used as stimuli. These experiments are in progress.

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Time Course of Short-Term Memory Depends on Associative Events

R. Menzel and M. Sugawa

Institut für Tierphysiologie – Neurobiologie – der Freien Universität, D-1000 Berlin

Memory of a recent learning trial is highly sensitive to new experience and to interference with experimental procedures [1, 2]. This early memory phase is referred to as short-term memory (STM). Retrograde amnesia induced by experimental procedures like electroconvulsive shock (ECS), cooling, or narcosis have been successfully used to characterize the time course of STM [3], although considerable controversy exists about the physiological status of the STM [4–6]. Little attention has been paid to the fact that additional learning trials during STM may facilitate memory consolidation and thus make the memory trace immune against amnestic treatment [3]. The mechanism of this effect has not yet been studied.

In honeybees the time course of STM is well defined because long-term memory (LTM) is established within minutes after one conditioning trial to an odor stimulus, and various experimental procedures induce the same retrograde amnestic gradient independent of the learning paradigm [7–9]. We show here that only additional associative events speed up memory consolidation but not additional exposures to the conditioned or unconditioned stimulus (CS, US) alone. The mechanism is a faster transfer from STM to LTM, but not an immediate access to LTM if STM is still occupied by the memory trace of the initial learning trial.

STM in honeybees is sensitive to new experience and to experimental procedures like narcosis, cooling, and ECS [7–9]. The time course of retrograde amnesia after one learning trial is 2.5 min (half time of effect), and the same for the three amnestic treatments. Similar time courses are found in different learning situations (classical and operant conditioning) and with different stimuli trained (visual and olfactory stimuli). Thus, STM resembles many characteristics of an early vulnerable memory phase in other animal species and humans [2, 3, 11] but is less sensitive to the test procedures and experimental arrangements. Particularly, it can be ruled out that ECS acts as a punishment [9].

The time course of retrograde amnesia changes after a session of several learning trials following each other quickly (massed learning trials). Erber [8] found that only the contribution of one learning trial is erased with ECS, if 2, 3, or 4 learning trials follow each other within a short time ($\leqq$1 min). This observation led to the hypothesis that STM is both time- and event-dependent. It is unknown, however, what kind of events are effective (repetition of the conditioned or unconditioned stimulus alone, or repetition of associative events), whether STM-LTM transfer is facilitated in such a way that LTM is formed faster, or whether the result of new learning trials reaches LTM immediately if STM is occupied. We have studied these questions with the proboscis conditioning paradigm [7–9, 12], since all relevant factors can be manipulated easily and accurately. Bees are fixed to a stage, and the extension of the proboscis is conditioned to odor stimuli. Bees extend the proboscis reflexively when the antennae are touched with a sucrose solution. If an odor is presented shortly before presenting the sucrose solution first to the antennae and then to the proboscis, a high proportion of the bees ($\geqq$70%) will respond to the conditioned stimulus (CS, odor) alone even after only one pairing with the unconditioned stimulus (US, sucrose solution to the proboscis).

In a first series of experiments we established that ECS delivered to the median-frontal head capsule erases the memory of a single learning trial, if the ECS follows the trial within 30 s. ECS is a rectangular AC (30 V, 60 μA, 50 Hz) delivered for 1 s 10 s after the last learning trial. This result resembles the finding with free-flying, color-trained bees [8, 9]. In a control experiment we demonstrated that ECS causes a transient reduction of retrieval of consolidated memory. This impairment of retrieval disappears within less than 40 min. Since a co-ordinated reflex can be released already a few minutes after ECS treatment, the retrieval block resides in the brain and effects specifically the activation of stored information. The amnesia is permanent. We have not found any recovery within the lifetime of the preparation (up to 3 days). If three massed learning trials are given within 30 s, and ECS follows 10 s after the last learning trial, no significant amnesia is found. These experiments confirm the earlier findings [8] with operant-conditioned animals that massed learning trials facilitate the establishment of a stable memory trace.

The next series of experiments address the question whether US-only or CS-only exposures also facilitate memory consolidation. Additional stimulation with CS after one trial learning does not change the susceptibility to ECS. Furthermore, two additional US-only trials following immediately on the one associative trial do not prevent the memory trace from being erased by the ECS. This result indicates that only ad-

ditional associative events help to establish a stable memory trace quickly. What kind of mechanism may facilitate memory consolidation by associative events? Is the transfer from STM to LTM speeded up or is LTM reached directly if STM is occupied by a recent association? To solve this question we trained the bee to two *different* odors in two successive learning trials. The two odors (geraniol, propionic acid) used in this series of experiments produce the same rate of response after one learning trial if conditioned separately. To avoid any bias with respect to the sequence of the two odors, we ran two groups of animals both for the experimental and the control group, one with geraniol first and propionic acid second, the other group vice versa. The ECS in this series of experiments was a sinusoidal AC of 18 Hz, ± 35 V and 70 μA. Sinusoidal current produces a longer-lasting retrieval block than the rectangular AC pulses. This effect is favorable for a better distinction between transient and long-lasting irreversible amnesia. The results are illustrated in Fig. 1. As the control group shows, the animals respond significantly better to the second trained odor irrespective which of the two odors (geraniol, propionic acid) is the first or the second. This result is known as the "recency effect" in serial learning experiments [11], and will not be analyzed further here. An ECS delivered 15 s after the second learning trial induces the expected strong retrieval block to both CS's. Hours later the response to the odor learned *first* recovers completely, whereas the response to the second odor stays significantly lower than in the control group. Again this effect is independent of which odor was conditioned first, and whether the first or the second trained odor was tested first. As in the experiments reported above and earlier [9], the ECS was unlikely to have acted as a punishment stimulus. As we know from appetitive conditioning, trace conditioning is only possible within ≤ 3 s after off-set of CS. However, here the time intervall between the second learning trial and ECS delivery is 15 s. The results indicate that the memory trace of the first associative trial is established firmly because it was followed by a second associative trial, whereas the trace from the second trial is susceptible to interference, and thus appears to have occupied STM when the animal was treated with ECS.

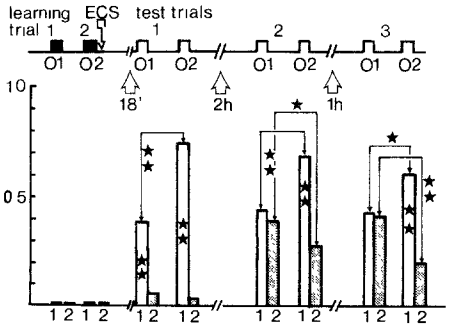

Fig. 1. Two groups of bees ($n=100$, each) are conditioned to two different odors (*O1*, *O2*: geraniol, propionate) in quick succession (within 30 s) and then either shocked (gray bars) or only sham-treated (open bars). ** highly significant differences ($P \leq 0.01$, χ^2 test), * low significant differences ($P \leq 0.05$)

Event dependence of STM is well known in memory studies in mammals [3, 11, 14, 15]. It was unknown, however, how specific the events have to be (e.g., nonassociative vs., associative events) and whether STM-LTM transfer is facilitated or whether the second trial establishes LTM directly. We show here for the honeybee that only *associative events* prevent the memory trace from being erased by ECS. Furthermore, we find that the content of the associations does not have to be exactly the same, and that consolidation is indeed speeded up by the second association. It will be of interest to find out how different the first learning trial can be from the second without losing its potential for fast consolidation.

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Responses of the Split Activity Components in Hamsters

D.J. Earnest

University of Rochester Medical Center, Department of Neurobiology and Anatomy, Rochester, NY 14642

F.W. Turek

Northwestern University, Department of Neurobiology and Physiology, Evanston, IL 60201

Most, if not all, circadian rhythms in rodents appear to be driven by a single master circadian pacemaker located in the bilaterally paired suprachiasmatic nuclei of the hypothalamus. Nevertheless, there is strong evidence that this pacemaker is composed of at least two circadian oscillatory components. Support for this hypothesis is based on the phenomenon of splitting – the dissociation of a single circadian rhythm under certain conditions into two dis-