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Retrograde Amnesia Production by the Intracisternal Injection of 20 μl of Saline in Rats

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KOBILER, D. AND C. ALLWEIS. *Retrograde amnesia production by the intracisternal injection of 20 μl of saline in rats.* PHARMAC. BIOCHEM. BEHAV. 6(3) 255–258, 1977. – The intracisternal injection of 20 μl of saline into a rat within an hour after training it in an active avoidance response was found to induce retrograde amnesia. The results obtained by combining this procedure with the intracisternal injection of 2,6 Diaminopurine (DAP), which inhibits RNA synthesis and prevents long-term memory formation when administered at the proper time and in the correct dosage, suggest the existence of a medium term memory (MTM) mechanism. MTM was normally evident up to 75 min after training but was demonstrable up to 210 min when LTM formation was prevented by DAP.

Amnesia Intracisternal injection 2,6 diaminopurine

IN AN earlier publication [6] we described the use of intracisternally administered solutions of 2,6 diaminopurine (DAP) to interfere with the formation of long term memory (LTM) in the rat. In most of the experiments we injected 10 μl of DAP solution before training. In a few experiments we injected a larger volume of solution (20 μl) intracisternally after training and found that control animals similarly injected with 20 μl of saline after training failed to remember a previously learned avoidance response. Further experimentation revealed that under certain conditions the mere intracisternal injection of a volume of 20 μl of an inactive solution induced retrograde amnesia. In this paper we describe the exploitation of this phenomenon to characterise different stages in the formation of LTM.

METHODS

Training and testing methods for the simple avoidance response have been described previously [6]. Briefly, the rat was placed repeatedly in the black side of a two-compartment box and trained by foot-shock to run to the white (safe) side within 5 sec. Trials in which the animal had to be shocked were scored as failures and training or testing was continued until an arbitrary criterion of n successful responses out of n or n + 1 trials was met. The final score (trials to criterion TTC) was then calculated as the total number of trials minus the number of criterion trials. Further details are given in [6].

The use of intracisternally injected DAP (60 μg per 10 μl of 0.15 M $NaHCO_3$ solution) to prevent the formation of long-term memory was also described in that paper.

The time course of changes in the incorporation of uridine-5-^{3}H into brain RNA brought about by DAP and the effects thereon of adenosine were determined in a separate series of animals as described previously [6]. The average results of such determinations were used in plotting our experimental results where appropriate. Quantitative results are presented in the following format: (TRAIN or TEST TTC ± STANDARD DEVIATION: Number of Animals).

In Experiments 2 and 3, tests for LTM formation were run at various convenient times greater than 4–5 hr after training. Since TTC values and their standard deviations do not change significantly with time later than 4–5 hr after learning under our experimental conditions [6], the average test TTC value for each group is ascribed to the entire period during which testing was performed. To further simplify the graphic presentation, groups differing in their average TTC by less than 0.4 trials (i.e. about one standard deviation) from the average values 0.7 (remembers) and 0.4 trials (i.e. about 0.2 of a standard deviation) from the average value 5.5 (does not remember) are assigned those nominal values. The exact data is given in the text where necessary.

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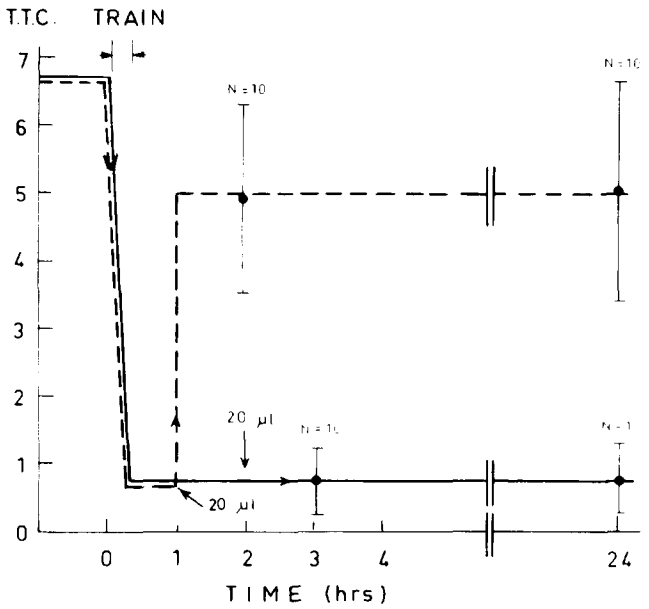

FIG. 1. Retrograde amnesia brought about by the intracisternal injection of 20 µl saline. ——— injected 120 min after training. - - - - -Injected 60 min after training. TTC = trials to criterion. The injection produced retrograde amnesia when given up to one hour after training (p = <<0.001, Mann-Whitney) but not when given after 2 hr.

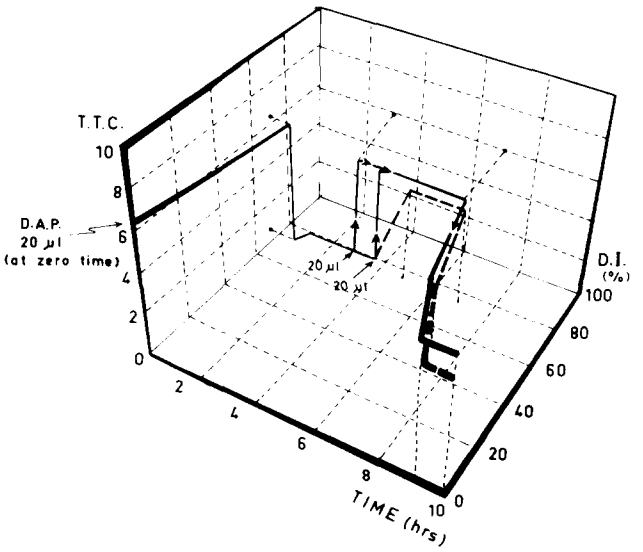

FIG. 2. The effect of rapid intracisternal injection of 20 µl saline on the retention of a learned avoidance response when RNA synthesis is slowed by DAP. (D.I. = decrease of incorporation of uridine-5-^{3}H into brain RNA expressed as a % of the uninhibited incorporation. The time course of inhibition was established in parallel groups of animals [6]. The downward branch of the trajectory 1 hr after DAP represents training. The arrows indicate the intracisternal injection of 20 µl saline into trained animals at either 3 or 4 hr after the DAP injection. The posttraining 20 µl-sensitive period is extended from about one hour in the absence of DAP (Fig. 1) to about 3½ hr (full line). Subsequently, memory is lost even though no 20 µl injection is given. (Broken line).

RESULTS

The Production of Retrograde Amnesia by Intracisternal Injection of 20 µl of Saline

Rats were trained in the active avoidance apparatus to a criterion of 5/5 correct responses. At various later times each rat received an intracisternal injection of 20 µl of saline under light ether anesthesia. The actual injection of fluid was done rapidly within five seconds.

The critical period during which the injection produced retrograde amnesia lasted up to 75 min after the start of training (Fig. 1). This period will be referred to as the 20 µl sensitive period. If the injection was done later no amnesia was seen.

This phenomenon did not occur when a smaller volume of fluid, 10 µl was injected within 1 hr of training (Test − 0.3 ± 0.49 TTC: N = 9).

The Effect of a Rapid Intracisternal Injection of 20 µl Performed During the Course of a DAP-Induced Slowing of RNA Synthesis

By combining a 20 µl saline injection with the administration of DAP, we have been able to learn more of the processes leading to LTM formation.

The purpose of this experiment was to see if DAP would alter the duration of the 20 µl-sensitive period. Animals received an injection of 20 µl (120 µg) of DAP solution. This dosage was enough to produce a prolonged decrease in RNA synthesis [6]. The animals were trained 60 min later, and the stability of the memory formed to a rapid intracisternal injection of 20 µl saline was tested at various times after. We found that providing that RNA synthesis was adequately slowed by previously administered DAP, then retrograde amnesia could be produced by saline injection at all times up to about three and a half hours after training. After this time, memory declined over a period of about an hour to the pretraining level (broken line) owing to the presence of DAP [6]. This extention of the duration of the 20 µl-sensitive period from about 75 min after the start of training in the absence of DAP to about three and a half hours after training with DAP is very important since it is the experimental basis on which our operative definition of Medium-term memory (MTM), will be based. Anticipating somewhat the remarks made in our discussion, we interpret these findings as follows. The intracisternal injection of 20 µl saline disrupts and rapidly terminates medium term memory in some unknown manner. If this 20 µl injection is made after long term memory has already been established, it has no discernible effect since the by now effective LTM mechanism masks the disruption of the MTM holding mechanism which is presumed to occur. If, however, the establishment of long-term memory is prevented by DAP, then the persistence of the 20 µl-injection-sensitive memory holding mechanism is demonstrable over a further two and a half hours approximately. We are here tentatively assuming that in the normal course of events medium-term and long-term memory overlap for a while until the medium-term memory delays to an ineffective level.

Determination of the Time Required to Form LTM Following Restoration of RNA Synthesis Previously Blocked by DAP

The following experiment was designed to determine how long it would take following the restoration of RNA synthesis with adenosine for LTM to be established. The

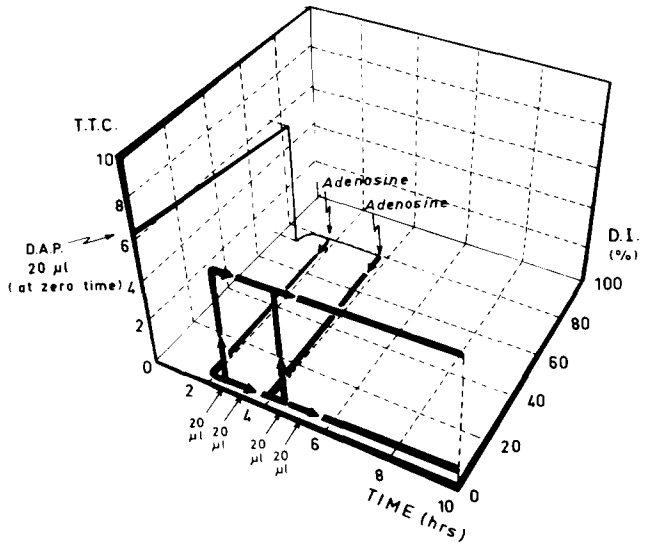

FIG. 3. Training under the influence of DAP was done as in Fig. 2. Following adenosine administration, the synthesis of RNA rapidly returns to normal and memory is retained for at least 10 hr in the absence of further experimental interference. The intracisternal injection of 20 µl of saline (after adenosine injection) was used to determine the time at which LTM has been formed since MTM is abolished by it whilst LTM is unaffected by it. Twenty µl injections made within 45 min after reinstating RNA synthesis abolish memory, (upward directed lines) whereas 20 µl injections made more than 45 min after the reinstatement of RNA synthesis have no effect on memory.

criterion used to determine the onset of LTM was the stability of the memory-holding mechanism to a 20 µl intracisternal injection.

We have previously shown that if RNA synthesis is decreased by intracisternally administered DAP, the establishment of LTM may be induced up to 3 hr after training by restoring brain RNA synthesis by the intracisternal injection of 120 µg of adenosine [6].

In this experiment, rats received a 20 µl dose of DAP at zero time and were trained about 1 hr later whilst the rate of RNA synthesis was decreased. One hundred-twenty or 240 min later, the rate of RNA synthesis was rapidly restored to normal by an intracisternal injection of adenosine. In both cases, 30 min after the adenosine injection the susceptibility of the memory trace to disruption by a 20 µl injection was determined. In both cases (120 + 30 min and 240 + 30 min) it was found to be disruptable (TEST – 6.14 ± 1.9 TTC; N = 7). But if the 20 µl test injection was made in both cases 60 min after the adenosine injection (i.e. 120 + 60 min and 240 + 60 min) then the 20 µl injections did not produce retrograde amnesia. TEST – 0.33 ± 0.5 TTC; N = 6). The critical point appeared to be about 45 min after the injection of adenosine.

It thus appears that about 45 min after reinstating RNA synthesis by administering adenosine a new memory trace which is resistant to disruption by a 20 µl injection of saline has been established.

DISCUSSION

To the best of our knowledge the production of retrograde amnesia by the intracisternal injection of a small volume of fluid within an hour after training has not previously been reported. Since the volume injected and rate of injection seem to be the critical factors involved, we deduce that the effect is brought about by a rise in the hydrostatic pressure of the CSF. One likelihood is that the transient rise in CSF pressure produces a transient fall in cerebral flood flow. The resulting brain anoxia might irreversibly alter the pattern of neural firing which at that time constitutes the only record of the training experience. This suggestion accords with the fact that anoxia-induced impairment of memory has been demonstrated in animals [8,9].

Whether or not this is the mechanism of action, the 20 µl injection has proved when applied either alone or in combination with other agents to be a useful tool in experiments designed to reveal the existence and duration of different processes involved in the establishment of LTM.

Most of the recent discussions of memory processes have hypothesized a two-stage process (LTM and STM) to explain the fact that the retrograde amnesia produced by ECS decreases with extension of the duration of the period between training and the administration of electroshock. Barondes and Cohen [3], on the basis of their own experiments with puromycin and experiments of Flexner *et al.* [4] suggested that the memory fixation process may be triphasic or multiphasic.

McGaugh has proposed a multi-trace hypothesis involving four different processes underlying memory and has discussed possible interactions between them [7]. His designation of the types of memory (differentiated by their degrees of permanence) as transient, short term, immediate, and long term is based partly on human experience and partly on animal experimentation.

Roy John [5] has also discussed the multiple trace theory, and infers from the available evidence the existence of an intermediate holding mechanism which is effective up to 180–360 min after an experience.

We find it helpful to orientate our discussion around 3 experimentally distinguishable trace-retaining mechanisms and 3 processes by which the trace appears to be transcribed from 1 retaining mechanism to the other [1].

1. STM – which is susceptible to ECS. Estimations of the duration of STM vary greatly with different experimental procedures.

2. MTM – which we define here on the basis of it's susceptibility to a 20 µl intracisternal injection. Its effective duration is seen to be limited to about 200 min after training when its masking by the onset of LTM is prevented by DAP.

3. LTM – which when formed is resistant to both 20 µl injection and to DAP, but whose formation is prevented by DAP. The three processes are those by which a learning experience is converted to STM; STM is converted to MTM; and MTM is converted to LTM.

The experiments described here relate particularly to the process which produces the transcription from MTM to LTM. The process whereby MTM is generated from STM as a result of a behavioral experience appears to be unaffected by interference with the synthesis of RNA [6] and proteins [2].

LTM, once established, is unaffected by the rapid intracisternal injection of 20 µl saline, but its establishment can be prevented by the administration at the correct time of an adequate intracisternal dose of DAP or other substances which interfere with the synthesis of RNA, or

proteins. Having found that MTM can be disrupted by the rapid intracisternal injection of 20 μ l saline (Fig. 2), we were able to show that the effective duration of the MTM trace (the termination of which is indicated by the spontaneous disappearance of memory when the establishment of LTM is prevented by DAP) is about 210 min. The duration of MTM in the absence of DAP cannot be determined because its latter part is normally masked by 20 μl-resistant LTM which is established about 60 min after the end of training. Since there is no way at present to demonstrate overlap between MTM and LTM and so prove unequivocally that both traces coexist at some time after training, the possibility that the establishment of LTM in some way terminates MTM is not excluded.

It remains to be seen whether the MTM trace itself induces the synthesis of the RNA which is essential for the establishment of LTM (sequential model) or whether another parallel process which is also sensitive to a 20 μl injection is initiated by STM and leads to the necessary RNA synthesis.

The minimal time required to achieve 20 μl-stable LTM after training (in the absence of DAP) was 75 min, whilst the time required to achieve 20 μl-stable LTM following reversal of DAP action by adenosine (Fig. 3) was only 45 min, after the injection of adenosine. This 30 min discrepancy suggests that a sequence of two distinct processes is involved in LTM formation following training. The first process, lasting about 30 min is insensitive to DAP. The second process is sensitive to DAP and therefore probably involves specific RNA synthesis. It takes only 45 min from the moment the second process is released from DAP restraint to achieve LTM. MTM must however be present whilst the second process is active in order for a long-term memory trace to be established.

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