Effect of inhibitory avoidance training on [3H]-glutamate binding in the hippocampus and parietal cortex of rats

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Abstract

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Glutamate receptors have been implicated in memory formation. The aim of the present study was to determine the effect of inhibitory avoidance training on specific [3H]-glutamate binding to membranes obtained from the hippocampus or parietal cortex of rats. Adult male Wistar rats were trained (0.5-mA footshock) in a step-down inhibitory avoidance task and were sacrificed 0, 5, 15 or 60 min after training. Hippocampus and parietal cortex were dissected and membranes were prepared and incubated with 350 nM [3H]-glutamate (N = 4-6 per group). Inhibitory avoidance training induced a 29% increase in glutamate binding in hippocampal membranes obtained from rats sacrificed at 5 min ($P<0.01$), but not at 0, 15, or 60 min after training, and did not affect glutamate binding in membranes obtained from the parietal cortex. These results are consistent with previous evidence for the involvement of glutamatergic synaptic modification in the hippocampus in the early steps of memory formation.

Key words
- Glutamate receptor
- Hippocampus
- Parietal cortex
- Long-term potentiation
- Emotional learning
- Memory

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It has been postulated that long-term potentiation (LTP) of glutamatergic synapses is a cellular mechanism underlying learning and memory. Glutamate receptors are involved in both the induction and the expression of LTP. LTP induction depends on activation of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor channel and the metabotropic glutamate receptor (mGluR), whereas its maintenance and expression depend on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor channels (1).

As with LTP, glutamate receptors play a role in both the formation and the expression of memory for the step-down inhibitory avoidance task. Memory formation for this task depends on glutamate receptor activation in several brain areas. Among other structures, the hippocampus is particularly important in the early phase of memory formation, while the posterior parietal cortex plays a late role in consolidation (4). Post-training infusion of the NMDA receptor antagonist aminophosphonopentanoic acid impairs retention of inhibitory avoidance in rats when given into the hippocampus immediately after training (2,3), or when given into the posterior parietal cortex 180 min after training (4). Intrahippocampal infusion of the mGluR antagonist methyl-carboxyphenyl glycine immediately after training (5) or of the AMPA receptor antagonist cyanonitroquinoxaline-dione (CNQX) up to 3 h after

training (6) impairs inhibitory avoidance retention, while infusions of glutamate or of the mGluR agonist aminocyclopentane dicarboxylate enhance retention (5). Infusions of CNQX into the hippocampus (4,7) or parietal cortex (4,8) prior to test impair inhibitory avoidance expression.

In membranes extracted from the dorsal hippocampus, there is an increase of the NMDA receptor subunit NMDAR1 at 30 min after inhibitory avoidance training, and an increase of the AMPA receptor subunit GluR1 and of the B_{max} of AMPA to AMPA receptors from 30 min to 3 h after training (9-11).

In order to further investigate the involvement of glutamate receptors in the hippocampus and parietal cortex in memory for inhibitory avoidance, we verified the effects of inhibitory avoidance training on [³H]-glutamate binding in membranes obtained from the dorsal hippocampus or parietal cortex of rats at different times (0, 5, 15 and 60 min) after training.

Adult male Wistar rats (3 month old) from our own breeding colony were used. The animals were housed in plastic cages, with water and food *ad libitum*, under a 12-h light/dark cycle at a constant temperature. They were divided into three experimental groups: group 1 (control group): the animals were left in their home cages until they were sacrificed by decapitation. Group 2 (trained group): the animals were placed on a 2.5 cm high, 7.0 cm wide platform at the left of a 50.0 x 25.0 x 25.0 cm training apparatus, whose floor consisted of parallel stainless steel bars spaced 1.0 cm apart. In the training session, immediately upon stepping down, the animals received a 0.5-mA footshock for 5 s. The animals were withdrawn from the apparatus and placed in their home cages until they were sacrificed by decapitation 0, 5, 15 or 60 min after the training session. Group 3 (shocked group): the animals were placed directly on the electrified bars of the apparatus described above, received the

shock for 5 s and were immediately withdrawn from the box. They stayed in their home cages until they were sacrificed by decapitation 0, 5, 15 or 60 min after the shock session.

Membrane preparations. The rats were sacrificed by decapitation and the CA1 area of the dorsal hippocampus and the junction between posterior parietal cortex I and II were immediately removed. The areas dissected in the hippocampus and parietal cortex were chosen on the basis of previous reports (2,4-8) on the effects of infusions of glutamate receptor antagonists on those areas. Membranes were prepared as previously described (12). Tissues were homogenized in 20 volumes of 0.32 M sucrose, 10 mM Tris/HCl and 1 mM $MgCl_2$ buffer, pH 7.4, with a glass homogenizer. The homogenate was centrifuged twice at 1,000 g for 15 min at 4°C and the pellets were discarded. The supernatants were pooled and centrifuged at 27,000 g for 15 min. The resulting pellet was lysed in a 10 mM Tris/HCl buffer, pH 7.4, for 30 min and centrifuged at 27,000 g for 15 min. The resultant pellet was washed three times in 10 mM Tris/HCl buffer, pH 7.4, and centrifuged at 27,000 g for 15 min. All steps were carried out at 4°C.

[³H]-Glutamate binding. [³H]-Glutamate binding assays were carried out as previously described (13). Membranes were incubated in 0.5 ml reaction mixture containing 25 mM Tris/HCl, pH 7.4, 5 mM $MgCl_2$ and 350 nM [³H]-glutamate (49 Ci/mmol; Amersham International, Amersham, Buckingham, UK). Incubation was carried out at 30°C for 15 min and the reaction was stopped by centrifugation at 27,000 g for 15 min. The pellet and the wall of the tube were quickly and carefully washed with ice-cold distilled water. SDS (0.1%) and scintillation liquid were added to the dry pellet and radioactivity incorporated was determined with a Wallac scintillation counter. All the assays were carried out in triplicate. Nonspecific binding was determined by adding 350 µM

nonradioactive glutamate to the reaction mixture in a parallel assay. Specific binding was considered to be the difference between total and nonspecific binding. Protein was measured by the method of Lowry et al. (14).

Statistical analysis. Results are reported as percent of control specific binding and were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test when necessary.

Results for specific [³H]-glutamate binding in membranes obtained from the hippocampus are shown in Figure 1A, and results for membranes obtained from the parietal cortex are shown in Figure 1B. Although training-induced changes in glutamate receptor subunits in the dorsal hippocampus have been reported in previous studies (9-11), we evaluated glutamate binding in this area in the present study in order to have a comparison basis for the results obtained in the parietal cortex. There was a transient increase in [³H]-glutamate binding in hippocampal membranes obtained 5 min after the training session (P<0.01) (Figure 1A). This increase was specific for inhibitory avoidance training because glutamate binding was not altered in the shocked group. Although we did not include a control group with rats allowed to freely explore the training box, previous reports have shown that free exploration of the box did not affect glutamate receptor binding when compared to shocked animals or to naive control animals (10,11). In contrast to hippocampal membranes, [³H]-glutamate binding in the parietal cortex was not affected by inhibitory avoidance training. However, there was a decrease in [³H]-glutamate binding in membranes obtained from the parietal cortex in the group given a footshock and sacrificed 15 min later (Figure 1B). Further experiments will be necessary to clarify the causes and the biological significance of this footshock-induced decrease in glutamate binding in the parietal cortex.

Our results are consistent with previous

pharmacological evidence showing a role of glutamate receptors in the hippocampus in the formation of memory for the step-down inhibitory avoidance task early after training (2-7). In addition, our results are in agreement with previous neurochemical evidence showing glutamate binding changes following training in different behavioral tasks. Classical conditioning of the rabbit eyelid response increases glutamate binding in synaptic membranes obtained from the hippocampus (15), and Tocco et al. (16) showed that classical conditioning increases AMPA receptor binding in the hippocampus of rabbit nictitating membranes. Chicks trained in a passive avoidance task show increased NMDA receptor-sensitive [³H]-glutamate binding in the left lobus parolfactorius and in the left intermediate medial hyperstriatum ventrale (17). In rats, there is an increase of the NMDA receptor subunit NMDAR1 at 30 min after inhibitory avoidance training, and an increase of the AMPA receptor subunit GluR1 and of the B_{max} of its receptors in
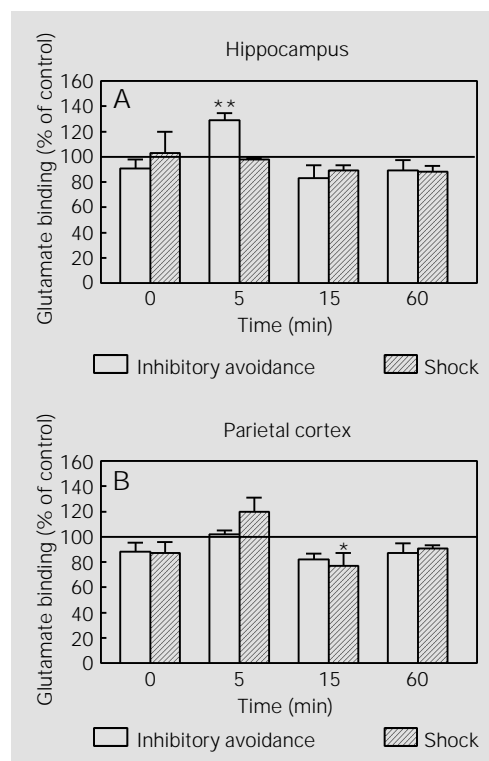

Figure 1 - Effect of inhibitory avoidance task or footshock on [³H]-glutamate binding. Hippocampal (A) or parietal cortex (B) membranes obtained from control rats or from rats submitted to shock or inhibitory avoidance and sacrificed 0, 5, 15 or 60 min after session were incubated with 350 nM [³H]-glutamate (N = 4-6 per group). Data are reported as mean ± SEM percentage of control values and were analyzed by one-way analysis of variance followed by Duncan's multiple range test when the F-test was significant. Statistically significant differences between control and experimental groups (**P<0.01; *P<0.05) are indicated. F value for comparison of hippocampus data between control and 5-min groups, $F_{(2,9)}$ = 24.36 (A). F value for comparison of parietal cortex data between control and 15-min groups, $F_{(2,9)}$ = 3.58 (B).

hippocampal membranes from 30 min to 3 h after training. (9-11).

It is possible that the increased [^{3}H]-glutamate binding found in the present study is associated with changes in NMDA receptors because, unlike the binding changes seen in AMPA receptors (11), it was a rapid and transient effect, similar to the amnestic effects of intrahippocampal infusions of NMDA receptor antagonists that impair inhibitory avoidance when given immediately after training, but not at 30-180 min after training (2-4). The lack of training-induced changes in glutamate binding in membranes obtained from the parietal cortex up to 60 min after training might be correlated with the delayed participation of NMDA receptors in the parietal cortex in the formation of memory for inhibitory avoidance (4).

In summary, the present study shows that inhibitory avoidance training induces a change in [^{3}H]-glutamate binding in hippocampal membranes, but not in membranes obtained from the parietal cortex at 5 min after training. It is consistent with previous pharmacological and neurochemical evidence showing a role for hippocampal glutamate receptors in the early stages of formation of memory for inhibitory avoidance.

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