Involvement of the hippocampus, amygdala, entorhinal cortex and posterior parietal cortex in memory consolidation

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Abstract

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A total of 182 young adult male Wistar rats were bilaterally implanted with cannulae into the CA1 region of the dorsal hippocampus and into the amygdaloid nucleus, the entorhinal cortex, and the posterior parietal cortex. After recovery, the animals were trained in a step-down inhibitory avoidance task. At various times after training (0, 30, 60 or 90 min) the animals received a 0.5-μl microinfusion of vehicle (saline) or 0.5 μg of muscimol dissolved in the vehicle. A retention test was carried out 24 h after training. Retention test performance was hindered by muscimol administered into both the hippocampus and amygdala at 0 but not at 30 min posttraining. The drug was amnestic when given into the entorhinal cortex 30, 60 or 90 min after training, or into the parietal cortex 60 or 90 min after training, but not before. These findings suggest a sequential entry in operation, during the posttraining period, of the hippocampus and amygdala, the entorhinal cortex, and the posterior parietal cortex in memory processing.

Introduction

The hippocampus and amygdala are connected with each other and with the entorhinal cortex, and the latter is connected with the posterior parietal area by afferent and efferent pathways (1,2). Lesions and pharmacological manipulations of the hippocampus, amygdala and entorhinal cortex are known to have profound effects on memory consolidation and retrieval (1,3-9). Lesions of the posterior parietal area in the rat have less dramatic, but also deleterious effects on spatial memory (10,11) and on at least some (11) but not all types of non-spatial memory (12). Single unit activity in the parietal cortex changes during different forms of learning in cats (13).

Muscimol is a specific agonist of gamma-amino butyric acid (GABA) type A receptors and exerts a strong amnestic effect when infused immediately after training into the

hippocampus or amygdala (5,14) and 1-3 h after training into the entorhinal cortex (3,4,7). This observation suggests a role for these structures in different phases of memory consolidation (3-8).

The objective of the present study was to determine the effect of muscimol infused bilaterally into different brain areas at different times after training on retention of inhibitory avoidance. The findings suggest a role of the hippocampus and amygdala immediately after training (which is in agreement with previous results reported by Izquierdo et al. (6)), a role of the entorhinal cortex starting 30 min later, and a role of the parietal cortex starting 60 min after acquisition.

Material and Methods

A total of 182 male Wistar rats (age, 3-4 months; weight, 220-350 g) were used. The animals were implanted under thionembutal anesthesia (30 mg/kg, *ip*) with 27-g guide cannulae aimed 1.0 mm above the following structures: the CA1 region of the dorsal hippocampus (A -4.3, L ±4.0, V 3.4) and the junction between the central and the lateral nuclei of the amygdala (A -2.3, L ±4.5, V 8.4) (N = 40), the surface of the entorhinal cortex (A -7.0, L 5.0, V 8.4) (N = 70), and the surface of the junction between the posterior parietal I and II regions (A -0.3, L ±6.9, V 4.5) (N = 72). The coordinates correspond to Figures 35, 27, 46 and 19, respectively, of the atlas of Paxinos and Watson (15).

Once recovered from surgery, the animals were trained in a step-down inhibitory avoidance task (3-5,8,9,16). The rats were placed on a 2.5 cm high, 7.0 cm x 25.0 cm platform facing a 42.0 x 25.0 cm grid of parallel 0.1-cm caliber stainless steel bars spaced 1.0 cm apart, and their latency to step down placing their four paws on the grid was measured. During the training sessions, immediately after stepping down, the animals received a 0.3-mA, 2.0-s scrambled footshock. During the test sessions no

footshock was given. The training-test interval was 24 h. Test minus training session step-down latency was taken as a measure of retention.

At the time of infusion, 30-g cannulae were fitted into the guide cannula. The infusion cannula was placed immediately (0 min) or 30 min after avoidance training into the hippocampus, amygdala, or amygdala and hippocampus, and 0, 30, 60 and 90 min after training into the entorhinal or parietal cortex. The tip of the infusion cannula protruded 1.0 mm beyond that of the guide cannula and was therefore aimed at a) CA1 in the dorsal hippocampus, and the junction between the central and the basolateral amygdaloid nucleus, b) the surface of the entorhinal cortex, and c) the surface of the posterior parietal cortex. The animals received bilateral 0.5-µl infusions of saline (0.9% NaCl) or muscimol (0.5 µg/side) dissolved in saline. The pH of the solutions was adjusted to 7.4 with 0.1 M sodium phosphate buffer. Infusions were manual and were carried out over a period of 45 s each, and the infusion cannula was left in place for an additional 15 s. Infusions were carried out first on the left side and then on the right side. Thus, the entire infusion procedure took about 4 min for the hippocampus and amygdala groups, and 2 min for the entorhinal and parietal cortex groups.

Two to 24 h after the end of the behavioral experiments all animals received a 0.5-µl infusion of 4% methylene blue through the infusion cannulae. Post-mortem verification of the cannula placements showed that in 36 of 40 animals implanted into the hippocampus and amygdala, in 68 of 70 animals implanted into the entorhinal cortex, and in 71 of 72 animals implanted into the parietal cortex, the infusion cannula tips reached the desired structures and were within a 0.5-mm radius of the aimed locations. The correct placements of the infusion cannula tips are shown in Figure 1.
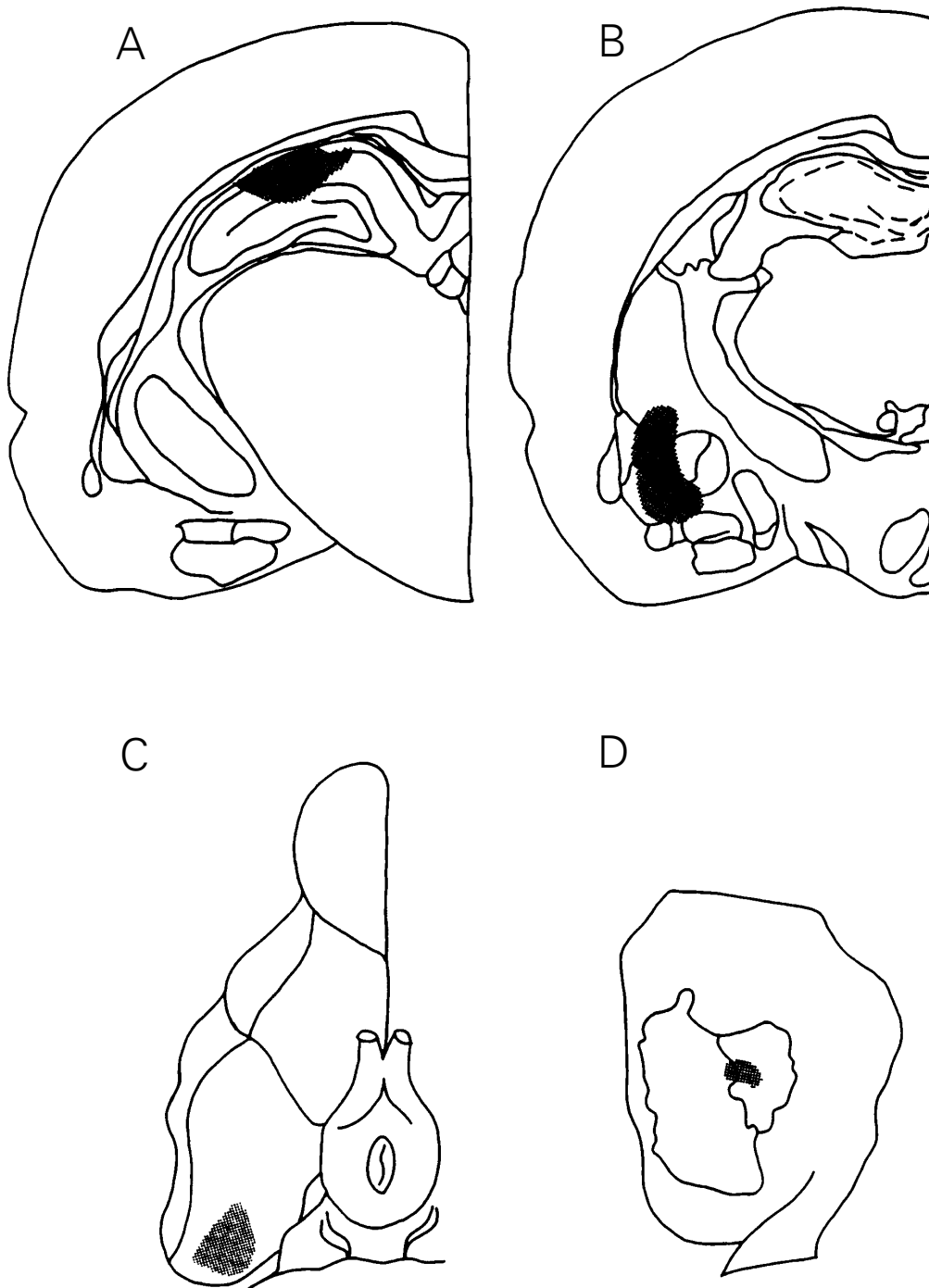
Statistical analysis of the behavioral data

Figure 1 - Schematic drawing of rat brain stions at planes A -4.3 and -2.3, respectively, of the atlas of Paxinos and Watson (15), showing the areas reached by the microinfusion cannula tips aimed at the CA1 region of the dorsal hippocampus (A) and between the central and the basolateral nuclei of the amygdala (B) (stippled). *C*, Schematic drawing of the lower aspect of the rat brain, showing the area reached by the microinfusion cannula in the entorhinal cortex (stippled). *D*, Schematic drawing of the lateral aspect of a rat brain hemisphere illustrating the subdivisions of the parietal cortex and the area reached by the microinfusions given into this area (stippled). Only placements considered to be correct are shown here (see Material and Methods).

was carried out only for the animals with correct cannula locations. Training step-down latency differences between groups were evaluated by Kruskal-Wallis analysis of variance for nonparametric data. Test minus training session latency scores of different groups were compared by the individual Mann-Whitney U-test (two-tailed).

Results

Training session step-down latency differences between groups were not significant (median 5.9 s, range 1.1 to 21.9 s, H [19,155] = 1.76, P>0.1).

The results of the retention test performance are shown in Figure 2. Muscimol caused full retrograde amnesia when infused

into the hippocampus and the amygdala immediately (0 min), but not 30 min after training. Muscimol was also amnestic when given into the entorhinal cortex 30, 60 or 90 min (but not 0 min) after training, or when given 60 or 90 min (but not 0 or 30 min) posttraining into the posterior parietal cortex.

Discussion

The present data show that, during the posttraining period, the hippocampus, amygdala, entorhinal and posterior parietal cortex are necessary for memory processing, and suggest that these structures enter into operation sequentially in the following order: first, immediately after training, the hippocampus and amygdala; 30 min later, the entorhinal cortex; 60 min after training, the parietal cortex. In the case of the entorhinal cortex, this is earlier than suggested by previous experiments in which posttraining intervals between 0 and 90 min were not studied (3,4,8), and in agreement with a study on the amnestic effect of protein kinase C inhibitors infused into this region 30 min after training (9).

The findings on immediate posttraining muscimol administration into both hippocampus and amygdala confirm those obtained previously when the drug was infused into each structure separately (5) or simultaneously into the two structures (8). Clearly, the present data do not discriminate between these two structures in terms of the amnestic effect of muscimol. The distinction, however, has been clearly made elsewhere (5,6, see 17). The hippocampus and amygdala enter into play at the time of training and in the period immediately after training, particularly in this task. The hippocampus is believed to be in charge of spatial, contextual and other cognitive aspects of memory of the training experience and the amygdala is believed to be in charge of the emotional aspects, particularly those of an aversive nature (6,17,18).

Figure 2 - Effect of muscimol infused bilaterally into both hippocampus and amygdala (HIPP + AMY), entorhinal cortex (ENTO) or posterior parietal cortex (PARIE) on retention test performance. Data are reported on the ordinates as median (interquartile range) test minus training session step-down latency, in seconds. The drug (0.5 µg in 0.5 µl per side) was amnestic when infused 0 min after training but not later in HIPP + AMY; 30-90 min after training, but not before, when given into ENTO, and 60-90 min after training, but not before, when given into PARIE. [a]P<0.002, U values 0 to 10; [b]P<0.02, U values 11 to 19 compared to controls (Mann-Whitney U-test, two-tailed) for 9 to 11 animals per group.
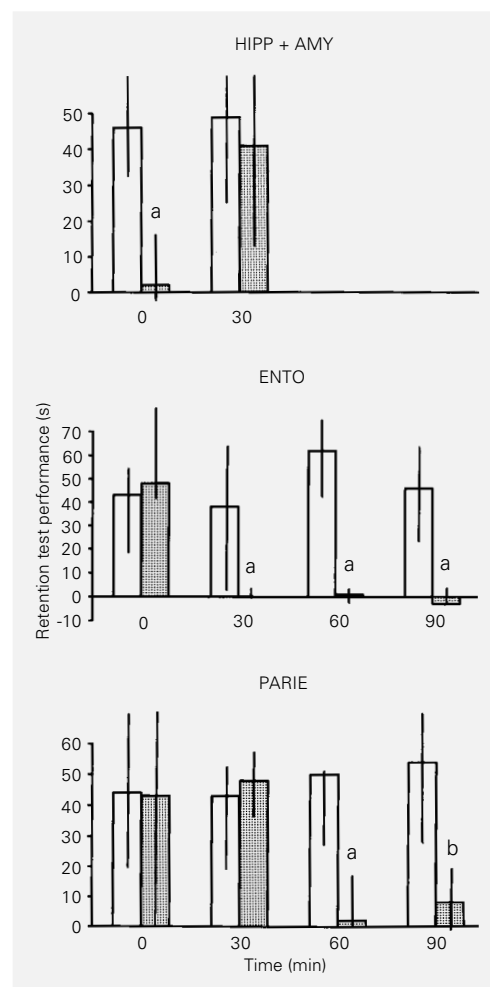
The findings about muscimol given into the entorhinal cortex 90 or 180 min after training confirm those of Ferreira et al. (3,4). The sequential involvement of the different brain regions in posttraining memory processing may be mediated by the anatomical connections among these brain regions (2). The reason for the 30-min delay between the interventions of the different areas is not known. Previous findings have suggested that post-acquisition activity in the hippocampus and amygdala, presumably long-term potentiation (LTP), must build up before it is able to trigger similar activity in the entorhinal cortex (3,4,7). Perhaps a similar explanation may account for the 30-min delay between the participation of the entorhinal cortex and that of the parietal cortex in memory processing. Indeed, a study similar to the present one was carried out using AP5 (D-2-amino-5-phosphonopentanoate), an antagonist of glutamatergic N-methyl-D-aspartate (NMDA) receptors, and the results were very similar to those reported here for muscimol: AP5 was amnestic when given early into both hippocampus and amygdala, and after 30 and 60 min when given into the entorhinal and parietal cortex, respectively (16). For evidence on the possible role of LTP in the hippocampus, amygdala and entorhinal cortex in memory consolidation, see Refs. 6 and 7.

The present findings illustrate the advantage of circumscribed drug infusion procedures over lesion procedures for the study of the role and timing of brain structures in learning and memory. During a few days, extensive synaptic rearrangements occur in areas (e.g., the hippocampus) to which lesioned fibers project (e.g., those emerging from the entorhinal cortex), and vicarious structures and systems enter into play (19). This fact precludes detailed interpretations of lesion studies in terms of the functions of either the injured region or its projection sites (7). On the other hand, localized microinfusions of drugs acting at specific receptor sites, such as muscimol, exert precise effects limited both in time (60 min) and space (0.5-mm radius) (20).

Previous lesion studies have failed to detect dramatic effects of posterior parietal cortex lesions on memory (e.g., 11,12), which was surprising in view of the multiple connections of this region with the entorhinal cortex (1,2). Lesion studies do not permit an accurate investigation of time-dependent processes. In contrast, the present experiments using the time-honored procedure of injecting drugs at different times after training (21) clearly point to an important role of the posterior parietal cortex in memory consolidation processes, starting 60 min after training.

Acknowledgments

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