# Electroconvulsive shock and lidocaine reveal rapid consolidation of spatial working memory in the water maze

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ABSTRACT Head trauma leading to concussion and electroconvulsive shock (ECS) in humans causes amnesia for events that occurred shortly before the injury (retrograde amnesia). The present experiment investigated the amnesic effect of lidocaine and ECS in 25 rats trained on a working memory version of the Morris water task. Each day, the escape platform was moved to a new location; learning was evidenced by a decrease in the latency to find the platform from the first to the second trial. "Consolidation" of this newly encoded spatial engram was disrupted by bilateral inactivation of the dorsal hippocampus with 1  $\mu$ l of 4% lidocaine applied as soon as possible after the first trial. When trial 2 was given after recovery from the lidocaine (30 min after the injection), a normal decrease in latency indicated that the new engram was not disrupted. When trial 2 was given under the influence of lidocaine (5 min after injection), absence of latency decrease demonstrated both the success of the inactivation and the importance of hippocampus for the task. To examine the role of events immediately after learning, ECS (30 or 100 mA, 50 Hz, 1.2 sec) was applied 0 sec to 45 sec after a single escape to the new platform location. A 2-h delay between ECS and trial 2 allowed the effects of ECS to dissipate. ECS applied 45 sec or 30 sec after trial 1 caused no retrograde amnesia: escape latencies on trial 2 were the same as in control rats. However, ECS applied 0 sec or 15 sec after trial 1 induced clear retrograde amnesia: escape latencies on trial 2 were no shorter than on trial 1. It is concluded that the consolidation of a newly formed memory for spatial location can only be disrupted by ECS within 30 sec after learning.

A period of amnesia for new events (anterograde amnesia) often follows severe head trauma, suggesting that at least some forms of information cannot be encoded until the brain recovers from injury. Loss of memory for events which precede a trauma (retrograde amnesia) has also been reported (1). The fact that memory for recent events appears to be more vulnerable than older memories (2-4) suggests that "consolidation" processes occurring after learning are essential to the stabilization of memory. While earlier work focused on a short-term "memory consolidation" process, typically lasting seconds, minutes, or hours, more recent work (5) has emphasized a longer-term process involving interactions between memory traces stored in several brain regions. In patients with damage to the medial temporal lobe, retrograde amnesia can be observed for periods lasting up to 15 years. These two aspects of consolidation-a short-term process and a much longer-term process-must be carefully separated and analyzed. The present report focuses on short-term consolidation processes; a report on long-term mechanisms is under preparation.

In the clinic (2), 20% of concussion cases suffer a memory loss for up to several days, 70% lose memories for <30 min

preceding the accident, and 10% report no amnesia at all. In a more naturalistic study, retrograde amnesia was assessed by interviewing football players within 30 sec after a concussion. They were able to remember details of the game, and what they were doing shortly before they got injured, but 5 min later the injured players could no longer recall such details (3). This confirmed the clinical observations and was interpreted as a failure of short-term memories getting stored or strengthened into long-term memory.

Electroconvulsive shock (ECS) has been used to simulate the amnesic effects of concussion. In animal studies [for example, using one trial inhibitory avoidance in the rat (6)] ECS has a graded amnesic effect: more severe for short delays between the first learning trial and the ECS. Delays up to 1 min show amnesic effects, and this time can be increased with higher levels of ECS current. In the present experiment we tested whether the rapid consolidation of newly acquired spatial memories could be disrupted by direct interference with the hippocampus, which is known to be essential for spatial learning (7, 8). In order to do so, we trained rats on a working memory version of the Morris water task (9), which can be acquired in only one trial. The rats must search for a submerged platform, the location of which varies from day to day. Even with delays of up to several hours between trials rats still show savings from the previous trial (9). By introducing a disruptive procedure after trial 1, and then waiting until the anterograde effects of the procedure dissipate before performing the second trial, we could test whether the engram formed during the first trial was consolidated or not. A consolidated engram would result in decreased latencies on the second trial, whereas a disrupted consolidation process would result in latencies on the second trial that were no shorter than those observed on the first.

In the first study we directly blocked the hippocampus with lidocaine, which is known to produce a retrieval deficit in another version of this task (10), as soon as possible after the first learning trial in the new location. In the second study we used ECS, a disrupting procedure which could be administered more quickly. Delays of 0-45 sec between the first trial and the ECS were used, and the strength of the engram was always tested 2 h after the administration of the ECS when the rats were found to be completely recovered from any retrieval impairments or anterograde deficits (11).

## MATERIALS AND METHODS

**Subjects.** Twenty-five male Long–Evans rats, weighing 250–270 g at the start of the experiment, were used. The rats were obtained from the Institute's breeding facility. Rats were grouped by five in large cages and had free access to food and water throughout the experiment. Each rat was handled for 3 days before pretraining. Eight rats were assigned to the group receiving lidocaine, eight rats were assigned to the group

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Abbreviation: ECS, electroconvulsive shock. <sup>‡</sup>To whom reprint requests should be addressed.

receiving 100 mA ECS (trial 1, ECS delay of 30 and 45 sec), and nine rats were assigned to the group receiving 30 mA ECS (trial 1, ECS delay of 0 and 15 sec). One rat from the lidocaine group was lost before the end of the study, so the data for the effects of lidocaine reflect information for only seven rats.

Behavioral Testing. Rats were pretrained in a reference memory version of the Morris water task (12) for 2 days before being switched to a working memory procedure. They were placed in a water tank and had to find a hidden platform which allowed them to escape from the water. In the reference memory version of the task, each rat received 12 trials a day for 2 days with the escape platform at the same location. In the working memory version of the task, the platform was put in a different location at the beginning of each day, and rats received eight trials at that location. Rats were trained on this task until their escape latencies dropped to 4-7 sec over the first four trials. In the lidocaine group this required training for 14 days over a period of 7 weeks, and in the ECS groups it required training for 9 days over a period of 3 weeks. A 2-h delay was imposed between trial 1 and trial 2 except when ECS was given at 0 or 15 sec; in these cases the delay was only used on the last 3 days of pretraining. Each rat participated in the various conditions of the working memory experiment and served as its own control.

## **Experiment 1: Lidocaine**

Surgery and Injection. The surgical procedure has been described in detail elsewhere (10). After rats were anesthetized with 50 mg/kg pentobarbital, their heads were shaven and cleaned with iodine before an incision was made allowing holes to be drilled in the skull. Ten-millimeter-long guide cannulae were implanted bilaterally into the dorsal hippocampus at the following coordinates: AP, 3.0 mm; ML,  $\pm 2.5$  mm; DV, 2.0 mm from the surface of the skull according to the atlas (13). Four anchoring screws were attached to the skull, after which dental acrylate was applied to hold the cannulae in place. An injection needle could be placed inside and protruded 1.5 mm beyond the tip of the guide cannulae in order to reach dorsal hippocampus. One microliter of a 4% lidocaine solution was delivered in 1 min in each hemisphere so that the injection procedure was completed in 2 min.

Experimental Design. We attempted to disrupt the newly encoded spatial engram by inactivating the dorsal hippocampus bilaterally as soon as possible after the first trial. The injection procedure started 1 min after the trial and lasted 2 min; thus, we assume that the dorsal hippocampus was inactivated after 3 min. Half of the rats were assigned to the experimental group on a given day, while the other half served as controls. The next day the assignment was reversed. The rats were tested on trial 2, after recovery from lidocaine (30 min after the injections were completed for a given rat), to test whether the drug disrupted consolidation of the engram. To control for the effectiveness of the injections two other conditions were run. In the first, rats were injected with lidocaine after trial 1 and were tested on trial 2 during the peak effectiveness of the lidocaine (8 min after trial 1). In the second, rats were injected with lidocaine before trial 1. In both of these cases trials 3 and 4 followed immediately after trial 2.

#### **Results and Conclusion**

**Histology.** The injection cannulae were located above the hippocampus for all the rats (Fig. 1). One rat had bilateral damage to the CA1 layer of about 1 mm of dorsal hippocampus: the exact placement of its injection cannula was omitted from the figure. Another rat had hematomas bilaterally. This was probably caused by mechanical damage during the injection procedure. The behavioral analysis showed that these rats performed at optimal levels in the control situations and were



FIG. 1. Location of the tip of the injection needles which were placed bilaterally in dorsal hippocampus. Plates from the atlas by Fifkova and Marsala (13). AD, antero-dorsal nucleus; CC, corpus callosum; FF, fimbria fornix; GL, corpus geniculatum laterale; HIP, hippocampus; HL, nucleus habenulae lateralis; HM, nucleus habenul ae medialis; LA, nucleus lateralis anterior; PVA, nucleus paraventricularis anterior; PVP, nucleus paraventricularis posterior; S, stria medullaris; ST, stria terminalis.

sensitive to lidocaine inactivation; therefore, their data were used in the analysis. Three other rats showed slight unilateral damage to the CA1 layer of the hippocampus; their data were also used in the analysis.

Behavioral Analysis. When lidocaine was applied immediately after the first trial in the working memory version of the Morris water task, and a second trial given after a 30-min delay to allow for the dissipation of the lidocaine effect, latency to find the platform did not differ from control values (Fig. 2). Therefore, lidocaine inactivation of the hippocampus 3 min after training did not disrupt consolidation of the engram formed on the first trial. In contrast, when the second trial was given after only a 5-min delay, during the peak effectiveness of the lidocaine, latencies were increased relative to control performance (Fig. 3) on trial 2 [t(7) = 1.89, P < 0.05]. However, these rats did decrease their latencies to the level of the control animals on trials 3 and 4. When lidocaine was injected before the first trial, acquisition across the trials was significantly impaired (Fig. 4) relative to control [t(27) = 1.70,P < 0.005].

## **Experiment 2: ECS**

Two thin metal plates (4  $\times$  4 mm), joined together with a spring, served as the ECS electrodes. These were placed on each rat's ears smeared with a drop of saline, and a single shock (100 mA for the application of the ECS at delays of 30 sec and 45 sec, and 30 mA for 0 and 15-sec delays) was applied for a



FIG. 2. Effects of lidocaine on consolidation of the spatial memory engram when applied immediately after a single trial in the working memory version of the Morris water task. The second trial was given after a 30-min delay (shaded area) which allowed elimination of the lidocaine effect. Trial 2 escape latencies did not differ from controls, showing that the consolidation process was not disrupted. Vertical bars denote SEM values. \*, Significantly different from control (P < 0.05).

duration of 1.2 sec. ECS elicited tonic clonic seizures. If a rat did not show convulsions from the ECS, it was excluded from the analysis. The shock was applied either 0, 15, 30, or 45 sec after the rat reached the platform. Each rat participated once at trial 1: ECS delays of 15, 30, and 45 sec, and twice at the delay of 0 s. For the 0- and 15-sec latencies, the electrodes were in place while the rats swam the first trial so that the shock could be delivered while the rats were on the platform. For the 30- and 45-s latencies, the electrodes were placed on the rats' ears after the animals had been removed from the pool. The



FIG. 3. Effects of lidocaine on retrieval of the spatial memory engram when applied immediately after a single trial in the working memory version of the Morris water task. The second trial was given after a 5-min delay which allowed the lidocaine solution to be at its peak effectiveness. Shaded area indicates trials during which the lidocaine was effective. Vertical bars denote SEM values. \*, Escape latencies measured on the second trial are significantly different from control (P < 0.05).



FIG. 4. Effects of lidocaine on acquisition of the working memory version of the Morris water task. Lidocaine was administered before the first trial. Shaded area indicates that the lidocaine was effective, without interruption, during all four trials. Vertical bars denote SEM values. \*, Escape latencies on the second, third and fourth trials are significantly different from control (P < 0.05).

rats were tested on trial 2, after the recovery from ECS (2 h after its application).

#### **Results and Conclusion**

There was an improvement in the latencies between trial 1 and trial 2 when ECS was applied 30 or 45 sec after the first trial and trial 2 was given 2 h later. When ECS was applied 0 or 15 sec after trial 1, there was a significant impairment in performance on trial 2. Fig. 5 shows that compared with the first trial, escape latencies under all control conditions dropped significantly on the second trial, 2 h later. ECS applied 45 sec after the first trial had no amnesic effects because escape latencies also decreased between the first and second trial [t(7) = 2.36],



FIG. 5. Effects of ECS on consolidation of the spatial memory engram when applied at various delays (0–45 sec) after a single trial in the working memory version of the Morris water task. The second trial was given after a delay of 2 h which allowed effects of ECS on retrieval to dissipate. The consolidation process was interrupted when ECS was applied <30 sec after trial 1. #, Trial 2 ECS is significantly different from trial 2 of controls (P < 0.05). \*, Significantly different from the first trial (P < 0.05). \*\*, Significantly different from the first trial (P < 0.01).



FIG. 6. Escape latencies on trial 2 expressed as a percentage of escape latencies on trial 1 as a function of the delay between trial 1 and ECS. Results show temporally graded retrograde amnesia.

P < 0.01]. This decrease was not significant when the ECS was applied 30 sec after the first trial. When ECS was applied 0 or 15 sec after the first trial, escape latencies did not decrease between the first and second trial, and trial 2 escape latencies significantly differed from those of controls [for 0 sec: t(16) = 2.11, P < 0.05; for 15 sec: t(8) = 2.22, P < 0.05]. This demonstrates that consolidation of a spatial memory trace can be disrupted with ECS only within 0–30 sec of acquisition. When latency on trial 2 is plotted as a proportion of latency on trial 1, the effects of ECS are seen to be a function of the delay between learning and the ECS treatment (Fig. 6). These data show a temporal gradient of consolidation of spatial memory, apparently completed within 45 sec of the acquisition of the spatial information.

### GENERAL DISCUSSION

ECS has been widely used in different clinical and experimental settings but has not often been used to test the time after acquisition of new spatial information at which the consolidation of spatial memory could be disrupted. The main difficulty is that animals generally acquire spatial tasks gradually, over many trials. An earlier study (11) showed retrograde effects of ECS similar to those found in the literature with humans. Rats were well trained on a reference memory version of the Morris water task, and ECS effects were observed over a period of 90 min. In our experiment we used an experimental design (9) which permitted us to test rats after a rapidly acquired spatial memory (one trial). In this situation a newly acquired spatial memory is insensitive to ECS disruption when it is applied >30 sec after acquisition. When the ECS is applied 0 or 15 sec after acquisition, rats completely lost the information they had previously acquired.

The data obtained under lidocaine inactivation of the hippocampus show that this task is dependent on an intact hippocampus. For rats trained under the lidocaine from trial 1 to trial 4, latencies decreased from trial 1 to trial 2 but remained higher than normal after trial 2. This pattern of performance suggests that a nonspatial search strategy was used, indicating that a functional hippocampus is typically essential for reaching asymptotic performance on the task. However, when lidocaine was administered after the first trial, an impairment was observed only on the second trial, while latencies on trials 3 and 4 reached asymptotic performance. In a reference memory version of this task, as previously noted (10), there are severe impairments when lidocaine is administered bilaterally into the hippocampus before training. In both the reference memory version and the working memory version of the task rats must learn not only about the location of the hidden platform, but also about the spatial arrangement of the experimental room. In the working memory version of the task, as used in the present study, learning about the experimental room takes place during the extensive pretraining required for acquisition of this task, and new learning is restricted to the location of the platform on the given day. Perhaps this difference between the two procedures accounts for why lidocaine inactivation of the hippocampus is more effective in disrupting retrieval of the reference memory task. The working memory version of the water task has the advantage of allowing us to look at immediate changes that occur after the acquisition of a limited amount of spatial information and should be useful in determining the neural mechanisms and systems involved in short-term consolidation.

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