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Increased hippocampal acetylcholine release during a working memory task

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Abstract

In this study we examined whether the food-reinforced alternation performance was associated with increased acetylcholine output in the dorsal hippocampus. Rats were trained to acquire the task using a T-maze. The control group consisted of rats introduced into the T-maze to run only on the day of dialysis. Acetylcholine release increased significantly in control rats only in the first 10 min after they were put into the T-maze. In trained rats acetylcholine output increased in the waiting cage as well as during trials in the T-maze. The increase in acetylcholine output in rats that had learned the task was significantly greater than in control rats.

Keywords: Hippocampus; Working memory; Acetylcholine release

The basal forebrain cholinergic projection system is involved in cognitive processes, and dysfunction in the septohippocampal or basal-cortical pathways system has been linked to learning and memory impairments (Olton et al., 1991).

It has recently been shown that memory performance, assessed in a passive avoidance task, was impaired in rats chronically treated with ethanol. Latency scores were significantly correlated with in vivo hippocampal basal release of acetylcholine (Melis et al., 1996).

In the present study extracellular levels of acetylcholine in hippocampus were monitored using in vivo brain microdialysis while rats performed an appetitive T-maze task, which involves spatial memory (Dunnett et al., 1982).

Male Sprague-Dawley rats (Nossan, Correzzana Italy) weighing 250–300 g were used. They were housed at a constant temperature of 22 ± 2°C and 60% relative humidity, food-deprived for 23 h/day to maintain 80% of normal body weight, but with water available ad libitum. The rats were trained to acquire a food-reinforced alternation task using a T-maze. The T-maze, made of Plexiglas, consisted of a central stem (10 (w) × 40 (l) × 20 (h) cm) with a start box (20 cm) and two arms (20 (w) × 60 (l) × 20 (h) cm) with a wire-mesh floor. The animals, after 3 days' habituation, were trained to make 12 consecutive trials (one session) in which they had to alternate between the right and left arms of the maze to obtain a shelled sunflower seed. On the first trial of each session, access to one of the arms was blocked, forcing the rat to enter only the opposite arms. On each of the next 11 trials, the food was placed in the arm opposite to that in the previous trial and both arms were unblocked (free-choice trials). A correct trial ended with the rat eating the food. An incorrect trial ended with the rat reaching the empty food cup. If the rat did not enter an arm within 3 min, the trial was not counted and the rat was allowed another attempt. After each trial, the rat was removed from the goal arm and placed in a waiting cage for 30 s. The rats were trained for 12–15 days, until they reached at least nine successful trials out of 11 for three consecutive sessions. Control rats were introduced into the T-maze only on the day of dialysis, but were handled in the same way and for the same time as trained rats.

Controls and trained rats were anesthetized with chloral hydrate (0.4 g/kg i.p.) and surgically implanted with a microdialysis probe in the dorsal hippocampus as previously described (Imperato et al., 1993). Two days after surgery aliquots for acetylcholine measurement were collected using a perfusion pump with 5 μl/min of Ringer solution containing 0.1 μM neostigmine and the extracellular concentration of acetylcholine was measured using high performance liquid chromatography (HPLC) with electrochemical detection (Damsma et al., 1985). 40 μl of

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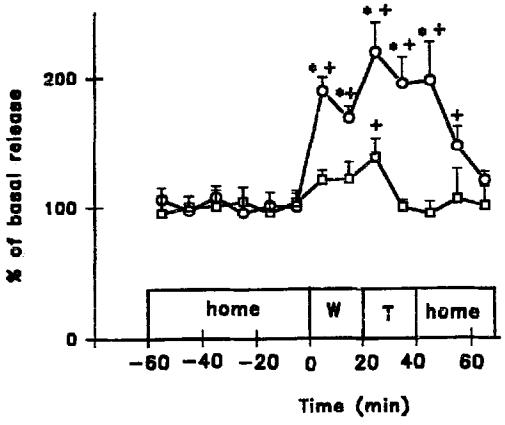

Fig. 1. Hippocampal dialysate concentration of acetylcholine. Data points represent means ± S.E.M. for six rats (○, trained; □, control). The average of basal values was 3.06 ± 0.25 and 3.4 ± 0.32 pmol/10 min for control and trained rats, respectively. +*, $P < 0.05$ (Newman-Keuls post hoc test) with respect to baseline levels and between trained and control rats in the corresponding period. W, waiting period; T, test period.

Ringer was injected into the analytical system at 10 min intervals. Baseline acetylcholine samples were collected in the home cage. After six baseline samples the rats were transferred to the waiting cage for 20 min and were then allowed to perform the task. When a trial session was completed the rats were placed in the home cage and samples for acetylcholine measurement were collected for another 30 min.

Fig. 1 shows the change in acetylcholine release in the hippocampus prior to, during and after the alternation task in control and trained rats. During the trial period acetylcholine release increased in trained rats, but did not do so to the same degree in control rats. Two-way analysis of variance (ANOVA) for repeated measures revealed a significant difference between groups ($F(1,7) = 21.41$; $P < 0.018$). The interaction between group and time ($F(7,56) = 4.613$; $P < 0.001$) was highly significant, suggesting that acetylcholine release in the groups followed a different trend.

The present results demonstrated that, when trained animals are waiting or performing the task, acetylcholine release in the hippocampus increases, suggesting the involvement of cholinergic septohippocampal pathways. In the control group when rats are exploring the T-maze there is a significant increase of acetylcholine release, probably linked to locomotor activity and/or attention. Indeed it has been shown that acetylcholine release increases in hippocampus and frontal cortex of rats during locomotor activity (Day et al., 1991). Inglis et al. (1994) have shown an enhanced ACh release in hippocampus and cortex during the anticipation and consumption of a palatable reward, but in the hippocampus there were no differences between control, naive, and trained rats, whereas in the frontal cortex the increase in ACh release in the trained rewarded rats was significantly greater than in the non-trained rewarded groups. However, using a working memory task, we have found that acetylcholine release in the hippocampus increases selectively during anticipation and when rats are performing the task, suggesting that cholinergic activity is important in processes of arousal, attention and memory. Furthermore our data support previous findings that the septohippocampal cholinergic system plays a major role in working memory, particularly in spatial working memory (Wan et al., 1994).

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