Physiol. Res. 51 (Suppl 1): S35-S47, 2002

Hippocampus-Dependent Retrieval and Hippocampus-Independent Extinction of Place Avoidance Navigation, and Stress-Induced Out-of-Context Activation of a Memory Revealed by Reversible Lesion Experiments in Rats

K. JEŽEK[1], M. WESIERSKA[2], A. A. FENTON[1,3]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Nencki Institute of Experimental Biology, Warsaw, Poland and [3]Department of Physiology and Pharmacology, State University of New York, Downstate Medical Center, New York, USA

Summary

The use of reversible lesion techniques in memory research was pioneered in the laboratory of Jan Bureš and Olga Burešova. We use the occasion of Jan's 75th birthday to briefly review the experimental utility of this approach. Two experiments from our current research are reported in which reversible lesioning methods are used to ask otherwise experimentally untenable questions about memory retrieval. The first experiment used intra-hippocampal injections of tetrodotoxin to temporarily inactivate the hippocampus during retrieval of a well-learned place avoidance navigation memory. This revealed that the hippocampus is necessary for place avoidance retrieval but that the extinction of place avoidance can occur independently of retrieving the memory and intact hippocampal function. The second experiment used KCl-induced cortical spreading depression in an interhippocampal transfer paradigm to demonstrate that a Y-maze memory that is learned by only one cortical hemisphere can be made to transfer to the other hemisphere by forcing the rat to swim, a unique stressful experience that occurred in a different apparatus, different behavioral context, and involved different behaviors than the Y-maze training. This demonstrates, we believe for the first time behaviorally, that memories can be activated outside of the behavioral context of their acquisition and expression in rats.

Introduction

Memories, once acquired may become stable through a process of consolidation and a good deal is known about the *neurobiology* underlying the acquisition and consolidation processes. But until a neural manifestation of the engram can be unambiguously identified the very existence of memory is only inferred when it is recalled and expressed by controlling behavior, a process called retrieval. The use of reversible lesions, pioneered in the Bureš laboratory[1], makes it possible to study the role of a brain region in a particular phase of the memory process without directly affecting the region's function in the rest of the memory process. For example, only with a post-training reversible lesion, is it possible to study the role of the hippocampus (Cimadevilla *et al*. 2001) or the basolateral amygdala (Vafaei *et al*. submitted) in the consolidation of a place avoidance memory (Bureš *et al*. 1997a, 1998) that is acquired and retrieved with a functional hippocampus.

This report will focus on the retrieval process asking questions that are only tractable using reversible lesioning techniques. In the first experiment, rats were trained in a place avoidance task that was designed to study open-field place navigation and the role of the hippocampus (Bureš *et al* 1997b). Reversible lesions

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

using tetrodotoxin (TTX) inactivations were used to study whether the hippocampus is necessary for retrieval of a place avoidance memory that was acquired by a functionally intact brain. Retrieval was tested in two ways, by giving the subjects an additional, reinforced training session as well as by giving the subjects an unreinforced extinction session. The results from both tests indicated that while the hippocampus is necessary for retrieving place avoidance memory, place avoidance behavior could be extinguished without a functional hippocampus and successful retrieval of the place avoidance memory.

The second experiment used cortical spreading depression (CSD) reversible lesions in an inter-hemispheric transfer of a lateralized memory paradigm that restricted learning of a Y-maze habit to one cortical hemisphere and then restricted retrieval of the memory to the other hemisphere. The memory content of the retrieving hemisphere was changed by inducing the Y-maze memory that was acquired with only one hemisphere to "transfer" to the other, untrained hemisphere. The transfer was induced by a stressful, forced swimming experience that occurred in a different place, a different apparatus, and thus a substantially different context from the Y-maze training. The interhemispheric transfer phenomenon directly demonstrates that retrieval alters the stored engram and that rats can activate a memory in an essentially different context than that of the learning. This "out-of-context activation of memory" (OCAM) is discussed as evidence that rats can recall memories for events in some respects like the autobiographical episodic recall of human experience.

Bureš and Burešová (1990) wrote a thorough review of the logic and application of reversible lesioning methods to which the reader is referred. This logic is taken as the departure point for the current studies.

Methods

Subjects

Male Long-Evans rats weighing between 300 and 450 grams were obtained from the Institute breeding colony. They were housed in groups of three or four at constant temperature (21 °C), and natural lighting. Food was rationed to keep the rats in Experiment 1 at 90 % of their free feeding weight. The rats in Experiment 2 were not food-deprived, and water was freely available to all animals. The treatment of the animals was approved by the Institute's Ethical Committee, and conformed to NIH guidelines and to the Animal Protection Law of the Czech Republic.

Surgical and reversible lesioning procedures

The surgical and inactivation procedures for TTX (Fenton and Bureš 1993, Cimadevilla *et al.* 2000) and CSD (Bureš *et al.* 1974) inactivation have been repeatedly described. The rats were anesthetized by thiopental (50 mg/kg).

For TTX inactivation, two stainless steel guide cannulae (22 gauge, 10mm long) were aimed with their tips 2 mm above the dorsal hippocampus (4 mm caudal from bregma and 2.7 mm lateral from the midline; Paxinos and Watson 1986). The cannulae were fixed to the skull using t-shaped bolts and dental acrylic. For place avoidance training, a 3 cm long, 0.5mm diameter stainless steel clip was used to make a low impedance connection through the skin on the back of the rat.

5ng of TTX in 1 µl of saline was injected into each hippocampus through the implanted cannulae. A 10 µl Hamilton syringe was attached to the injection needle (12 mm long, 30 gauge) via a short piece of polyethylene tubing. The 1 µl solution was delivered over 1 min, the injection needle was left in place for another 1 min before it was slowly withdrawn. All the animals received a habituating injection of TTX a week after surgery. The animals were injected then returned to their cages and observed for signs of physical impairment but none were observed.

In the second experiment, two trephine holes (4 mm diameter) were made over both fronto-parietal cortices without damaging the dura and each was fitted with an aluminium well (4 mm in diam., 5 mm high) that allowed free access to the dura in the course of the experiment. This assembly was fixed to the skull with dental acrylic. Both wells were filled by saline-soaked cotton and covered with a metal cap to protect the exposed dura from dessication.

The next day, the CSD was elicited (approximately 50 % of animals left, 50 % right in each group) by placing a filter paper soaked in 25 % KCl on the exposed dura above one hemicortex. Before and after the training, the effectiveness of CSD was judged by the unilateral cortical motor impairment in the placing reactions (Bureš *et al.* 1974; 1983). After the training session, the filter paper was removed and the dura was washed with saline and protected from dessication. The hemisphere in which CSD was elicited during Y-maze training will be refered to as the "untrained" side, and the

other, undepressed side will be referred to as the "trained" side.

Experiment 1a and 1b: Effects of hippocampal inactivation on the retrieval and extinction of place avoidance navigation.

Two experiments were performed using TTX inactivation to determine whether the hippocampus is necessary for the retrieval of a well-learned place avoidance memory. Rats were trained to avoid a place where they are shocked while foraging for scattered food in a circular arena. After the avoidance performance was asymptotic for several days, retrieval was tested during bilateral hippocampal inactivation by TTX injections into the dorsal hippocampi. In the first experiment, memory retrieval was tested by giving the rats an additional reinforced training session. In the second experiment retrieval was tested by giving the rats an unreinforced extinction session.

Place avoidance apparatus and position tracking.

The arena was an elevated (50 cm) 80 cm diameter circle covered by an electrically conductive mesh. It was centered in a 5 m X 4 m room with many visual landmarks. A feeder mounted 2 m above the arena dropped 20 mg pasta pellets to random places in the arena at 10 s intervals. A television camera mounted above the arena was used to record the position of the rat by tracking a LED that was held between the rat's shoulders by a latex harness. The television signal was analyzed by a custom spot-tracker in a PC. The rat's position was tracked with a spatial resolution of 0.4 cm and a temporal resolution of 100 ms.

Place avoidance training and analysis.

Over 3 days, the rats were trained in a daily 20 min session to forage continuously for the scattered food. At least a week after surgery, this training resumed for 3 days and then daily place avoidance training sessions began. A counter-balanced cable conducting current for the tracking LEDs and shock was attached to the rat. The strain from the cable was transmitted to the latex vest on the rat. The shock wire was clipped to the shock electrode pin with a miniature alligator clip. An unmarked "to-be-avoided" 60° sector of the arena was defined in one quadrant of the arena by the tracking system. During place avoidance training, as before, the rat was put on the arena to forage for 20 minutes but whenever it entered the to-be-avoided sector for more than 0.5 s, a 50 Hz current (<0.6 mA) was delivered for 0.5 s across the rat's paws and the arena floor. The shock was repeated after 3 s if the animal did not leave the prohibited area. The shock was intended only to be unpleasant, and the amplitude was adjusted for individual rats so that the rats escaped quickly and continued to forage over the unpunished surface of the arena.

Three parameters were used to assess place avoidance performance. Since a non-spatial, but effective avoidance can be achieved by immobility, the total distance moved during the session (D) was taken as a measure of locomotor activity. The number of times the rat entered the to-be-avoided area (N) measured the avoidance of the to-be-avoided place within the whole session. The time to first enter the to-be-avoided area (T) is also reported. This measures place avoidance memory retention from the previous session and is not affected by within-session learning. Of the three measures, only T is not influenced by the presence or absence of the shock reinforcement, and it was therefore used to compare performance on training and extinction sessions.

The effects of the TTX inactivation on memory retrieval were assessed by paired-t tests comparing performance in the session before the TTX inactivation with the performance during the inactivation. Repeated-measures ANOVAs followed when appropriate, by Newman-Keuls test were used to assess performance across 3 or more sessions. Means $\pm$ S.E.M. are reported and statistical significance was accepted for $p<0.05$.

Experiment 2. Swim-induced out-of-context activation of memory revealed by the interhippocampal transfer of a lateralized memory.

Y-maze apparatus.

A Y-shaped maze with 50 cm long, 12 cm wide and 25 cm high arms (120° angle between the arms) was used. It was placed on a 70 cm table. The floor was an electrifiable metal grid, which could deliver a foot-shock (0.5 mA, 50 Hz, 0.5 s) to the start and error arms of the maze. The goal arm was protected from shock. The goal position was set according to the experimental design (see below) and remained stable for each rat on each training day. The start arm was always the same.

Y-maze procedure and analysis

Figure 4 shows the experimental design. The typical experiment lasted 2 consecutive days. On Day 1, the rats were brought to the room in their home cages. The cages were placed in a different part of the room but at a similar height as the Y-maze. The animals remained

in the room until each animal in the cage was trained. One at a time, the animals were allowed to explore the maze for 10 min, without any shock. Then, if needed, unilateral CSD was elicited in some groups. After successful CSD hemidecortication was confirmed, the Y-maze training started. The rat was placed in the start arm and after 5 s, given a footshock every 3 s until it escaped to the goal arm. After approximately 30 s was spent in the goal arm, the animal was removed and placed at the start to begin the next trial. A correct response was recorded when the rat escaped directly from the start- to the goal-arm. When the animal entered the error arm by at least half a body length, an incorrect response was scored. The animal was always allowed to correct an error. If it did not escape within 60 s it was put in the goal arm by the experimenter and an incorrect response was scored.

Trials on Day 1 continued until the criterion of 9 correct responses in the last 10 trials (9/10) was met and the animal completed a further 30 trials.

On Day 2, 150 minutes before the training, the home cage containing 3 or 4 rats was brought to the experimental room and placed on in the same place as on the previous day. Rats from group Lat-Swim were given the unique, emotional experience of having to swim in partial darkness, without the possibility of escape for 20 min in a bucket of water (30 cm in diam., water 25 cm deep, 27 °C). The bucket was placed on the floor of the room and was covered so the Lat-Swim animals could not look around the room while they were in the bucket. After 20 min. had elapsed the rat was removed from the water, gently dried with paper towel and returned to its home cage. Rats in the Lat-NoSwim group received the identical treatment with the exception of being put in the bucket of water and being dried. All animals waited in the room until training 2 hours later. Unilateral CSD was elicited in the opposite hemicortex as on Day 1 and shortly afterwards the Day 2 training in the Y-maze started: the rats were trained in the same way as before, but the goal arm was now on the opposite side (i.e. in the previous day's error arm). When the rat reached the criterion of 3 correct responses in the last 3 trials the session was terminated. Since rats from both the Lat-Swim and Lat-NoSwim groups were housed in the same cage, and the bucket of water was in the same room as the Y-maze, all animals spent the same time with the experimenter in the experimental room regardless of whether or not they were given the swimming experience. A third group of animals (OnlySwim) were forced to swim 2 hours before the Day 1 training. This group was a control for the effects of the swim on Y-maze acquisition. They did not receive the Day 2 procedures.

The number of to-criterion errors on both days was analyzed. The scores are presented as the mean number of to-criterion errors ± S.E.M. The criterion for analysis was set at 3 correct responses out of 3 (3/3). Comparisons between two groups were based on a t-test and comparisons between more than two groups used a one-way ANOVA. A paired t-test was used for within group comparisons. The significance level was set at $p<0.05$.

Results

Experiment 1a. Effect of hippocampal inactivation on reinforced retrieval of place avoidance navigation.

Eight rats were trained over 9 days. On days 1-7 (Intact Training), nothing was injected and a daily place-avoidance session was given. On day 8 (TTX Training), both hippocampi were inactivated by TTX and one hour later, a place-avoidance session was given. On day 9 (Intact Extinction), nothing was injected and the rat was allowed to forage in the absence of shock.

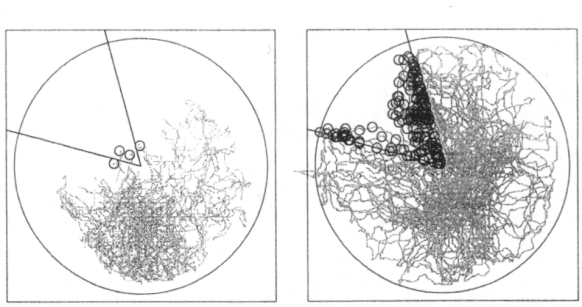

Fig. 1. *Expression of a well-learned place avoidance is good when the hippocampi are functionally intact (left). The tracks of this rat on the arena (large circle and through the room (square))shows it only entered the to-be-avoided sector in the North-East once and received one shock (small circle) after which the rat retreated from the sector. Note that whenever the rat approached the border of the to-be-avoided sector it retreated. In contrast, when the hippocampi were inactivated by TTX (right) the same rat entered the to-be-avoided sector more frequently and received many shocks. Notice the rat no longer retreated when it approached the to-be-avoided place nor did it escape away from the sector when it was shocked. Under TTX the rat seems so disoriented that it moves further within the to-be-avoided area when it is shocked.*

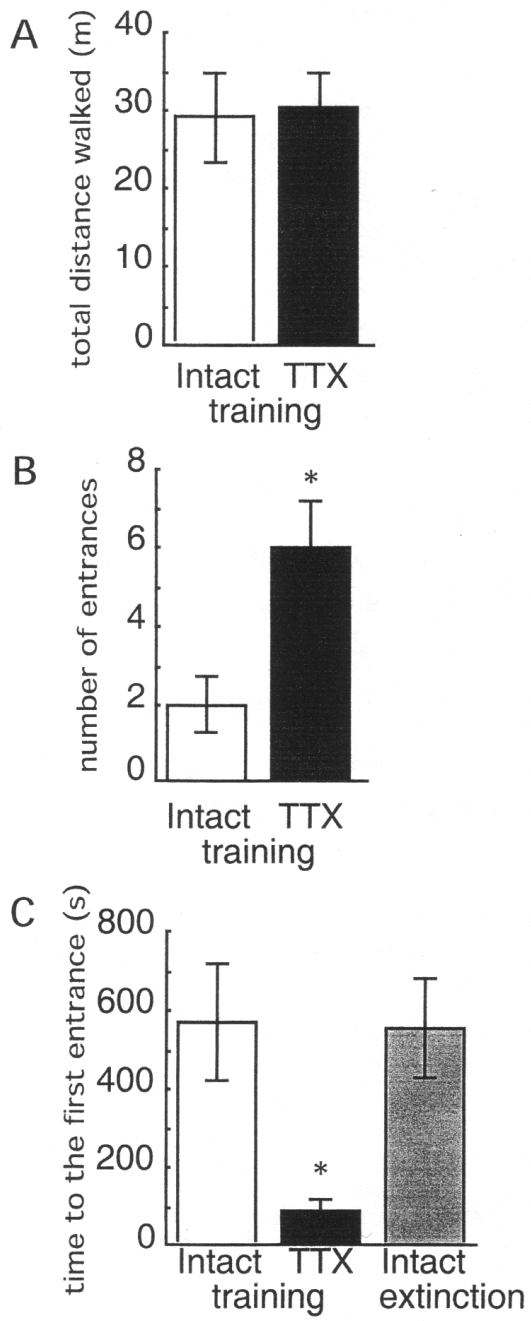

Fig. 2. TTX *inactivation of the dorsal hippocampi did not alter locomotion (A) but it impaired place avoidance performance in a training session by causing the rats to enter the to-be-avoided place more often (B) and sooner (C) than when the hippocampi were functionally intact. (C) After the TTX inactivation, place avoidance performance recovered to the pre-inactivation level (C). *p<0.05 relative to Intact-training.*

Figure 1 shows the tracks from a representative rat on the sessions before and during hippocampal inactivation. The rat shows a good avoidance of the to-be-avoided place before but not after the TTX was injected. The TTX did not change the amount the rats walked (Fig. 2A; t7=0.86, p=0.2). But the TTX injection impaired performance measured by N (Fig. 2B; t_7=3.1, p=0.02) and T (Fig. 2C; t_7=3.5, p=0.01).

The rats recovered from the TTX inactivation because performance on the extinction test the next day was good (Fig. 2C). The ANOVA on T + post-hoc testing confirmed that the performance after the TTX was at the same level as before the TTX injection ($F_{2,14}$=5.6; p=0.02) but better than during the inactivation. In summary, injecting TTX into the hippocampi impaired retrieval of a well learned place avoidance memory, but the disturbance was completely reversible since the next day, performance was at the same level as if the inactivation had never occurred.

Experiment 1b. Effect of hippocampal inactivation on unreinforced retrieval (extinction) of place avoidance navigation.

This experiment asked whether the place avoidance memory had to be retrieved in order for it to be extinguished. After learning the place avoidance task with functional hippocampi, the dorsal hippocampus was inactivated during an extinction session. The next day, after the hippocampi had recovered from the inactivation, retention of the avoidance memory was tested with the hippocami functionally intact. In the second extinction session, the rats should perform as if they did not have the first extinction session if the hippocampi are either necessary for learning that the shock was no longer present in the environment, or if the place avoidance memory must be retrieved for the avoidance behavior to be extinguished.

Nine rats were trained to their performance asymptote over 6 to 10 days. The next day, they were injected in each hippocampi with 5 ng TTX and 1 hour later given a 20-minute extinction session with no shock. The day after that, they were given a second extinction session (they were not injected). Only the time to enter the to-be-avoided place was used to compare place avoidance performance on the reinforced acquisition and the unreinforced extinction sessions. As in Experiment 1a, the robust avoidance during the intact acquisition session was disrupted by the TTX injection, however unlike in Experiment 1a, performance did not return to the pre-injection level in the second extinction session (Fig. 3; $F_{2,16}$=3.6, p=0.05). Despite the rats inability to retrieve the place avoidance memory during TTX inactivation, the avoidance behavior was extinguished by

the extinction session under TTX inactivation of the hippocampi.

Experiment 1 discussion.

Experiments 1a and b show that a functionally intact hippocampus is necessary for the retrieval of a well-learned place avoidance memory but that neither the successful retrieval of the memory, nor the functional integrity of the hippocampus is required for the extinction of the avoidance behavior.

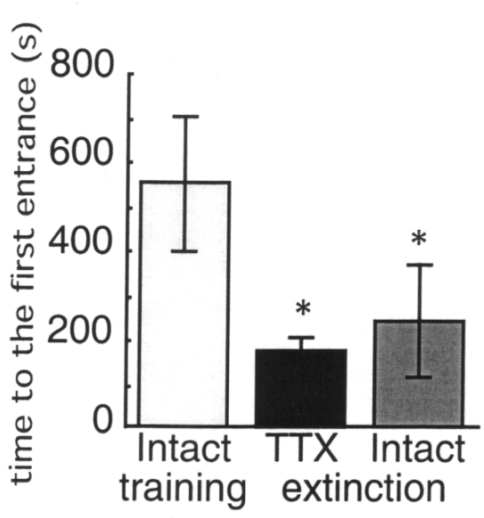

Fig. 3. *TTX inactivation of the dorsal hippocampus impaired retrieval of place avoidance on the extinction retrieval test. However, in contrast to the post-inactivation effect of the inactivation during training (Fig. 2C), the hippocampal inactivation did not prevent place avoidance from being extinguished during the extinction session. *p<0.05 relative to Intact performance.*

It is not surprising that the hippocampus is necessary for retrieval of a place avoidance memory. The task was designed to be sensitive to hippocampal damage, and like a similar allothetic active avoidance task (Cimadevilla *et al*. 2000) it requires intact spatial cognition. There was also no evidence of within session learning during the hippocampal inactivations of the present experiments even though the rats could have adopted a non-spatial passive avoidance strategy of not moving to avoid the punishing shocks. Their locomotion was however unchanged by the hippocampal inactivation. Presumably, for hungry rats, the merits of the passive avoidance strategy were outweighed by the benefits of foraging.

Rats, trained in the identical conditions of the present experiments, were shown to form separate place avoidance memories that are defined in the dissociable spatial coordinate frames of the room and the arena (Fenton *et al*. 1998). The memories can be acquired, expressed, and extinguished independently (Bureš *et al*. 1997a, 1998). The room frame avoidance depends on allothesis, navigation in this case, according to distant room frame information. The arena frame memory depends, at least in part, on idiothesis, navigation by internal self-motion information supported by occasional reference to stable cues on the arena (Mittelstaedt and Mittelstaedt 1980; reviewed by Gallistel 1990; see Fenton and Bureš 2000). Allothesis has repeatedly been shown to depend on a functional hippocampus (Jarrard 1980, Morris *et al*. 1982, Sutherland *et al*. 1982, Fenton *et al*. 1994) whereas it is unclear whether idiothesis depends on an intact hippocampus (Alyan and McNaughton 1999, Whishaw and Jarrard 1996). Since hippocampal inactivation abolished any place avoidance behavior, these data support the view that idiothesis supported by allothetic updating is hippocampus-dependent.

Expression of the place avoidance memory was not necessary for the extinction of the place avoidance behavior. Since place avoidance memory is hippocampus-dependent and the hippocampus was inactivated, this demonstrates that place avoidance memory need not be activated for its expression to be extinguished. This adds to the body of evidence that extinction does not require the direct modification of an engram. Rather, extinction is an indication that a novel, separate memory has been learned and this extinction memory competes with the initial reinforced memory for control of the subject's behavior (Prado-Alcalá *et al*. 1994, Quirk *et al*. 2000).

Experiment 2. Swim-induced out-of-context activation of memory revealed by the interhippocampal transfer of a lateralized memory.

The brain is an anatomically bilaterally symmetric organ, and especially in rodents, there appears to be little hemispheric specialization (For review Bureš *et al*. 1988). Nonetheless, experimental methods can cause some memories to form in only one hemisphere, a process called "lateralization". The most direct way to lateralize a memory is to lesion the other hemisphere during learning. When the lateralizing lesion is a reversible lesion, it is commonly observed that the memory remains lateralized even after the inactivating effect has terminated. Whether or not the memory has

remained lateralized can be verified in a retrieval test that is given after switching the side that is inactivated so that the "trained" side, the hemisphere that was functional during lateralized training is now inactive and the "untrained" side, the hemisphere that was inactive during the lateralized training is now active (see Fig. 4). Under these conditions, if the subject cannot perform the task, the memory is said to be lateralized; if performance is intact, the lateralization procedure must have either failed or the memory must have spontaneously spread to the untrained hemisphere during the post-training period when both hemispheres were functional.

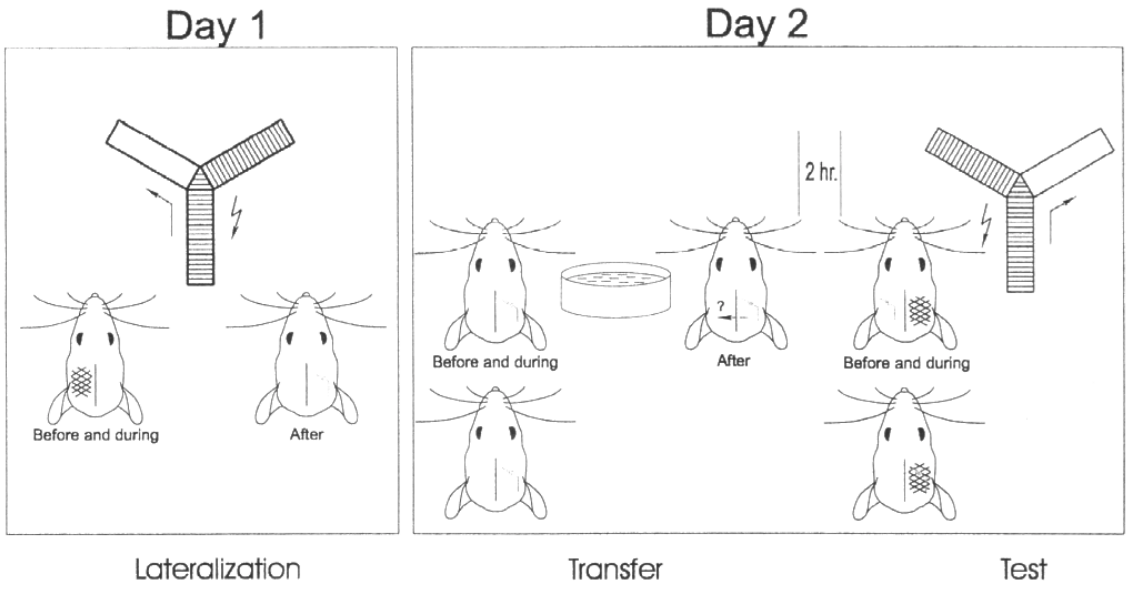

Fig. 4. *Design of Experiment 2: On Day 1 both groups formed the lateralized memory engram. On Day 2, the Lat-Swim group (top) was given a 20 min forced swim, whereas the Lat-NoSwim group was not (bottom). Two hours later the side of inactivation was switched to verify the lateralization. The originally trained hemisphere was blocked and the opposite habit was learned under inactivation of the trained hemisphere.*

Typically, in lateralization experiments, the cortically lateralized engram does not spontaneously spread to the untrained hemisphere; it remains lateralized, or in plain words, it remains dependent on the trained hemisphere for it's expression. In many experiments with rats, Bureš, Burešová and their colleagues (see Bureš *et al*. 1974; 1988) exploited lateralized memories to describe under what conditions memories could be spread and even combined. A basic result was that in order for a lateralized cortical memory to spread to the untrained hemisphere, a process called "interhemispheric transfer" or "transfer" for short. The lateralized memory had to be activated while the untrained hemisphere was functional. Activating the memory required returning the subject to the testing apparatus for one or a few training trials (which itself is not able to form a substantial new memory). These trials are called the "transfer experience" and whether they could induce the memory transfer is verified by switching the side of the inactivation and testing performance with the trained hemisphere inactivated and the untrained hemisphere functional.

A particularly interesting transfer experience is to return the rat to the testing apparatus and give it a brief, unreinforced extinction test. When this transfer experience induces memory transfer it is especially convincing that memory activation (required for retrieval during the extinction experience) causes the lateralized memory to transfer to the untrained hemisphere, because the extinction experience cannot reinforce the conditioned behavior. On the contrary, unreinforced extinction should weaken the expression of a conditioned behavior.

Since memory activation is required for transfer, and transfer experiences do not seem to occur spontaneously (the rat must be returned to the testing environment) this is taken as strong evidence that rats do not have spontaneous rehearsal of their experiences, which is to say they do not spontaneously reactivate, or replay their memories. In contrast, these are common characteristics of human memory. People typically remember their experiences; remembering is often likened to "reliving" an experience and it does not require

explicit or direct external prompting by returning to the context of the stored experience. This is especially true for dreaming.

Although the lateralization and subsequent transfer of memory is an experimentally induced phenomenon that may not occur naturally, it has the potential to unveil some of the properties of memory that would otherwise not be observable. Once characterized one may then seek to find ways to identify the discovered properties in natural phenomena. In this quest, we have used an interhemispheric transfer paradigm to explore whether rats can be induced to activate a specific memory without being returned to the experimental apparatus, and without being made to execute any of the behaviors that were associated with acquiring the memory. The paradigm is derived from a classic interhemispheric transfer study by Goldowitz *et al.* (1972), which shows that a stressful, unique experience that is contextually unrelated to Y-maze learning has the ability to organize the relative strengths of two separately lateralized Y-maze memories. We adapted the paradigm to ask if a lateralized Y-maze memory can be induced to transfer by a forced swim (a stressful, unique experience) that is unrelated to the Y-maze learning. If the swim is a transfer-inducing experience it would indicate that the lateralized Y-maze memory was activated outside of the learning context, and this demonstration would pave the way for developing a paradigm to systematically study whether the swim-induces the Y-maze memory to be replayed, and even possibly remembered by the rat.

Experiment 2. Does a stressful swimming experience induce interhemispheric transfer of a lateralized Y-maze memory?

The Day 1 training under unilateral functional decortication caused a Y-maze memory to form in the trained side and the lateralized engram did not spontaneously spread to the untrained hemisphere. This was tested on Day 2 by switching the CSD inactivation to the trained side during acquisition of the opposite habit. There was no difference in the number of to-criterion errors on Days 1 and 2 (Lat-NoSwim: Day 1=0.7±0.33, Day 2=0.9±0.35, t_9=0.45, p=0.7; Fig. 5A). Since Y-maze performance on Day 2 was the same as when the rat was naive, this verified that the Day 1 Y-maze memory was lateralized. If the Day 1 memory had not been lateralized, Day 2 performance would indicate there was a reversal effect (e.g. turn left on Day 1; then turn right on Day 2) by an increase in the Day 2 errors. This is exactly what was observed in the Lat-Swim group (Fig. 5A). The forced swim two hours before Day 2 training increased the Day 2 error score of the Lat-Swim group relative to Day 1 (Day 1=0.88±0.19, Day 2=2.3±0.40, t_{24}=3.60, p=0.001) and relative to the Lat-NoSwim group (t_{33}=2.11, p=0.04). This suggests the forced swim caused the Day 1 engram to transfer to the untrained side and this caused the reversal effect in Day 2 performance.

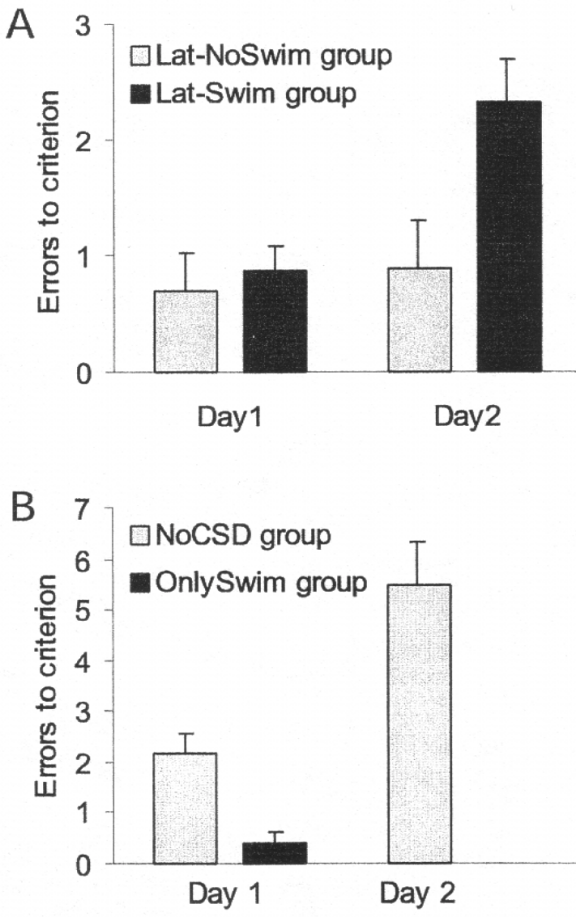

Fig. 5. *The number of to-criterion errors during acquisition on both days. A) the swim experience increased the error score on Day 2. B) the swimming on Day 1 did not itself cause the learning impairment (Only-Swim group). The reversal effect in animals without a lateralized Day 1 engram (NoCSD group). *paired t-test p<0.05 relative to Day 1 score, p<0.05 relative to Day 2 score in Lat-NoSwim group.*

An alternative explanation is that the swimming-induced increase in Day 2 errors is due to a non-mnemonic effect of swimming such as tiredness or hypothermia. But this explanation is rejected since in the OnlySwim group, swimming before training had no effect (Day 1=0.4±0.22) compared to the Day 1 performance of the Lat-Swim and Lat-NoSwim groups

(one-way ANOVA, $F_{2,42}$=0.94, p=0.40; Fig. 5B). Thus, the data indicate that the swimming experience caused the Day 1 engram to transfer to the untrained side and this was manifest as the reversal effect.

Another group of rats was trained according to the same Day 1 and Day 2 schedule but, no CSD was elicited (and no swim) in order to compare with the magnitude of the transfer-induced reversal effect. As expected, the Day 2 error score was significantly higher than on Day 1 (Day 1=2.2±0.31, Day 2=5.5±0.85, t_5=4.56, p<0.001; Fig. 5B). This increase in errors expresses the Y-maze reversal effect that occurs naturally. It was greater than the transfer-induced reversal that was observed in the group Lat-Swim (t_{29}=3.48, p=0.02).

Experiment 2 discussion.

Typically, when a subject returns to the original learning context, memories that were learned there are activated. For a certain period of time, referred to as the reconsolidation period (Spear and Mueller 1984), such a "reactivated" engram becomes labile and sensitive to interference (Przybyslawski and Sara 1997). Several studies have shown, that presenting contextual reminder cues can strengthen the memory that is subsequently expressed, compared with non-reminded rats, which in contrast show forgetting. This is called the contextual cue reminder effect and it is believed that contextual reminding serves as an additional training trial, which refreshes the memory trace (Sara 2000).

In the current experiment, a stressful, unique experience with no direct relation to the original learning, caused an analogous increase in memory expression. The lateralized memory which does not spontaneously spread to the untrained side until it is retrieved, after the forced swim induced a reversal effect in the untrained hemisphere during learning the opposite Y-maze habit. Without the swim, learning the opposite habit was as if the animals were naive. The latter confirmed that the Day 1 memory was lateralized because learning the opposite habit under the same conditions but without CSD also caused a reversal effect, i.e. an increase in to-criterion errors. The Day 2 learning impairment in the Lat-Swim group therefore was caused by the swimming experience. Hypothermia interferes with learning and memory and fatigue with its expression, but we excluded the possibility that the effect of swim on the subsequent acquisition could be caused only by such non-mnemonic factors. The OnlySwim group, which had received the identical swimming experience before training was not impaired. This demonstrates, that a stressful event with both hemispheres intact activated the lateralized memory, and the activation induced the memory to transfer to the untrained side. The next day's learning with the trained side inactivated therefore occurred with the untrained side not being naïve to the Y-maze and the reversal effect happened. We call this swim-induced phenomenon out-of-context activation of memory (OCAM).

We point out that in the NoCSD group, although the Day 2 errors were nearly two times higher, than what was caused by OCAM, the magnitude of the reversal effect reflecting the differences between Day 1 and 2 are similar (see Fig. 5). The fewer errors during the lateralized learning occurs because that rats tend to escape to the depressed side due to a sensory contralateral sensory neglect.

Temporary unilateral inactivation of the brain and the subsequent lateralization of memory does not normally occur. Some forms of epileptic seizures can remain localized and thus cause a temporary functional blockade of some cortical and limbic areas, but given that these are pathological states, it is not currently known if OCAM can be demonstrated for a non-lateralized memory in an intact brain.

General Discussion

. The two experiments have some common features. First, the expression of a memory changed after an experience in which the memory was not expressed behaviorally. In Experiment 1, the retrieval of a well-learned place avoidance was blocked by bilateral hippocampal inactivation. However, as seen in the second extinction test in Experiment 1b, the avoidance was modified after experiencing that the shock was absent during the first extinction session, we emphasize, even though the memory was not expressed at this time.

This is interesting from the point of view of lateralized spatial memory paradigms. It has been repeatedly shown that learning under unilateral hippocampal blockade is robust and leads to the formation of a spatial memory trace (the location of the platform in the water maze) in the trained side, whereas when the animals are tested with the trained side inactivated, they manifest no retrieval (Fenton and Bureš 1993, 1994).

The unilateral acquisition does not differ in its rate from intact learning. This allowed Fenton *et al.* (1995) to train rats to learn two different lateralized memories for two different target locations in the water

maze by using repetitive alternating inactivations of one and then the other hippocampus during training. This is particularly interesting from the point of view of Experiment 1. The absence of shock during bilateral hippocampal blockade leads to the extinction of a well-formed hippocampus-dependent memory. In contrast, in the water maze experiment, the unilateral acquisition of target location A during hippocampal inactivation of the other hippocampus (which contained the memory for location B), did not lead to any interference due to the reversal of the goal locations. The lateralized memories were not weakened and there was no reversal effect although different places were reinforced. Thus it seems that when the retrieval of the memory is blocked by hippocampal inactivation and the animal is given an extinction trial, the subsequently expressed conditioned behavior is weaker, whereas when the animal is given a session with reinforcement whether in the correct (Exp. 1a) or in a different place (Fenton *et al.* 1995), the subsequently expressed conditioned behavior is not weakened. A parsimonious explanation is that the extinction learning is hippocampus-independent and induces the formation of a memory that the reinforcement is no longer present. The performance on the next day with both hippocampi recovered is then a result of the competition between the two engrams, one coding for the reinforced behavior, the other more recent memory coding that there is no longer reinforcement present. The trace representing the extinction situation is the more recent memory and thus dominates in the subsequent session (Goldowitz *et al.* 1972). Similarly, the reinforcement in the same or in a different location than the one which is coded by the blocked engram can maintain the hippocampus-independent engram coding that there is reinforcement in the environment. During the retrieval with both hippocampi the two engrams both lead to the reinforced behavior.

Rather than forming a new memory, another explanation for extinction is that the original engram itself is modified. Spatial learning is a complex process dependent on different structures (Whishaw 1998). and the corresponding memory has a complex character with several components where only some of them are hippocampus-dependent (Packard and McGaugh 1992). It is possible that during the hippocampal blockade, those which are not hippocampus-dependent can be retrieved and this activation can allow their modification. The current data do not support this view since the behavior under hippocampal inactivation did not manifest even partial retrieval in both the reinforcement (Exp. 1a) and extinction (Exp. 1b) protocols.

Accepting the possibility that the memory trace had been retrieved and therefore transfered during the swimming experience, we can speculate about how this was related to the original learning context. The acquisition took place a day before in the Y-maze apparatus using electric foot-shock motivation. Those qualities we consider as parts of a learning context, and as a reminder their presentation can strenghten the memory. Then, there is a variety of stimuli (e.g. the experimenter, the transport from the vivarium to the experimental room, the experimental room itself, the aversiveness of the stimulus, etc.), which are not directly contextually connected, but can lead to stimulus generalization and thus to be associated with the acquisition experience. Their presentation itself, as we have seen in our Lat-NoSwim group, however, is not sufficient to induce the engram transfer and thus, we have no evidence for its activation.

The swimming experience induces a stress reaction that releases a cascade of stress hormones, which interfere with mechanisms of learning and memory (for review Cahill and McGaugh 1998). The administration of adrenaline or corticosterone after an acquisition trial leads to a dose dependent memory enhancement. From this point of view, an alternative explanation is that the arousal caused by the forced swim made the animals more sensitive to the recognition of features of the learning context such as the experimental room and the experimenter. However, this itself did not activate the trace in a way that induced the transfer. Since memory recall is usually elicited by a triggering experience, such as a direct query, or a recollection or experience of some feature of the memory, it is possible that during arousal, the animals from the Lat-Swim group are more attentive to such triggering cues and this elicits the reminder effect, which causes the transfer to the untrained hemisphere. We are in the process of trying to identify what triggering features could explain why the Y-maze memory was activated by the forced-swim.

The importance of Exp. 2 Iies in the experimental evidence that rats can activate the memory trace independently of the experimental environment where it had been acquired. As presented here, this phenomenon has been shown behaviorally. An analogous process (memory reactivation outside the learning context) in rats has been described by electrophysiological means during sleep (Wilson and McNaughton 1994). Skaggs and McNaughton (1996) and

Louie and Wilson (2001) have shown that the place cell activity patterns expressed during exploration in an environment reoccur in the subsequent periods of slow wave- (SWS) and rapid eye movement (REM) sleep, respectively. Such "replay" during SWS and REM can provide a powerful way to stabilize the memory trace (Bliss and Collingridge 1993, Buzsaki 1989) and is a natural physiological process, which does not need to be unique to spatial memory. However, there it is a logical difficulty in connecting the place cell replay during sleep and the data from memory lateralization experiments. On one hand, there is a memory replay during sleep, on the other hand, there is no spontaneous transfer of a lateralized engram and only its activation can induce the transfer. Given that, it is apparent, that the sleep replay itself is not sufficient to induce the memory transfer (otherwise the memory would be transferred after post-acquisition sleep). Thus place cell sleep replay and OCAM represent distinct types of memory reactivations that are contextually independent of the acquisition. In neither case do we know whether those memories were consciously retrieved or not, but they may be indicative of a phenomenon which is a characteristic for human episodic memory- the ability to retrieve information independently of the initial learning context.

Though reversible brain lesions do not represent a natural condition, they provide a powerful tool for exploring memory processes in the central nervous system. None of the experiments described above could have be done without the possibility to selectively inactivate a specific brain structure for a limited time with a subsequent full recovery.

Appendix

[1] Jan Bureš' science is notable for his uniquely creative experimentation, his ability to inspire, and to develop lasting collaborations. This strikes anyone who visits his multifaceted, internationally populated laboratory. In 1991 one of us (AF) had just completed an undergraduate degree in biology at McGill in Canada, and when he wandered into the Bureš laboratory virtually ignorant of both Jan and neuroscience, Jan's bold curiosity and complementing creativity was intoxicating. A naive understanding of cognitive mapping from O'Keefe and Nadel 1978 led AF to choose "teach rats to read a map" from Jan's long list of possible experiments. Though utterly unsuccessful, the belief that one could understand the mechanisms of cognition through experimentation was irreversibly conditioned in nine months of innumerable tries at remedial rodent map reading. MW was already a PhD in neurophysiology from the Nencki Institute of Experimental Biology, in Poland when in 1986 she started her collaboration with the Bureš and Burešova lab. In 1997 Jan convinced AF to return to Prague to run the lab. That summer, during the first of AF's regular commutes between New York and Prague, the place avoidance navigation paradigm (Bureš *et al*. 1997) was developed and his collaboration with MW began. MW's now visits Prague in the summer and then in the winter to investigate the neural bases of place avoidance navigation. KJ came to the Prague lab from the Charles University, Czech Republic during 1999, the third year of his PhD training. Jan proposed that AF offer him a one year project which turned out to be "test if rats put memories into time order". That experiment was based on an interhemispheric transfer of a lateralized memory study (Goldowitz *et al.* 1972) but it was utterly unsuccessful because the swimming procedure revealed the interesting phenomenon that rats can activate memories outside of the context in which they are learned. KJ has now joined the lab and investigates episodic memory phenomena in rats.

Jan Bureš' leadership is powerful and his leadership technique is almost imperceptible. His humility is dominating and since it leaves little opportunity for expressing gratitude, we take this opportunity to express ours. Thank you Dr. Bureš for creating in the Laboratory of Neurophysiology of Memory an oasis of optimism, nourished by an atmosphere of excited creativity and excellence. Thank you for teaching innovation by your unrelenting effort to build new experimental tools. We admire your energy, cherish your humanity and are eager to continue the passionate arguments and the scheming to trick nature into revealing the workings of the brain.

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Reprint requests

Dr. André Fenton, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic. Fax: +420 2 4106 24 88. email: fenton@biomed.cas.cz