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Time-Dependent Aspects of CO_2 Induced Amnesia and Hippocampal Monoamine Metabolism in Rats

G. VAN EYS, H. RIGTER[1] AND B. E. LEONARD

Pharmacology Department, Scientific Development Group
Organon, Oss, the Netherlands

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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(5) 787–793, 1975. – The time course of amnesia for a one-trial passive avoidance response after treatment with carbon dioxide (CO_2) was studied. Amnesia developed gradually over the first 4 hr following the amnesic treatment. Once established, amnesia remained during a 4 week test period. Previously, we reported that acquisition of the passive avoidance response was attended with a rise in the hippocampal concentration of serotonin 24 hr later and that this rise was not observed when acquisition was followed by amnesic treatment. In the present study, it was found that a rise in hippocampal serotonin parallelled the transient retention of the avoidance response 2 hr after amnesic treatment. However, 2 weeks after acquisition and amnesic treatment no changes in hippocampal monoamine metabolism could be detected. Hippocampal noradrenaline did not correlate with avoidance and amnesia.

Retrograde amnesia Carbon dioxide Hippocampus Passive avoidance Serotonin Noradrenaline

IN previous studies, we showed that changes in brain monoamine metabolism parallelled carbon dioxide (CO_2) induced retrograde amnesia for a passive avoidance response. These changes were most pronounced in the hippocampus, a region of the brain which is thought to be associated with memory consolidation processes [21,30] and behavioral inhibition [6]. Most conspicuously, acquisition of the passive avoidance response was attended with a rise in the hippocampal levels of serotonin 24 hr later. This rise was not observed when acquisition was immediately followed by the application of CO_2 [13,25]. Subsequently, it was found that as the period between acquisition and CO_2 treatment was increased the degree of amnesia decreased. This amnesia gradient was also attended with changes in hippocampal serotonin metabolism; the concentration of serotonin increased as the interval between acquisition and amnesic treatment increased [25].

These data suggest that a correlation may exist between the observed changes in hippocampal monoamine metabolism on the one hand and avoidance and amnesia on the other hand. However, in these studies all behavioral and biochemical measurements were made 24 hr after acquisition. The present investigation was undertaken to determine whether this correlation would also hold for other acquisition-test intervals. In Experiments 1 and 2, we studied the effect of variations in the acquisition-test interval on amnesia; in Experiment 3, the effect of these variations on hippocampal monoamine levels was assessed.

EXPERIMENT 1

One prominent theory states that amnesia results from a disruption of the memory consolidation process. According to this theory, an amnesic treatment of sufficient intensity will prevent consolidation of memory items, thereby causing permanent amnesia [9, 15, 16, 17]. However, the results of other investigations do not substantiate this view. Thus, in a number of studies it has been found that memory recovers following amnesic treatment [11, 19, 22, 29]. Experiment 1 was undertaken to study possible changes in amnesia over a 4 week period.

Method

Animals. One hundred and fifty male rats of an inbred Wistar strain weighing 220–225 g were used. They were obtained from TNO-Zeist, the Netherlands, 2 weeks before the start of the experiment. The animals were trained in a step-through passive avoidance apparatus of the type described by Ader *et al.* [1].

Procedure. The animals were randomly divided into 15 groups of 10 rats. They were trained according to the procedure described by Leonard and Rigter [13]. They were given 3 pretraining trials on Day 1 of the experiment and a single acquisition trial on Day 2. During the acquisition trial a 0.5 mA foot shock (FS) was given for 3 sec to 10 groups of rats (FS groups). Five FS groups were subjected to

[1] Reprint requests should be sent to H. Rigter, Pharmacology Department, Scientific Development Group, Organon, Oss, the Netherlands.

amnesic treatment with CO_2 immediately on termination of the acquisition trial (FS-CO_2 groups) whereas the other 5 groups received sham amnesic treatment (FS-$NoCO_2$ groups). The method of administration of CO_2 was described previously [13,24]. The rats were treated with 100 percent CO_2 for 30–35 sec. Rats receiving sham amnesic treatment were placed in an air-filled box. The remaining 5 groups were not subjected to foot shock at the time of acquisition and did not receive the CO_2 treatment (NoFS-$NoCO_2$ groups).

The retrieval test was given either 24 hr, 48 hr, 1 week, 2 weeks or 4 weeks following acquisition. For each acquisition-test interval one NoFS-$NoCO_2$, one FS-CO_2 and one FS-$NoCO_2$ group were used. The results were analyzed by means of the Yates test [28]. The test scores were divided into 3 classes: (1) latencies of 0–10.0 sec (no avoidance); (2) latencies of 10.1–299.9 sec (incomplete avoidance); (3) latencies of 300.0 sec (complete avoidance).

Results

Figure 1 shows that the step-through latencies of all NoFS-$NoCO_2$ rats were shorter than 10.0 sec irrespective of the duration of the acquisition-test interval. However, a slight but significant increase of these latencies was apparent after an interval of 2 and 4 weeks ($p<0.02$, compared to the NoFS-$NoCO_2$ groups tested after 24 or 48 hr; two-tailed Mann-Whitney U test). The mean latency was 2.0; 1.6; 1.7; 3.2; and 2.9 sec for groups which received the test 24 hr; 48 hr; 1 week; 2 weeks and 4 weeks following acquisition.

All FS-$NoCO_2$ groups showed passive avoidance: the differences between these and the corresponding NoFS-$NoCO_2$ groups were significant (Table 1). Passive avoidance slightly decreased as the interval between acquisition and test increased: the FS-$NoCO_2$ groups which were tested after 2 and 4 weeks significantly differed from the FS-$NoCO_2$ groups which were tested after 48 hr: $z = 2.23$, $p<0.05$.

All FS-CO_2 groups displayed amnesia: the differences between these and the corresponding FS-$NoCO_2$ groups were significant (Table 1). Spontaneous recovery of memory could not be detected : the FS-CO_2 groups which were tested between 48 hr and 4 weeks after acquisition did not significantly differ from the FS-CO_2 group which was tested after an interval of 24 hr ($z \leqslant 1.49$, $p>0.05$).

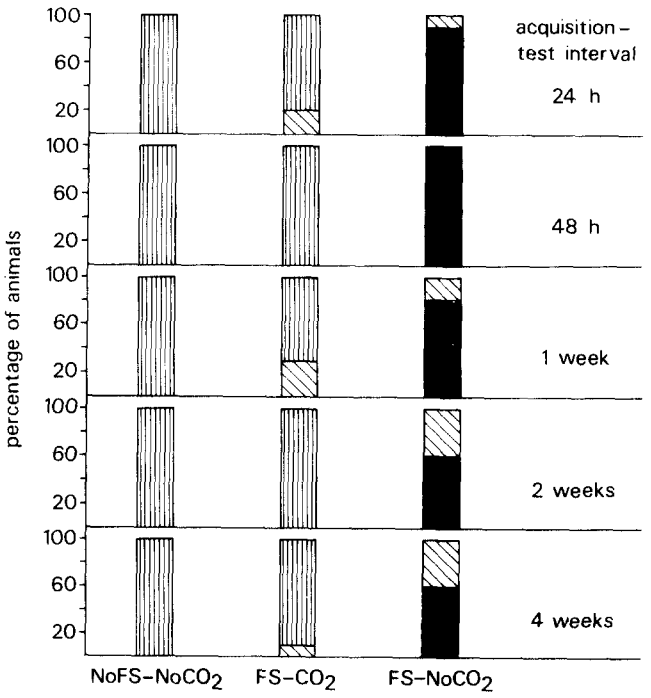

FIG. 1. Absence of spontaneous recovery of memory following treatment with CO_2. The figure shows the latencies of groups of rats (10/group) at the test trial which was given 24 hr, 48 hr, 1 week, 2 weeks or 4 weeks after acquisition. The scores were divided into 3 classes: (1) 0–10.0 sec (no avoidance); (2) 10.1–299.9 sec (incomplete avoidance); (3) 300.0 sec (complete avoidance). FS: foot shock; NoFS: no foot shock; CO_2 : CO_2 treatment; $NoCO_2$: no CO_2 treatment.

EXPERIMENT2

In most studies on amnesia, retrieval is tested 24 hr after acquisition and the application of the amnesic agent. Under these circumstances, amnesia is generally apparent. However, there are several reports that animals which are tested within a few hours after acquisition and amnesic treatment show adequate retrieval. Complete amnesia only appears between 1–6 hr after acquisition and amnesic treatment [2, 4, 7, 10, 18, 20]. The present experiment was undertaken to see whether this occurred when CO_2 was used as the amnesic agent.

Method

One hundred and seventy male Wistar rats (210–240 g) were used. The animals were randomly divided into 17 groups of 10 rats each. They were trained in the passive avoidance task according to the schedule described in Experiment 1. Two groups received neither foot shock nor amnesic treatment (NoFS-$NoCO_2$ groups). Four groups did not receive foot shock but were subjected to CO_2 treatment immediately on termination of the acquisition trial (NoFS-CO_2 groups). Seven groups were given foot shock immediately followed by CO_2 treatment (FS-CO_2 groups) whereas the remaining 4 groups received foot shock followed by sham amnesic treatment (FS-$NoCO_2$ groups). The NoFS-$NoCO_2$ groups were tested 30 or 1440 min after acquisition; the NoFS-CO_2 groups, 30, 120, 240 or 1440 min, respectively; the FS-CO_2 groups 30, 60, 120, 180, 240, 300 or 1440 min, respectively, while the FS-$NoCO_2$ groups received the retrieval test 30, 120, 240 or 1440 min following acquisition.

Results

The step-through latencies of the NoFS-$NoCO_2$ and NoFS-CO_2 groups are given in Table 2. At the retrieval test, all animals in the two NoFS-$NoCO_2$ groups entered the chamber within 10.0 sec; the time between the acquisition and the test trial did not affect the latencies of these groups (the NoFS-$NoCO_2$ 30 min vs the NoFS-$NoCO_2$ 1440 min group: U = 37, not significant, two-tailed Mann-Whitney U test). The locomotor behavior of NoFS-CO_2 rats was disturbed 30 min after CO_2 treatment: the step-through latencies of these animals were significantly increased (the NoFS-CO_2 30 min vs the NoFS-$NoCO_2$ 30 min group : U = 3.5, $p<0.002$); locomotor behavior had recovered 120 min

TABLE 1

STATISTICAL EVALUATION OF STEP-THROUGH LATENCIES OF GROUPS OF RATS TESTED AT DIFFERENT TIMES AFTER ACQUISITION

Group	Acquisition Test Interval	Statistical Value (z)	
NoFS-NoCO$_2$	24 hr	1.49‡	4.36†
FS-CO$_2$	24 hr	4.13†	
FS-NoCO$_2$	24 hr		
NoFS-NoCO$_2$	48 hr	0.00‡	4.46†
FS-CO$_2$	48 hr	4.46†	
FS-NoCO$_2$	48 hr		
NoFS-NoCO$_2$	1 week	1.87*	4.36†
FS-CO$_2$	1 week	4.02†	
FS-NoCO$_2$	1 week		
NoFS-NoCO$_2$	2 weeks	0.00‡	4.10†
FS-CO$_2$	2 weeks	4.10†	
FS-NoCO$_2$	2 weeks		
NoFS-NoCO$_2$	4 weeks	1.02‡	4.00†
FS-CO$_2$	4 weeks	3.86†	
FS-NoCO$_2$	4 weeks		

FS = foot shock NoFS = no foot shock CO_2 = CO_2 treatment $NoCO_2$ = no CO_2 treatment
Statistical values are computed by means of the one-tailed Yates test.
*$p<0.05$ †$p<0.0001$ ‡not significant

after CO_2 treatment (the NoFS-CO_2 120 vs either NoFS-$NoCO_2$ group : $U \geqslant 37.5$, not significant) (Fig. 2).

All FS-$NoCO_2$ groups showed passive avoidance, irrespective of the time of test (Fig. 2): the differences between these groups were not significant ($z \leqslant 1.53$). The FS-CO_2 group which was tested 1440 min after acquisition displayed amnesia (compared to the FS-$NoCO_2$ 1440 min group: $z = 4.00$, $p<0.0001$). Amnesia decreased as the time between acquisition and test decreased. The FS-CO_2 groups which were tested 240 and 300 min after acquisition did not significantly differ from the FS-CO_2 1440 min group ($z = 1.53$ and 0.52, respectively). However, the FS-CO_2 groups which were tested 30, 60, 120 or 180 min after acquisition showed less amnesia than the FS-CO_2 1440 min group ($z = 2.26$, $p<0.05$; $z = 2.00$, $p<0.05$ and $z = 2.69$, $p<0.01$, respectively), although some degree of amnesia was present (FS-CO_2 30 min vs FS-$NoCO_2$ 30 min : $z = 1.92$, $p<0.05$; FS-CO_2 120 min vs FS-$NoCO_2$ 120 min : $z = 2.39$, $p< 0.01$).

EXPERIMENT 3

Previously, we showed that CO_2 induced amnesia for the step-through passive avoidance response was attended with

TABLE 2

STEP-THROUGH LATENCIES OF GROUPS OF RATS NOT SUBJECTED TO FOOT SHOCK

Group	Acquisition Test Interval (min)	Latency at Trial*	
		Acquisition	Test
NoFS-NoCO$_2$	30	1.6 ± 0.2	1.8 ± 0.2
NoFS-NoCO$_2$	1440	1.7 ± 0.2	1.7 ± 0.2
NoFS-CO$_2$	30	1.4 ± 0.1	7.1 ± 1.7†
NoFS-CO$_2$	120	1.4 ± 0.1	3.3 ± 1.5
NoFS-CO$_2$	240	1.5 ± 0.2	1.9 ± 0.2
NoFS-CO$_2$	1440	1.7 ± 0.2	1.6 ± 0.1

*Mean seconds ± standard error of the mean

†Significant increase compared to the corresponding NoFS-NoCO$_2$ group ($p<0.002$, Mann Whitney U test)

NoFS = no foot shock CO_2 = CO_2 treatment $NoCO_2$ = no CO_2 treatment

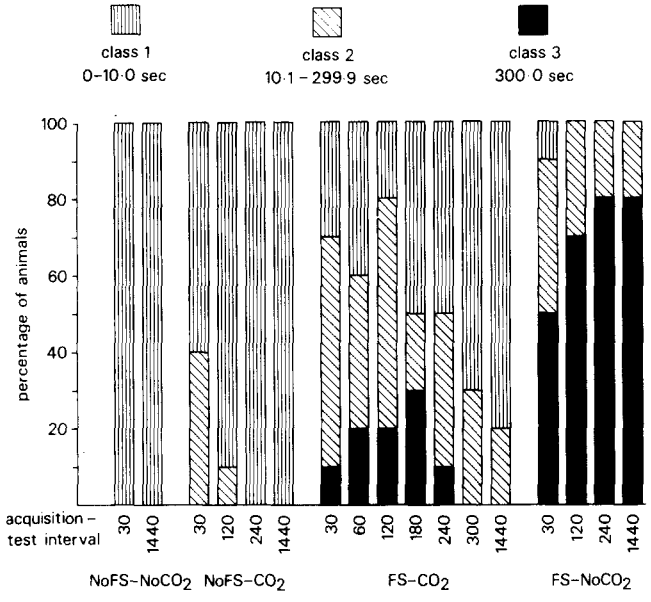

FIG. 2. Delayed onset of amnesia following treatment with CO_2. The figure presents the latencies of groups of rats which were tested 30, 60, 120, 180, 240, 300 or 1440 min after acquisition. The scores were divided into 3 classes: (1, vertical bars) 0–10.0 sec (no avoidance); (2, diagonal bars) 10.1–299.9 sec (incomplete avoidance); (3, solid bars) 300.0 sec (complete avoidance). FS: foot shock; NoFS: no foot shock; CO_2: CO_2 treatment; $NoCO_2$: no CO_2 treatment.

changes in hippocampal monoamine metabolism [13]. In Experiment 1 it was shown that this amnesia remained present over a 4 week period. In Experiment 2 it appeared that the amnesia only gradually developed after application of the amnesic treatment. The present experiment was undertaken to examine if changes in hippocampal monoamine metabolism parallelled these behavioral phenomena. Groups of rats were killed either 2 hr, 24 hr or 2 weeks after the acquisition trial. The interval of 2 hr was chosen since it was shown in Experiment 2 that after this time lapse most FS-CO_2 rats still displayed some degree of retrieval. However, after 24 hr and 2 weeks amnesia was present (Experiment 1).

Method

Twelve groups of 20 male Wistar rats (200–230 g) were used. The experiment was run in 5 randomized blocks. Each block contained 48 rats, 4 in each group. The animals were trained in the step-through passive avoidance apparatus as described in Experiment 1. At the time of acquisition trial, 3 groups were subjected to foot shock followed by sham amnesic treatment (FS-$NoCO_2$ groups) and 3 groups were given foot shock immediately followed by CO_2 treatment (FS-CO_2 groups). The remaining 6 groups did not receive foot shock: 3 of these groups were subjected to CO_2 treatment (NoFS-CO_2 groups) whereas the other 3 groups were given sham amnesic treatment (NoFS-$NoCO_2$ groups). The rats were not subjected to a retrieval test but instead were killed by decapitation either 2 hr, 24 hr or 2 weeks following acquisition. For each time interval, one NoFS-CO_2, one FS-CO_2 and one FS-$NoCO_2$ group were used. The behavioral part of the experiment and the killing of the rats took place between 09:30 and 11:00 hr a.m. to

TABLE 3

CHANGES IN THE CONCENTRATIONS OF HIPPOCAMPAL SEROTONIN AND NORADRENALINE IN GROUPS OF RATS KILLED AT DIFFERENT TIMES AFTER ACQUISITION AND/OR AMNESIC TREATMENT

	Group			
Time Killed	NoFS-NoCO$_2$	NoFS-CO$_2$	FS-CO$_2$	FS-NoCO$_2$
Serotonin				
2 hr	2.46	2.51	2.92*	3.12*
		+2% (−9, +13)	+19% (+6, +32)	+26% (+13, +41)
24 hr	2.01	1.89	1.73	2.30*
		−5% (−19, +11)	−14% (−27, +2)	+16% (0, +34)
2 weeks	1.89	1.95	1.92	1.85
		+3% (−15, +25)	+1% (−17, +22)	−2% (−19, +18)
Noradrenaline				
2 hr	0.14	0.15	0.15	0.16*
		+7% (−6, +17)	+7% (−7, +16)	+14% (+1, +25)
24 hr	0.18	0.19	0.18	0.20
		+6% (−6, +19)	0% (−13, +11)	+10% (−2, +24)
2 weeks	0.16	0.16	0.16	0.15
		−4% (−15, +8)	−4% (−14, +7)	−8% (−18, +3)

All statistical comparisons made between the treated groups and the NoFS-NoCO$_2$ group.
*$p<0.05$; the 95 percent confidence intervals are given in parentheses. All values expressed as μg/g wet weight of hippocampus.
FS = foot shock NoFS = no foot shock CO_2 = CO_2 treatment $NoCO_2$ = no CO_2 treatment

minimize any differences which could result from changes in monoamine metabolism associated with the circadian rhythm [14]. Following decapitation, the hippocampi were rapidly dissected and frozen on solid carbon dioxide. The hippocampi from 4 rats of the same group were pooled for the biochemical assays. The tissue was homogenized in 7 ml 0.01 N HCl to which 0.7 ml 10 percent (w/v) sodium edate had been added. Following centrifugation (800× g; 20 min), aliquots of the clear supernatant were removed for the determination of noradrenaline [5]. The pellet and the remainder of the supernatant fraction were extracted with butanol for the determination of serotonin [26].

Results

The results are presented in Table 3. In agreement with our previous data [13,25] the concentration of serotonin was significantly raised in the FS-NoCO$_2$ group which was killed 24 hr after acquisition. This rise did not occur in the corresponding NoFS-CO$_2$ and FS-CO$_2$ groups. There were no significant changes in the concentration of noradrenaline.

The FS-NoCO$_2$ group, which was killed 2 hr after acquisition, also showed an increased concentration of serotonin. However, in contrast to the corresponding 24 hr group, the concentration of noradrenaline was also raised. The FS-CO$_2$ group which was killed 2 hr after acquisition had an increased concentration of serotonin; the concentration of noradrenaline was unchanged.

No changes occurred in either serotonin or noradrenaline in any of the groups which were killed 2 weeks after acquisition.

DISCUSSION

In Experiment 1, spontaneous recovery of memory for a passive avoidance response following treatment with CO_2 could not be detected even up to 4 weeks after the acquisition trial. This finding does not exclude the possibility that under different conditions recovery of memory may occur. It fact, it has been found in this and other laboratories that some recovery of memory takes place when the animals are subjected to repeated test trials [11, 23, 24, 29].

In the second experiment, it was shown that CO_2 induced amnesia for a passive avoidance only developed gradually over the first 4 hr after the amnesic treatment. Although CO_2 affected locomotor behavior shortly following treatment, it is improbable that this effect influenced the finding of delayed onset of amnesia: locomotor behavior of NoFS-CO_2 rats was normal 2 hr after CO_2 but at that time FS-CO_2 animals still showed retention. The finding of delayed onset of amnesia is in keeping with numerous other reports [2, 4, 7, 10, 18, 20]. The basis of this phenomenon is not known. Recently, it was hypothesized that memory consolidation is comprised of two parallel processes, one of a short-term and the other of a long-term nature. Amnesic treatment may affect the long-term but not the short-term conponent [10].

In Experiment 3, we determined changes in hippocampal monoamine metabolism at different intervals following acquisition and amnesic treatment. Previously, we reported that changes in hippocampal serotonin levels parallelled the CO_2 induced amnesia for the passive avoidance response. Acquisition of the passive avoidance response was attended with a rise in the concentration of serotonin 24 hr later and this rise was not observed when acquisition was immediately followed by CO_2 treatment [13]. In a subsequent study, we showed that a gradient of hippocampal serotonin concentrations parallelled the amnesia gradient 24 hr after acquisition [25]. Experiment 3 corroborated the previous finding that acquisition of the passive avoidance response was attended with a rise in hippocampal serotonin 24 hr later and that this rise did not occur when acquisition was followed by amnesic treatment. A correlation between the behavioral and biochemical measures was also found at an acquisition-test interval of 2 hr: FS-NoCO$_2$ as well as FS-CO_2 animals showed passive avoidance behavior 2 hr after acquisition (Experiment 2); at that time both groups showed a rise in the concentration of serotonin (Experiment 3). Taken together, the results of our studies indicate that within 24 hr following acquisition and amnesic treatment a rise in hippocampal serotonin is found in conjunction with passive avoidance (in the FS-NoCO$_2$ group [13]; when the interval between acquisition and the CO_2 treatment gets longer [25]; and at an acquisition-test interval of 2 hr). This rise in serotonin does not occur in amnesic animals. However, a rise in serotonin does not correlate with the presence of passive avoidance behavior *per se*: 2 weeks after acquisition changes in hippocampal levels of serotonin could not be detected (Experiment 3) although FS-NoCO$_2$ rats still displayed passive avoidance at that time (Experiment 2).

At least two explanations for our data can be offered: (1) A rise in hippocampal serotonin is directly or indirectly related to passive avoidance behavior and the absence of such a rise is associated with amnesia. The fact that this correlation is found within the first 24 hr following acquisition but not 2 weeks later may be interpreted as indicating that hippocampal serotonin metabolism is directly or indirectly associated with memory consolidation rather than memory retrieval. This would be in accordance with the statement of Allen *et al.* [3] that serotonin plays an essential role in the formation of memory for passive avoidance responses. However, such a conclusion is premature as long as uncertainty exists about the number and the nature of the processes involved in acquisition and amnesia [10, 17, 22, 27]. (2) The parallel changes in hippocampal serotonin metabolism on the one hand and passive avoidance on the other hand may be purely co-incidental. It is possible that the foot shock induces a transient rise in hippocampal serotonin and that the CO_2 treatment curtails this rise. However, it is unlikely that stress induced release of ACTH and corticosteroids causes the change in serotonin metabolism since administration of ACTH 1 hr prior to acquisition did not alter serotonin metabolism in NoFS or FS-CO_2 rats [12]. Moreover, recent pharmacological experiments suggest that the changes in hippocampal serotonin are indeed correlated with avoidance and amnesia. Thus, $ACTH_{4\text{-}10}$, a peptide which attenuates amnesia when administered prior to the retrieval test [24], also induces a rise in hippocampal serotonin in FS-CO_2 rats [12].

In Experiment 3, hippocampal noradrenaline levels did not correlate with avoidance and amnesia. No changes occurred in noradrenaline in any of the groups 24 hr or 2 weeks after acquisition. The FS-NoCO$_2$ group which was killed 2 hr after acquisition had a significantly increased concentration of noradrenaline. In the corresponding FS-CO_2 group, which similarly showed avoidance (Experiment 2), this rise did not occur. Gold *et al.* [8] suggested that noradrenaline modulates the formation of memory for a passive avoidance response. Our data do not support this view.

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