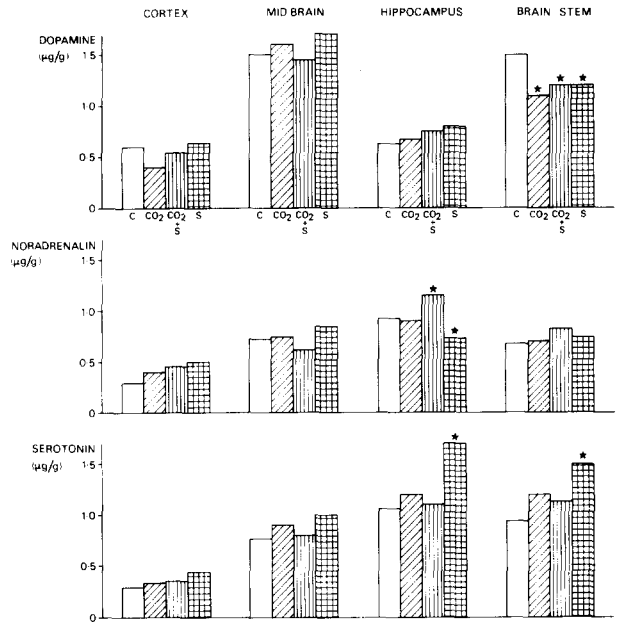

FIG. 3. Effect of treatment with foot shock and/or CO_2 at the time of the acquisition trial on the concentration of dopamine, noradrenaline and serotonin in different brain areas at the time of the retrieval trial. Details otherwise as given in Fig. 2.

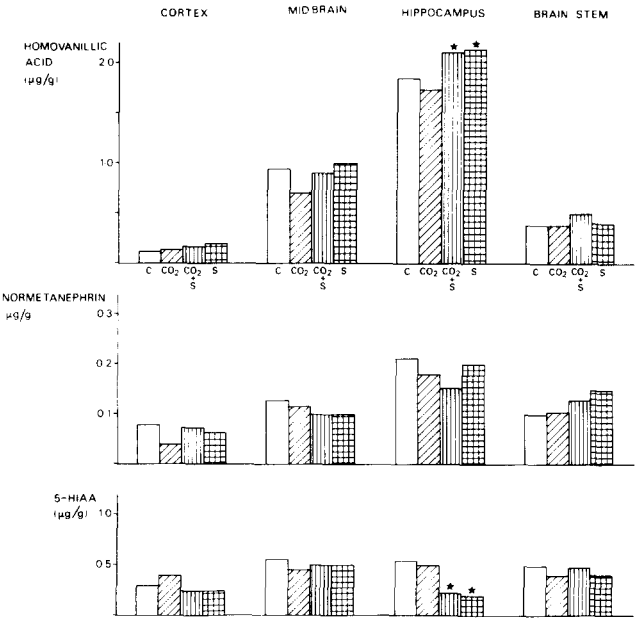

FIG. 4. Effect of treatment with foot shock and/or CO_2 at the time of the acquisition trial on the concentration of homovanillic acid, normetanephrine and 5-hydroxyindole acetic acid (5-HIAA) in different brain areas at the time of the retrieval trial. Details otherwise as given in Fig. 2.

increased in the FS-NoCO$_2$ group but did not change from control values in the FS-CO$_2$ group. Both the FS-NoCO$_2$ and the FS-CO$_2$ groups had reduced 5-HIAA concentrations in the hippocampus.

DISCUSSION

It is apparent from the results of Experiment 1 that CO_2 is an effective amnesic agent. Eight out of 10 FS-CO$_2$ animals did not show avoidance behaviour at the retrieval test. The CO_2 treatment did not affect the step-through latencies of NoFS-CO$_2$ animals. These findings do not assure, however, that CO_2 induced amnesia is based on a disturbance of some sort of memory process. Some investigators have suggested that amnesia may be due to a disruption of performance [6,25]. Additional findings from our laboratory argue against this possibility. Thus, it was shown that as the interval between acquisition and the application of CO_2 gets longer, so the degree of amnesia declines thereby resulting in an amnesia gradient [23]. Moreover, CO_2 is also able to induce amnesia for an appetite-motivated response [22]. Taken together, these data indicate that CO_2 induced amnesia is not due to an aspecific disturbance of performance but results from an interference with some memory process.

The results of Experiment 2 show that changes in amine metabolism in foot shock and/or CO_2 treated animals mainly occurred in the brain stem and the hippocampus. There were no changes in the cortex. Pilot experiments also demonstrated that amine concentrations in the cerebellum were unchanged. The only significant change in the midbrain concerned a rise in tyrosine in FS-CO$_2$ as well as FS-NoCO$_2$ rats.

Dopamine concentrations were reduced in the brain stem of foot shock and/or CO_2 treated rats. There were no corresponding changes in homovanillic acid. This result is difficult to explain. If this effect was correlated with possible stress-inducing properties of CO_2 and foot shock, one should have expected a greater fall in dopamine concentration in the FS-CO$_2$ group compared to the groups which received foot shock or CO_2 alone. This was not the case.

Changes in serotonin metabolism in the brain stem and hippocampus parallelled the behavior at the retrieval test. In both areas, the FS-NoCO$_2$ group showed an increase in serotonin whereas such an increase did not occur in the FS-CO$_2$ group. In the hippocampus, the rise in serotonin in the FS-NoCO$_2$ group was correlated with a fall in the concentration of 5-HIAA. This may be indicative of a decreased turnover of serotonin. Essman [10] suggested that amnesia is associated with an increased whole-brain concentration of serotonin which could be causative of an impaired protein synthesis, at least in the case of electroshock-induced amnesia. Our findings do not support this hypothesis: we found a change in the concentration of serotonin only in the rats which had been subjected to foot shock. In the group of rats in which amnesia was induced, there was no significant change in the concentration of this amine. The use of CO_2 as the amnesic agent may be responsible for the differences between our results and those of Essman [10].

Noradrenaline concentrations were only changed in the hippocampus. The decrease in the concentration of noradrenaline was correlated with a fall in the concentration of its extraneuronal metabolite, normetanephrine, which may be indicative of an increased turnover of the amine. In con-

trast, there was a significant rise in the concentration of noradrenaline in the FS-CO_2 group, this effect being correlated with a fall in the concentration of normetanephrine which is suggestive of a decreased turnover of the amine.

It is possible that the observed changes in brain amine metabolism are an indirect consequence of increased adrenocortical activity as a result of the stress to which the animals were exposed during the acquisition trial. It is now well established that stress induced changes in adrenocorticosteroid levels can have a profound effect on brain amine metabolism. This can be due to a direct effect of stress on the turnover and release of brain amines [13,20]. It is also possible that a rise in liver tryptophan pyrolase activity can occur as a consequence of a stress-induced increase in the secretion of adrenocorticosteroids; this could lead to a reduction in brain serotonin metabolism [11,24]. However, in the present paradigm it has been found that 24 hr after the acquisition trial NoFS-NoCO_2, NoFS-CO_2, FS-CO_2 and FS-NoCO_2 groups of rats do not differ with respect to plasma corticosterone concentration [22].

The finding that the most marked changes in amine metabolism occurred in the hippocampus is in agreement with the view that disturbance of the function of this area plays an essential role in the causation of amnesia. However, it is premature to implicate serotonin and noradrenaline in learning and memory. The present results suggest a correlation between changes in brain stem and hippocampal amine metabolism and amnesia but the nature of the correlation remains obscure. Moreover, a correlation with amnesia does not necessarily mean an involvement in memory. The theoretical basis of amnesia is uncertain. McGaugh and Dawson [16] proposed that amnesic agents disrupt memory consolidation. However, other investigators have suggested that amnesic agents affect the retrieval of consolidated memory rather than the consolidation process itself [17,28].

The present results do not justify the conclusion that the amnesic effect of CO_2 is associated with changes in brain amine metabolism. We may deal with independent effects. In subsequent studies, we examined this possibility by determining the changes in brain amine metabolism which occur following alterations in the parameters of the amnesia paradigm. Thus, we found that the amnesia gradient is parallelled by a hippocampal serotonin gradient [23]. Furthermore, it appeared that amnesia developed gradually over the first 4 hr following the amnesic treatment; this was parallelled by a gradual change in hippocampal levels of serotonin [31]. These additional data support the view that a correlation exists between changes in brain amines, particularly serotonin, metabolism and amnesia. The nature of this correlation, however, remains obscure.

ACKNOWLEDGMENT

We gratefully acknowledge the skillful assistance of Mr. H. Müller and Mr. C. Versteegen.

REFERENCES

1. Ader, R., J. A. W. M. Weijnen and P. Moleman. Retention of a passive avoidance response as a function of the intensity and duration of electric shock. *Psychon. Sci.* **26:** 125–128, 1972.
2. Andén, N. E., B. E. Roos and B. Werdinius. On the occurrence of homovanillic acid in brain and cerebrospinal fluid and its determination by a fluorometric method. *Life Sci.* **2:** 448–458, 1963.
3. Anton, A. H., and D. F. Sayre. A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.* **138:** 360–375, 1962.
4. Anton, A. H. and D. F. Sayre. The distribution of dopamine and dopa in various animals and a method for their determination in diverse biological material. *J. Pharmac. exp. Ther.* **145:** 326–336, 1964.
5. Anton, A. H. and D. F. Sayre. Distribution of metanephrine and normetanephrine in various animals and their analysis in diverse biological material. *J. Pharmac. exp. Ther.* **153:** 15–29, 1966.
6. Chorover, S. L. and P. H. Schiller. Re-examination of prolonged retrograde amnesia in one-trial learning. *J. comp. physiol. Psychol.* **61:** 34–41, 1966.
7. Curzon, G. and A. R. Green. A rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac.* **39:** 653–655, 1970.
8. Engel, J., L. C. F. Hanson, B. -E. Roos and L. -E. Strömbergsson. Effect of electroshock on dopamine metabolism in rat brain. *Psychopharmacologia* **13:** 140–144, 1968.
9. Essman, W. B. Drug effects and learning and memory processes. In: *Advances in Pharmacology and Chemotherapy,* edited by S. Garattini, A. Goldin, F. Hawking and I. J. Kopin. New York: Academic Press, 1971, pp. 241–330.
10. Essman, W. B. Retrograde amnesia and cerebral protein synthesis: Imitation and inhibition by 5-hydroxytryptamine. *Totus Homo,* **4:** 61–67, 1972.
11. Green, A. R. and G. Curzon. The effect of tryptophan metabolites on brain 5-hydroxytryptamine metabolism. *Biochem. Pharmac.* **19:** 2061–2068, 1970.
12. Hess, S. and S. Undenfriend. A fluorometric procedure for the measurement of tryptamine in tissues. *J. Pharmac. exp. Ther.* **127:** 175–177, 1959.
13. Hinesley, R. K., J. A. Norton and M. H. Aprison. Serotonin, norepinephrine 3,4-dihydroxyphenylethylamine in rat brain parts following electroconvulsive shock. *J. Psychiat. Res.* **6:** 143–152, 1968.
14. Ladisich, W., N. Steinhauff and N. Matussek. Chronic administration of electroconvulsive shock and norepinephrine metabolism in the rat brain. *Psychopharmacologia* **15:** 296–304, 1969.
15. Leonard, B. E. and S. R. Tonge. The effect of some hallucinogenic drugs upon the metabolism of noradrenaline. *Life Sci.* **8:** 815–825, 1969.
16. McGaugh, J. L. and R. G. Dawson. Modification of memory storage processes. *Behav. Sci.* **16:** 45–63, 1971.
17. Miller, R. R. and A. D. Springer. Induced recovery of memory in rats following electroconvulsive shock. *Physiol. Behav.* **8:** 645–652, 1972.
18. Milner, B. The memory defect in bilateral hippocampal lesions. *Psychiat. Res. Rep.* **11:** 43–52, 1959.
19. Nielson, H. C. Evidence that electroconvulsive shock alters memory retrieval rather than memory consolidation. *Expl. Neurol.* **20:** 3–20, 1968.
20. Nielson, H. C. and R. M. Fleming. Effects of electroconvulsive shock and prior stress on brain amine levels. *Expl Neurol.* **20:** 21–30, 1968.
21. Paolino, R. M., D. Quartermain and N. E. Miller. Different temporal gradients of retrograde amnesia produced by carbondioxide anaesthesia and electroconvulsive shock. *J. comp. physiol. Psychol.* **62:** 270–274, 1966.
22. Rigter, H. *Amnesie in de rat.* Ph. D. thesis, University of Utrecht, 1973.

23. Rigter, H., G. van Eys and B. E. Leonard. Hippocampal monoamine metabolism and the CO_2 induced retrograde amnesia gradient in rats. *Pharmac. Biochem. Behav.* **3**: 781–785, 1975.
24. Ross, D. P. and F. McGintry. The effect of steroid hormones on tryptophan metabolism. *Adv. Steroid Biochem.* **1**: 97–136, 1970.
25. Schneider, A. M., A. Malter, and C. Advokat. Pretreatment effects of a single ECS and footshock plus ECS on step-down latencies of trained and untrained rats. *J. comp. physiol. Psychol.* **68**: 627–630, 1969.
26. Snyder, S. H., J. Axelrod and H. Zweig. A sensitive and specific fluorescence assay for tissue serotonin. *Biochem. Pharmac.* **14**: 831–835, 1965.
27. Taber, R. I. and A. Banuazizi. CO_2 induced retrograde amnesia in a one-trial learning situation. *Psychopharmacologia* **9**: 382–391, 1966.
28. Thompson, C. I. and L. B. Grossman. Loss and recovery of long-term memories after ECS in rats: evidence for state dependent recall. *J. comp. physiol. Psychol.* **78**: 248–254, 1972.
29. Tonge, S. R. and B. E. Leonard. The effects of some hallucinogenics upon the metabolism of 5-hydroxytryptamine in the brain. *Life Sci.* **8**: 805–814, 1969.
30. Uchida, T. and R. D. O'Brien. The effects of hydrazine on rat brain 5-hydroxytryptamine, norepinephrine and γ-aminobutyric acid. *Biochem. Pharmac.* **13**: 725–730, 1964.
31. Van Eys, G., H. Rigter and B. E. Leonard. Time-dependent aspects of CO_2 induced amnesia and hippocampal monoamine metabolism in rats. *Pharmac. Biochem. Behav.* **3**: 787–793, 1975.
32. Waalkes, T. P. and S. Udenfriend. A fluorometric method for the estimation of tyrosine in plasma and tissues. *J. Lab clin. Med.* **50**: 733–736, 1957.
33. Yates, F. The analysis of contingency tables with groupings based on quantitative characters. *Biometrika* **35**: 178–181, 1948.
34. Zornetzer, S. F., R. B. Chronister and B. Ross. The hippocampus and retrograde amnesia: localization of some positive and negative memory disruptive sites. *Behav. Biol.* **8**: 507–518, 1973.