

Sequential Role of Hippocampus and Amygdala, Entorhinal Cortex and Parietal Cortex in Formation and Retrieval of Memory for Inhibitory Avoidance in Rats

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Abstract

The hippocampus and amygdala, the entorhinal cortex and the parietal cortex participate, in that sequence, both in the formation and in the expression of memory for a step-down inhibitory avoidance task in rats. Bilateral infusion of AP5 or muscimol caused retrograde amnesia when given 0 min after training into both hippocampus and amygdala, when given or 180 min after training into the entorhinal cortex, or when given 180 min after training into the parietal cortex. Therefore, memory formation requires the sequential and integrated activity of all these areas mediated by glutamate NMDA receptors in each case. Pre-test administration of CNQX 1 day after training into hippocampus and amygdala, 1 or 31 days after training in entorhinal cortex, or 1, 31 or 60 days after training in the parietal cortex temporarily blocked retention test performance. Therefore, 1 day after training, all these brain structures are necessary for retrieval; 1 month later, the hippocampus and amygdala are no longer necessary for retrieval but the entorhinal and parietal cortex still are; and 60 days after training only the parietal cortex is needed. In all cases the mechanisms of retrieval require intact glutamate AMPA receptors.

Introduction

The hippocampus, amygdala and entorhinal cortex are interconnected by two-way pathways and, in addition, the entorhinal cortex is linked to the posterior parietal and prefrontal cortex (Witter *et al.*, 1989; Hyman *et al.*, 1990; see also Green and Adey, 1956). All these structures play a role in memory, probably in an integrated fashion (Hyman *et al.*, 1990; Ferreira *et al.*, 1992a, b; Izquierdo *et al.*, 1993b; Willner *et al.*, 1993; Jerusalinsky *et al.*, 1994).

The hippocampus processes spatial, contextual and other cognitive data (Izquierdo *et al.*, 1993b; Bechara *et al.*, 1995; Eichenbaum, 1996). The amygdala processes mainly emotional or aversive information (Bechara *et al.*, 1995; Cahill *et al.*, 1995; McGaugh *et al.*, 1995). The entorhinal cortex processes many types of memory (Thompson, 1976; Ferreira *et al.*, 1992a, b; Squire, 1992; Wigg and Bilkey, 1994; Suzuki, 1996) and possibly integrates information processed by the amygdala and hippocampus during and after training (Ferreira *et al.*, 1992a; Willner *et al.*, 1993; Jerusalinsky *et al.*, 1994). Lesions of the non-sensory parietal cortex hinder spatial and non-spatial memories (Thomas and Gash, 1990; Kesner *et al.*, 1992; Compton *et al.*, 1994), and unit activity changes were described both in parietal and in entorhinal cortex during spatial and non-spatial learning (Markowitsch and Pritzel, 1987; Nakamura *et al.*, 1995). Further, imaging studies in humans (McCarthy, 1995) and glucose uptake observations in

animals (Bontempi *et al.*, 1991, 1996; Meunier *et al.*, 1991; Sif *et al.*, 1991; McIntosh and González-Lima, 1994) suggest an involvement of the associative parietal cortex, among other cortical regions, in a variety of tasks.

Humans with bilateral temporal lobe damage are unable to form new declarative memories, but conserve those acquired some time prior to the lesion (Corkin *et al.*, 1985). This has suggested that the temporal lobe structures are involved in memory formation and early storage and then memories move to extra-temporal cortical storage sites (Squire, 1992; Skaggs and McNaughton, 1996), one of which could be the parietal cortex (Zanatta *et al.*, 1996).

Here we report on the involvement of hippocampus and amygdala, entorhinal cortex and parietal cortex both in memory formation in the post-training period, and on retrieval measured 1, 31 or 60 days after training. A step-down inhibitory avoidance task was chosen, for two reasons: (i) it is acquired in one very brief session (Gold, 1986; Izquierdo *et al.*, 1992, 1993a,b); (ii) its pharmacology is well known and was recently found to be very similar to that of various forms of long-term synaptic plasticity (Izquierdo and Medina, 1995, 1996, 1997; Maren and Baudry, 1995; Medina and Izquierdo, 1995; Bernabeu *et al.*, 1996). In particular, post-training intrahippocampal, intra-amygdala or intra-entorhinal infusion of the glutamate receptor

antagonist D-2-amino-5-phosphono-pentanoic acid (AP5), or of the GABA_A receptor agonist muscimol, was found to cause retrograde amnesia for this and other tasks (Ferreira *et al.*, 1992a, b; Izquierdo *et al.*, 1992). Further, the pre-test infusion of the glutamate α -amino-3-hydroxy-5-methyl-4-isoxazolone propionic acid (AMPA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), but not that of AP5 or muscimol, into hippocampus and amygdala (Bianchin *et al.*, 1993; Izquierdo *et al.*, 1993a,b) or entorhinal cortex (Jerusalinsky *et al.*, 1994; Quillfeldt *et al.*, 1994) hinders retrieval of this task 1 day after training.

Here we study the effect of post-training AP5 and muscimol infusions, and of pre-test CNQX infusions, into hippocampus and amygdala, entorhinal cortex and parietal cortex at different post-training and training-test intervals than those that were studied before.

Materials and methods

Subjects

A total of 700 male Wistar rats (age, 3–4 months; weight, 230–340 g) from our own breeding colony were used. The animals were housed in plastic cages, four to five to a cage, with water and food *ad libitum*, under a 12 h light/dark cycle (lights on at 7.00 a.m.) at a constant temperature of $23 \pm 1^\circ\text{C}$.

Surgery

All animals were bilaterally implanted under deep thionembutal anaesthesia with 30-g guide cannulae in: (i) *both* dorsal CA₁ region of the hippocampus *and* the junction of the central and the lateral nuclei of the amygdala; (ii) entorhinal cortex; or (ii) posterior parietal cortex. The cannulae were fixed to the skull with dental acrylic. In all, 21 rats died of postoperative causes and 25 lost their acrylic headpieces. Stereotaxic co-ordinates were according to the atlas of Paxinos and Watson (1986), as follows: (a) hippocampus, A -4.3 , L ± 4.0 , V 3.4 ; (b) amygdala, A -2.3 , L ± 4.5 , V 8.4 ; (c) entorhinal cortex, A -7.0 , L 5.0 , V 8.4 ; (d) the junction between the posterior parietal cortex I and II regions, A -0.3 , L ± 6.9 , V 4.5 .

Infusion procedure and control of cannula placements

After recovery from surgery, the animals were submitted to inhibitory avoidance training (and some of them also to free exploration of the training apparatus, see below). At the appropriate time, 0–270 min post-training, 10 min prior to testing or 10 min before training (depending on the experiment, see below), 27 g infusion cannulae were fitted into the guide cannulae and drug, saline or vehicle infusions were carried out. The infusions were carried out first on one side and then on the other. They were performed manually using a microsyringe attached to the cannulae with a polyethylene tube. Infusions were carried out slowly over 30 s, and then the infusion cannula was left in place for another 15 s. Therefore, the entire procedure took slightly over 90 s in the animals implanted in entorhinal or parietal cortex, and 180 s in those implanted in the amygdala and hippocampus (Izquierdo *et al.*, 1992; Jerusalinsky *et al.*, 1994; Zanatta *et al.*, 1996).

At 2–6 h after the end of the behavioural procedures, 0.5 μl of a solution of 4% methylene blue in saline was infused as indicated above into each implanted site. Animals were killed by decapitation 1 h later and their brains were withdrawn and stored in formalin for histological localization of infusion sites as explained elsewhere in detail (Ferreira *et al.*, 1992a; Izquierdo *et al.*, 1992; Jerusalinsky *et al.*, 1994). Cortical infusion placements were checked first by

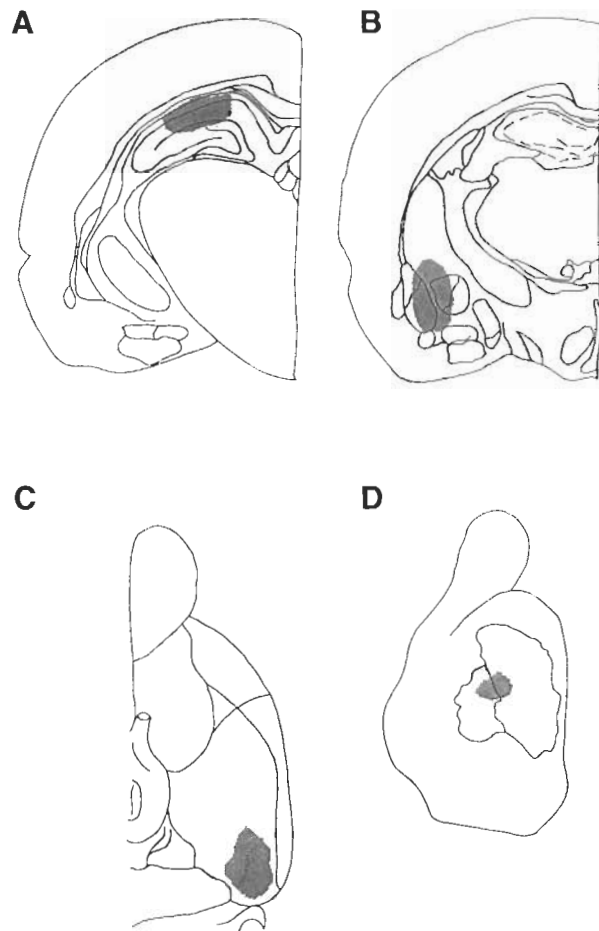


FIG. 1. (A) and (B) Drawings of planes A -4.3 and -2.3 of the atlas of Paxinos and Watson (1986), showing, stippled, the extent of the area reached by the infusions in the dorsal hippocampus and amygdala respectively. (C) Ventral view of the brain showing the extent of infusions into entorhinal cortex. (D) Lateral view of the brain (Zilles, 1985) showing the extent of infusions into posterior parietal cortex.

macroscopic examination according to the atlas of Zilles (1985). In the cortical infusions, histological sections showed that the depth of the spread of the dye never exceeded 1.5 mm; in the intrahippocampal and intra-amygdala infusions, the spread never exceeded a 1.0–1.5 mm³ radius. This agrees with previous observations of Martin (1991) using 0.5 μl infusions of radiolabelled lidocaine and muscimol into brain tissue; he measured both the spread of these compounds and their effect on 2-deoxyglucose uptake, and found a clear overlap between the two sets of data. Results of the anatomical verifications are shown in Figure 1. Infusion placements were correct in 96 of the 108 rats implanted in both hippocampus and amygdala (89%), 265 of the 269 rats implanted in entorhinal cortex (98.5%) and 263 of the 277 animals implanted in parietal cortex (95%).

Behavioural procedures

Step-down inhibitory avoidance (Izquierdo *et al.*, 1992, 1993a; Jerusalinsky *et al.*, 1994; Quillfeldt *et al.*, 1994) was carried out as follows. The animals were placed on a 2.5 cm high, 7.0 cm wide platform at the left of a 50.0 \times 25.0 \times 25.0 cm training apparatus, whose floor was a series of parallel 0.1 cm calibre stainless steel bars spaced 1.0 cm apart. Latency to step down placing the four paws on the grid was measured with an automatic device. In training sessions,

immediately upon stepping down, the animals received a 2.0-s scrambled footshock. Intensity of the footshock was 0.3 mA for the animals that were to be tested 1 day later, and 0.7 mA for the animals that were to be tested 31 or 60 days later, in order to ensure good retention levels at these longer intervals; previous data suggest that this does not qualitatively change the task (see Gold, 1986; Bianchin *et al.*, 1993). In test sessions no footshock was given and the step-down latency was cut off at 180 s; i.e. values ≥ 180 s were counted as 180 s.

Three separate experiments were carried out.

The first experiment examined the effect on memory of AP5 (5.0 g/side) or muscimol (0.5 g/side) infused bilaterally 0, 30, 60, 90, 180 or 270 min after training into both hippocampus and amygdala ($n = 56$), into the entorhinal cortex ($n = 163$), or into the posterior parietal cortex ($n = 163$). Drugs were dissolved in saline to an infusion volume of 0.5 l, and control animals received infusions of 0.5 l of saline. A 0.3 mA training footshock was used. Training-test interval was 24 h.

The second experiment studied the effect of CNQX (0.5 g/side) given bilaterally into both hippocampus and amygdala ($n = 40$), into entorhinal cortex ($n = 84$), or into parietal cortex ($n = 81$) on test session performance measured 1, 31, or 60 days after training (Quillfeldt *et al.*, 1996). CNQX was dissolved in 0.5 l of 20% dimethylsulfoxide in saline, and control animals received an equal volume of the vehicle. Two test sessions were carried out: the first, 10 min after the infusions, and the second, 90 min later, a time at which the material infused had presumably diffused away from the cannula site (Martin, 1991).

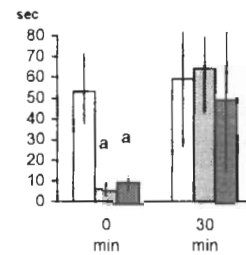
The first two experiments, because of their design, required a comparison of drug effects across structures and at different times. Therefore, they required the repetition of some observations carried out in previous work: (i) effect of muscimol (and AP5) given into both amygdala and hippocampus immediately post-training (Jerusalinsky *et al.*, 1994); (ii) effect of muscimol and AP5 given into entorhinal cortex 0, 90 and 180 min after training (Ferreira *et al.*, 1992a, b); and (iii) effect of pre-test CNQX given into all these structures (Izquierdo *et al.*, 1993a; Jerusalinsky *et al.*, 1994) 1 day after training. As will be seen, the new findings reported here corroborate those of the previous papers in all cases.

The third experiment examined the possibility that CNQX could influence general performance, sensory or motor aspects (Quillfeldt *et al.*, 1994). Eighteen rats were implanted with cannulae in the entorhinal cortex and 19 with cannulae in the posterior parietal cortex. They received a bilateral infusion of CNQX or vehicle into the parietal or the entorhinal cortex; 10 min later, they were allowed to explore the training apparatus for 3 min. Crossings of three imaginary lines that divided its floor into four equal 12.5×25.0 cm segments were counted. On the following day, the same animals were randomly reassigned to saline or CNQX groups, were trained 10 min after the infusions in the inhibitory avoidance task using a 0.3 mA footshock, and were tested for retention 24 h later.

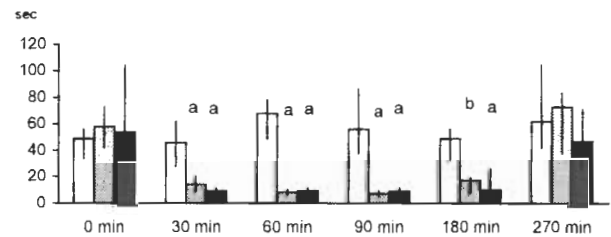
Statistics

Due to the 180 s ceiling used for test session latencies, inhibitory avoidance data were analysed by non-parametric procedures. Training session step-down latency differences among groups were evaluated by a one-way Kruskal-Wallis analysis of variance. Median training latency for all animals was 6 s, range 1–25 s, $H(632) = 9.33$, $P > 0.1$. Comparisons between training and test session latencies within each group, and comparisons of test session values of different groups were by individual Mann-Whitney *U*-tests, two-tailed.

HIPPOCAMPUS+AMYGDALA



ENTORHINAL



PARIETAL

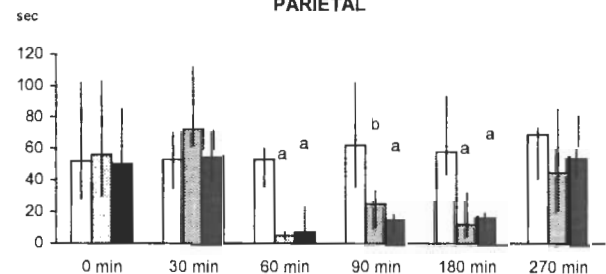


FIG. 2. In this and following figures, retention of step-down inhibitory avoidance is expressed as median (interquartile range) test session latency, in s (ordinates). Animals received bilateral infusions of saline (white columns), AP5, 5.0 g (light grey columns) or muscimol, 0.5 g (dark grey columns) infused into both dorsal hippocampus and amygdala, entorhinal cortex and parietal cortex 0–270 min after training. $n = 9$ –10 in the hippocampus + amygdala groups, 8–10 in the entorhinal cortex groups, and 8–11 in the parietal cortex groups. Post-training AP5 and muscimol were amnesic when given 0 but not 30 min after training in hippocampus + amygdala, 30–180 min but not 0 or 270 min after training in entorhinal cortex, and 60–180 but not 0–30 or 270 min after training in parietal cortex. In this and following figures, ^aSignificant difference from control, $P < 0.002$; ^bSame, at $P < 0.02$ level; all test session values not marked by ^a or ^b are significantly different from training session latencies at $P < 0.002$ level (Mann-Whitney *U*-tests, two-tailed).

Results

Post-training effects of AP5 and muscimol on retention

Median training latency for all animals in this experiment was 7 s, range 1–24 s. Differences among groups were not significant: $H = 15.46$, $df = 41$, $P > 0.1$.

The effects on test session performance of post-training AP5 and muscimol infusions are shown in Figure 2. The immediate (0 min) post-training bilateral infusion of any of the two substances into both hippocampus and amygdala caused retrograde amnesia for the inhibitory avoidance task. Treatments given 30 min post-training into these two structures had no effect. Infusions of AP5 or muscimol

into the entorhinal cortex 30, 60, 90 or 180 min after training were amnesic; when given 0 or 270 min after training they had no effect. Infusions of AP5 or muscimol into the parietal cortex 60, 90 or 180 min after training caused retrograde amnesia for the avoidance task; when given 0, 30 or 270 min post-training they had no effect.

Effect of pre-test CNQX on retrieval

Median training latency for this experiment was 6 s, range 1–23 s. Differences among groups were not significant: $H = 7.49$, $df = 15$, $P > 0.1$.

The effect of pre-test CNQX infusions on retention test performance is shown in Figures 3, 4 and 5. One day after training, the pre-test infusion of CNQX into hippocampus and amygdala, entorhinal cortex or parietal cortex hindered retrieval (Fig. 3). At 31 days from training, the infusion of CNQX into hippocampus and amygdala was no longer effective, whereas infusion of the drug into either entorhinal cortex or parietal cortex still disrupted retrieval (Fig. 4). At 60 days from training, the infusion of CNQX into entorhinal cortex was no longer effective, whereas when given into the parietal cortex the drug blocked retention test performance (Fig. 5).

In all cases, retention test scores returned to normal in the second test session, 90 min after the infusion of CNQX. Therefore, the effect of the drug was temporary in all cases. Differences in retention scores between the two test sessions were not significant in control groups. Differences among groups in performance in the second test were not significant.

Effect of CNQX on acquisition of the avoidance task and on exploratory activity

Results of the experiment on the effect of CNQX on exploratory activity and on acquisition of the avoidance task are shown in Figures 6 and 7 respectively.

Median training latency for the third experiment (pre-training infusions) was 4 s, range 1–24 s, $H = 1.09$, $df = 1$, $P > 0.1$. The drug had no effect on these variables when given either into the entorhinal or the parietal cortex, which suggests that it had no major influence on sensory or motor performance variables in either case.

Discussion

The findings lead to three major conclusions. First, all the structures studied play a role in post-training memory processing, in sequential order. Second, they also play a role in retrieval, and the duration of this role is also sequential. Third, both after the training session and at the time of testing at short training-test intervals, all the structures appear to act in an integrated fashion: the inhibition of any one of them hinders consolidation and retrieval, respectively (see also Izquierdo *et al.*, 1992, 1993a, b; Bianchin *et al.*, 1993; Jerusalinsky *et al.*, 1994; Quillfeldt *et al.*, 1994).

In the present study no attempt was made to separate effects of drugs given into the hippocampus from those of drugs given into the amygdala. Previous studies had shown that AP5 and muscimol given immediately post-training into either structure (Izquierdo *et al.*, 1992) or into both simultaneously (Jerusalinsky *et al.*, 1994) cause retrograde amnesia. Also, previous studies had shown that, in order effectively to hinder retrieval, CNQX must be given prior to testing into both structures and not into each separately (Bianchin *et al.*, 1993; Izquierdo *et al.*, 1993a). In the present paper, the focus was on the timing of these effects relative to those upon the entorhinal and parietal cortex. Aside from the fact that the hippocampus and amygdala are known to participate simultaneously both in the early

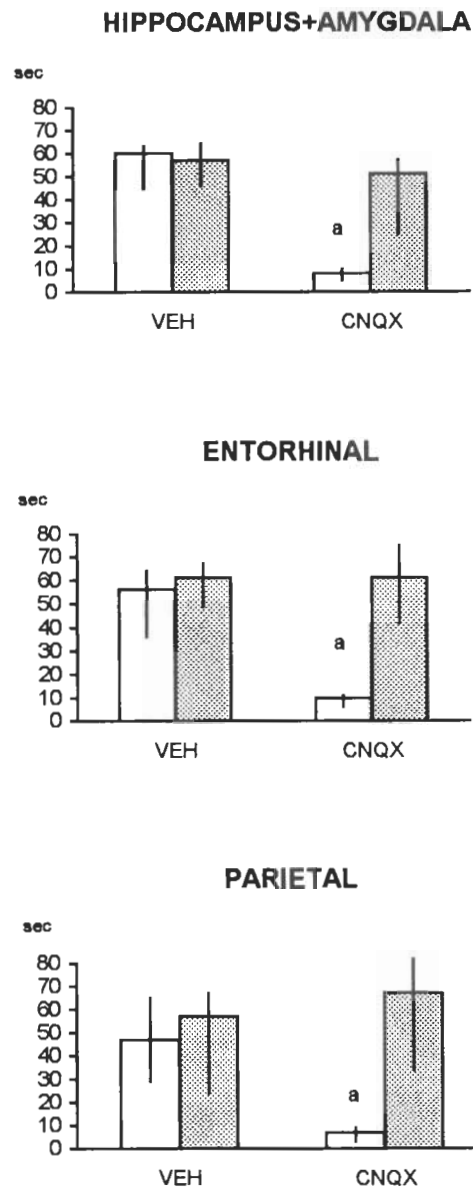


FIG. 3. Effect on retention test performance of a bilateral infusion of vehicle (VEH) or CNQX, 0.5 g, into both dorsal hippocampus and amygdala, into the entorhinal cortex or into the parietal cortex, in animals that were tested 1 day after training. Two test sessions were carried out: the first, 10 min after the infusions (white columns); the second, 90 min after the infusions (light grey columns) $n = 12$ (VEH) and 11 (CNQX) in hippocampus + amygdala groups; 12 in both entorhinal cortex groups; 14 (VEH) and 13 (CNQX) in parietal cortex groups. CNQX hindered retention test performance in the first test session when given into any of these structures. Test session values returned to normal after 90 min.

stages of memory formation (Izquierdo *et al.*, 1992) and in the retrieval of this task at short training-test intervals (Bianchin *et al.*, 1993; Izquierdo *et al.*, 1993a), there are important differences concerning their role in memory. As mentioned in the Introduction, the hippocampus is more related to the cognitive aspects (Izquierdo *et al.*, 1993b; Bechara *et al.*, 1995; Eichenbaum, 1996) and the amygdala to emotional aspects (Izquierdo *et al.*, 1993a, b; Cahill *et al.*, 1995; Bechara *et al.*, 1996) of this or other tasks.

Several of the findings in this paper replicate and therefore confirm those published elsewhere: the effect of immediate post-training

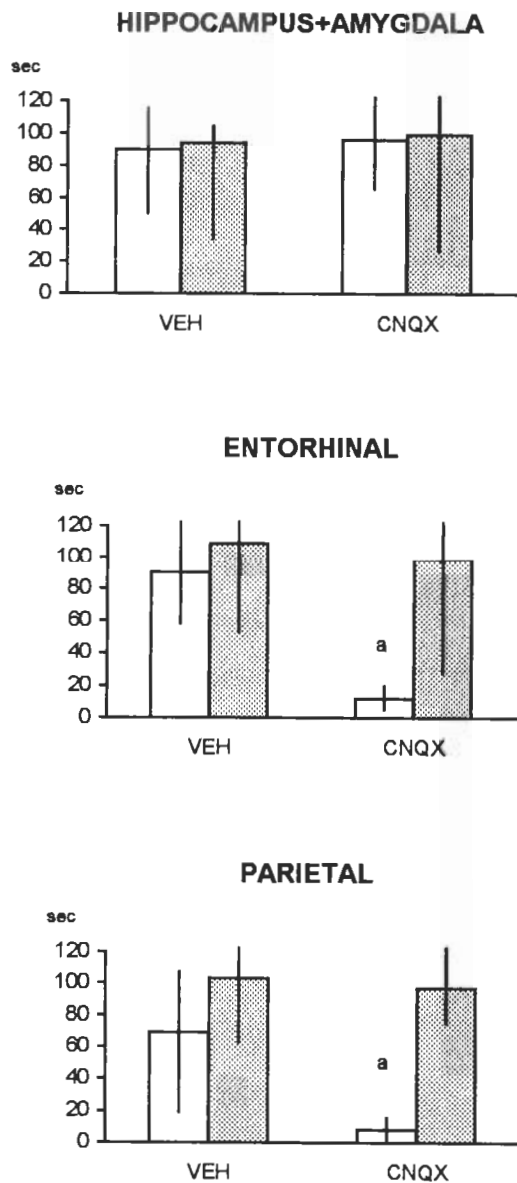


FIG. 4. Same as Figure 3 but for animals tested 31 days after training. $n = 10$ in all hippocampus + amygdala groups, 12 (VEH) and 14 (CNQX) in entorhinal cortex groups; 15 in both parietal cortex groups. CNQX was ineffective when given into hippocampus + amygdala, but hindered performance in the first test session when given into either entorhinal or parietal cortex.

muscimol given into both hippocampus and amygdala (Jerusalinsky *et al.*, 1994), or 0, 90 and 180 min post-training into entorhinal cortex (Ferreira *et al.*, 1992a, b), or the effect of pre-test CNQX given into hippocampus and amygdala (Izquierdo *et al.*, 1993a) or entorhinal cortex (Jerusalinsky *et al.*, 1994) 1 day after training. Those groups had to be reinvestigated in order to permit a comparison with the other time intervals studied here, and/or with the data on the parietal cortex.

Post-training memory processing

The hippocampus and amygdala enter into play immediately after training, the entorhinal cortex 30 min later, and the parietal cortex 60 min after training. In all cases, the role of these structures in

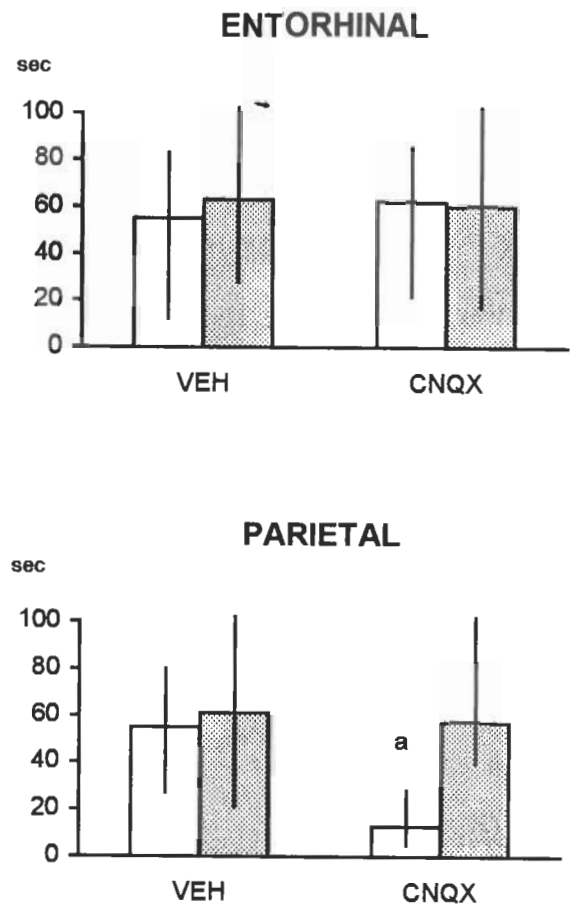


FIG. 5. Same as Figure 4 but for animals tested 60 days after training. $n = 14$ (VEH) and 16 (CNQX) in the entorhinal groups; $n = 14$ in both parietal cortex groups. CNQX hindered retention performance in the first test only when given into parietal cortex.

memory processing is sensitive to muscimol and requires glutamate *N*-methyl-D-aspartate (NMDA) receptors. The time window of intervention is shorter for the hippocampus and amygdala (<30 min) than for the entorhinal or parietal cortex (>180 and >120 min respectively).

The present results confirm a preliminary report on the late onset of the post-training effect of AP5 in the entorhinal and parietal cortex (Zanatta *et al.*, 1996). They also confirm previous findings on the immediate post-training effect of AP5 and muscimol given separately into hippocampus or amygdala (Izquierdo *et al.*, 1992), or of muscimol given into the two structures simultaneously (Jerusalinsky *et al.*, 1994). The present findings show that the drug effects in the entorhinal cortex start earlier than what previous papers had suggested; in those papers intervals between 0 and 90 min had not been studied (Ferreira *et al.*, 1992a, b).

Reasons for the delay between the post-training intervention of the hippocampus and amygdala and that of the entorhinal cortex, and between that of the entorhinal and that of the parietal cortex are not known. It is logical to think that the sequential role of these structures in post-training memory processing obeys their anatomical connections (Witter *et al.*, 1989; Hyman *et al.*, 1990; Iijima *et al.*, 1996; Suzuki, 1996). Neurochemical findings suggest that long-term potentiation generated in hippocampus and amygdala at the time of training and sustained over several minutes could lead to a build-up of activity in the entorhinal cortex (Jerusalinsky *et al.*, 1994; Izquierdo

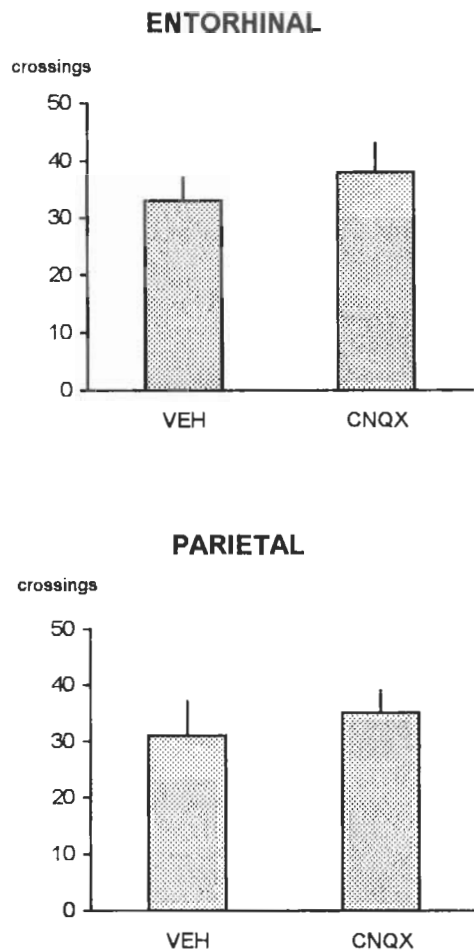


FIG. 6. Mean (standard deviation) number of crossing responses over 3 min of free exploration of the training apparatus in animals that received, 10 min prior to this, vehicle (VEH) or CNQX, 0.5 g, bilaterally into the entorhinal or parietal cortex. $n = 9$ in the entorhinal groups; $n = 8$ (VEH) and 9 (CNQX) in the parietal cortex groups. The drug had no effects on this measure of exploratory activity ($P > 0.1$ in t -tests).

and Medina, 1995, 1996). This hypothesis could also be applied to the delayed link between the entorhinal and the parietal cortex. Skaggs and McNaughton (1996) have suggested that the replay of behaviourally generated hippocampal neuronal firing sequences during sleep might play a role in the transmission of information from that structure to other regions of the brain.

Delays similar to those described here and shown in Figure 1 have been described for the uptake of ^{14}C -glucose or deoxyglucose by different brain regions of the mouse following different training procedures (Bontempi *et al.*, 1991, 1996; Sif *et al.*, 1991), which also suggests long-term interchanges. Rapid interactions between entorhinal cortex and hippocampus have also been described (Green and Adey, 1956; Iijima *et al.*, 1996), and may play a role in gating the entry of information from the former into the latter (Iijima *et al.*, 1996) or *vice versa* (Adey, 1961; see also Jerusalinsky *et al.*, 1994). A role of the entorhinal cortex at the time of training has been shown to be necessary for the post-training late intervention of this structure in memory consolidation (Willner *et al.*, 1993).

Retrieval

All the structures studied play a role at the time of retention testing, and for this intact glutamate AMPA receptors are necessary. The role

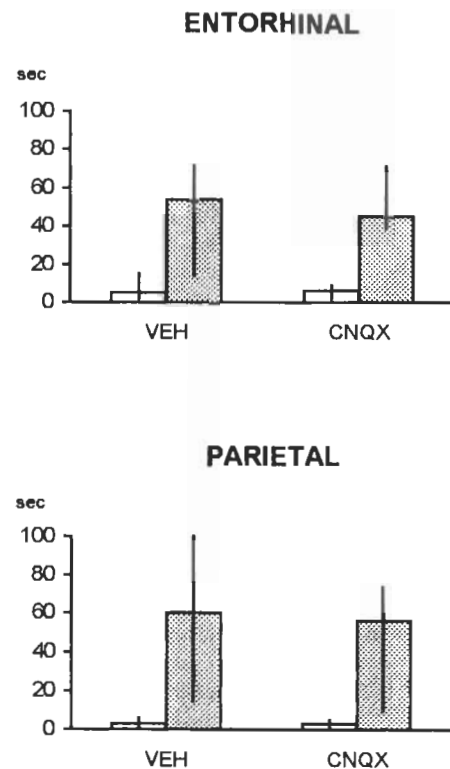


FIG. 7. Median (interquartile range) training (white columns) and test session (light grey columns) in animals that received bilateral vehicle (VEH) or CNQX, 0.5 g infusions into entorhinal or parietal cortex, 10 min prior to training. Training-test interval, 24 h. Training footshock: 0.3 mA. n per group as in Figure 6. There were no significant drug effects at $P = 0.1$ level in either training or test session scores. In all cases, training-test latency differences were significant at $P < 0.002$ level (Mann-Whitney U -tests, two-tailed).

of the hippocampus and amygdala on retention test performance lasts less than a month, that of the entorhinal cortex lasts at least a month, and the role of the parietal cortex lasts at least 2 months. CNQX given into hippocampus and amygdala blocks retrieval 1 day [and, as shown elsewhere (Bianchin *et al.*, 1993), 20 days after training]; but not 31 days after training. CNQX given into the entorhinal cortex blocks retention test performance 1 or 31, but not 60, days after training. CNQX given into the parietal cortex blocks retention test performance 1, 31 or 60 days after training. The effect of CNQX into these two cortical areas was apparently not due to an influence on performance variables, since the drug did not affect either acquisition of the avoidance task or free exploration of the training apparatus (see also Quillfeldt *et al.*, 1994 for a similar negative finding on the entorhinal cortex). Intra-amygdala or intrahippocampal CNQX has no effect on exploratory activity (Izquierdo *et al.*, 1993a; Quillfeldt *et al.*, 1996).

Lidocaine infusion into the prefrontal and insular, but not perirhinal, cortex has been reported to hinder long-term retrieval (Liang and Liao, 1995). Unlike CNQX which acts specifically upon glutamate AMPA/kainate receptors, lidocaine would be expected to affect *all* cells and fibres of passage as well, and its use may therefore yield spurious results (Martin, 1991; Izquierdo *et al.*, 1993a, b; Izquierdo and Medina, 1996, 1997). The use of receptor-specific drugs is to be preferred over that of local anaesthetics in localized brain infusion experiments (Zanatta *et al.*, 1996).

General aspects of the organization of memory

The findings with post-training AP5 and with pre-test CNQX infusions are coherent with the possibility that memory uses mechanisms similar to those of long-term potentiation (Izquierdo *et al.*, 1992, 1993a, b; Izquierdo and Medina, 1995; Maren and Baudry, 1995). It seems likely that the brain uses similar biochemical cascades for generating and establishing long-lasting synaptic plastic changes, be they those of memory or those of long-term potentiation (Izquierdo and Medina, 1996, 1997). Therefore, the most parsimonious conclusion from the present findings is that glutamatergic NMDA synaptic transmission is necessary first in hippocampus and amygdala, later in the entorhinal cortex, and finally in the parietal cortex for post-training memory processing; and that regular glutamatergic AMPA transmission is necessary in the same structures later on, for memory expression.

The findings on pre-test CNQX effects certainly suggest, but do not demonstrate that memories are effectively stored in any of the structures studied. They merely indicate that different brain structures are *involved* or play a *major role* in the circuits relevant to memory formation of step-down inhibitory avoidance at different times after original learning, and that the same structures are *involved* or play a *major role* in the circuits relevant to retrieval of this task, in the same order, at 1, 31 or 60 days from original training. Our data suggest that different sets of brain areas are in charge of memory maintenance over time, and this implies that they may therefore mediate the eventual modifications that can then occur. New learnings, retrieval, fabrication, forgetting, dreaming, lesions (Izquierdo, 1989) and emotional states (Izquierdo and Medina, 1996, 1997) are known to modify memories long after acquisition.

Prominent post-training increases of ^{14}C -glucose or deoxyglucose uptake have been reported in various portions of the cingulate, frontal, parietal and pyriform cortex, in various nuclei of the thalamus and the hypothalamus, in the cerebellum, etc. (Bontempi *et al.*, 1991, 1996; Sif *et al.*, 1991; McIntosh and González-Lima, 1994; Meunier *et al.*, 1996). The regions involved and the times at which they show changes vary with the task and the session. These regions are connected to the hippocampus, entorhinal and parietal cortex (Witter *et al.*, 1989; Hyman *et al.*, 1990). Glucose uptake or other imaging methods (e.g. McCarthy, 1995) are not suitable for a survey of the regions that participate in memory processing (McIntosh and González-Lima, 1994; Bontempi *et al.*, 1996). The localized infusion technique may, if properly used, serve to indicate the neurotransmitter mechanisms or biochemical processes involved in each case.

In fact, both methods have changed our view of memory processing completely in the past few years. The old notion that some forms of learning depend on just one structure of the brain (the hippocampus, the amygdala, the cerebellum; see Izquierdo and Medina, 1995, 1997 for references) is simply no longer tenable. That notion was based largely on lesion studies or on the analysis of effects of systemic or intracerebroventricular injections. Lesions lead to complex synaptic rearrangements (Cotman *et al.*, 1981; Freund, 1996) and to the entry into play of vicarious circuits or systems (see Izquierdo, 1989). Non-localized injections are of course totally unspecific as to the structures really involved in the effects (Izquierdo, 1989; Izquierdo and Medina, 1995, 1996, 1997). Anatomically linked networks of interacting structures (McIntosh and González-Lima, 1994; Bontempi *et al.*, 1996) have now been shown to participate even in tasks as simple as step-down inhibitory avoidance (see above, and Tassoni *et al.*, 1992) and T-maze discriminative learning (Meunier *et al.*, 1991). Intra-entorhinal and entorhinal-hippocampal rapid reverberation of electrical activity has recently been described using imaging

techniques of high temporal resolution (Iijima *et al.*, 1996). This suggests various forms of interaction between these two structures in learning processing (see also Adey, 1961; Jerusalinsky *et al.*, 1994), and renders the notion of an independent operation of both structures in memory obsolete. Current evidence indicates that, in various species, different structures participate in memory processes through interacting serial and parallel cascades of biochemical processes regulated at different steps by diverse modulatory systems (Izquierdo and Medina, 1995, 1997; Rose, 1995; Bernabeu *et al.*, 1996).

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Abbreviations

AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolone propionic acid
AP5	D-2-amino-5-phosphono-pentanoic acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
GABA _A	γ -amino butyric acid type A
NMDA	<i>N</i> -methyl-D-aspartate

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