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Effects of Intraseptally Injected Glutamatergic Drugs on Hippocampal Sodium-Dependent High-Affinity Choline Uptake in "Naive" and "Trained" Mice

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MARIGHETTO, A., J. MICHEAU AND R. JAFFARD. *Effects of intraseptally injected glutamatergic drugs on hippocampal sodium-dependent high-affinity choline uptake in "naive" and "trained" mice.* PHARMACOL BIOCHEM BEHAV 49(3) 689-699, 1994.—We have previously reported that spatial reference memory (RM) training-induced alterations in hippocampal cholinergic activity as measured by sodium-dependent high-affinity choline uptake (SDHACU). Each training session was found to induce an immediate (30 s) increase in SDHACU followed (30 s to 15 min posttest) by a deactivation and long-lasting inhibition (15 min to 24 h) of this cholinergic marker. The present experiments were designed to assess the role of septal glutamatergic receptors in this posttraining cholinergic deactivation. In the first experiment, the effects of intraseptal injections of different doses of glutamic acid and glutamatergic antagonists (kynurenic acid, KYN, and AP5) on hippocampal SDHACU were studied in awake but otherwise resting (i.e., naive) mice. The results showed that glutamic acid at the lowest dose used (5 ng) produced a decrease in SDHACU, whereas both glutamatergic antagonists produced a dose-related increase in this cholinergic marker. It was concluded that septal glutamatergic receptors mediate a tonic inhibitory input on the cholinergic cells. Hence, in a second experiment the effect of intraseptal injections of KYN (5 ng) on the training-induced changes in hippocampal cholinergic activity were assessed following variable amounts of radial maze RM training. Trained mice were injected 20 min before the first or the ninth training session and killed 30 s or 15 min posttraining for determination of SDHACU. KYN slowed the posttesting cholinergic deactivation (disinhibition), this effect being more marked in good learners than in bad learners. The present findings suggest that septal glutamatergic receptors mediate an inhibitory input on the cholinergic cells, and that this input could play a role in memory consolidation.

High-affinity choline uptake Septum Septo-hippocampal pathway Glutamatergic-cholinergic interactions
Spatial reference memory Mouse

THERE is increasing evidence that central cholinergic neurons play a role in the cognitive processes involved in learning and memory. However, the exact role of acetylcholine (ACh) is still unresolved because a large body of evidence derived from pharmacological studies based on the systemic administration of cholinergic drugs has given rise to conflicting hypotheses [for reviews, see (13,19)]. The absence of consensus is understandable in view of the differential involvement of each of the central cholinergic pathways in different aspects of cognition as outlined by lesion studies. Overall, the lesion of one particular cholinergic pathway will tend to reproduce the behavioral effects of lesioning the area of projection of these cholinergic fibers (10,32). This strongly suggests that cholinergic modulation of the target area would have an important influence on specific cognitive processes occurring in this area. The septo-hippocampal cholinergic pathway is one of the major subcortical inputs to the hippocampus and is likely to participate in various learning and memory processes. Lesions of the medial septum or fornix have been found to lead to behavioral impairment in a wide variety of tasks, including habituation [e.g., (36)], spatial memory [e.g., (18,24,33)], and/or working memory [e.g., (25,30)]. Local application of

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drugs designed to interfere acutely with cholinergic activity have reproduced some but not all of the behavioral effects of septal lesions [see (35)]. For example, intraseptal injection of a GABAergic agonist was found to have differential and sometimes opposite effects on the same task, depending on the time of treatment (34). A pretraining injection produced an impairment both in a spatial task and in passive avoidance, whereas posttraining injections had no effect on passive avoidance but improved subsequent retention of spatial learning. This suggests that the septo-hippocampal cholinergic contribution to memory processes may be both task and phase dependent.

One way of addressing this issue is by investigating the dynamic alterations in cholinergic transmission contingent on specified forms and phases of memory processing. If the septo-hippocampal cholinergic neurons are specifically involved in certain aspects of memory, their activity should reflect memory processing as a function of both the type and the phase of memory currently being tapped. The kinetics of sodium-dependent high-affinity choline uptake (SDHACU) in brain samples has been found to be positively correlated with the level of presynaptic cholinergic activity at the time of killing. Therefore, the measure of SDHACU in hippocampal synaptosomes can be used as a dynamic in vitro neurochemical index of the in vivo neuronal activity of the septo-hippocampal cholinergic neurons at the particular time of sacrifice (1,26). By using this technique, several authors have shown that memory testing induces alterations in hippocampal cholinergic activity (9,14,37,46). In a previous study (29), we observed that radial arm maze training produced significant changes in hippocampal SDHACU in mice killed at different intervals after the end of testing with respect to naive controls. Specifically, spatial discrimination training produced an acute increase (at 30 s posttest) followed by a secondary decrease and long-lasting inhibition (from 15 min to 24 h and even several days posttest) of hippocampal cholinergic activity. This secondary decrease and the long-lasting inhibition of the septo-hippocampal cholinergic pathway were likely to be specific to Reference-Memory (RM) because testing-induced decreases in SDHACU have only been observed following spatial (9) and nonspatial (39) RM training. In contrast, short-term working memory (WM) tasks have been found to result in a long-lasting increase in hippocampal SDHACU (29,46). Moreover, the time course of the secondary decrease in SDHACU induced by RM training was found to be dependent on both degree of training and individual performance (9,29). Taken together, these observations led us to postulate that: a) an active inhibitory mechanism mediates the decrease and long-lasting inhibition of septo-hippocampal cholinergic neurons following RM but not WM training; b) post-RM training inhibition of cholinergic activity is involved in the consolidation of permanently valuable information (RM).

The aim of the present study was to identify, by infusing drugs in the septal area, the septal receptors that might mediate the observed posttraining deactivation of the septo-hippocampal cholinergic system. Pharmacological identification of the inhibitory mechanism supposedly involved in the testing-induced decrease in hippocampal SDHACU would then enable us to assess the behavioral significance of this change by local application of drugs designed to interfere selectively with this process in animals submitted to spatial RM testing.

Numerous receptors have been identified in the septal area, and intraseptal injection of drugs has indicated that some receptors mediate a tonic or phasic regulation of septo-hippocampal cholinergic activity (6). It is now well established that intraseptal injection of a GABAergic agonist results in a dose-related decrease in hippocampal markers of cholinergic activity (2,17). The effects of GABAergic antagonists, however, are still unclear [cf., (6,40) vs. (47)], as is the case for other mediators identified in this region. The experiments reported here were designed to examine the involvement of septal glutamatergic receptors in the training-induced decrease in hippocampal cholinergic activity. Their involvement in this process was surmised from the following considerations: a) the glutamatergic fibers that project from the hippocampus to the lateral septum (22,23) have synapses on GABAergic neurons (20,21,28). These GABAergic neurons might thus constitute a relay between the lateral glutamatergic synapses and the cholinergic cells in the medial septal area (4), suggesting that glutamatergic receptors mediate an indirect (GABAergically mediated) inhibition on the septo-hippocampal cholinergic system. This idea is supported indirectly by electrophysiological studies showing that electrical stimulation of fimbria fibers results in a polysynaptic hyperpolarization of medial septum cells that are thought to be cholinergic (8,12,31); b) The secondary post-RM training decrease in SDHACU was found to result in a long-lasting (24 h to 9 days) inhibition of the septo-hippocampal cholinergic path, suggesting that a long-term potentiation (LTP)-like mechanism might be responsible for this training-induced cholinergic alteration. It has been demonstrated that a long-lasting increase in synaptic efficiency in the lateral septum can be produced by high-frequency stimulation of fimbria fibers, both in vitro (43,45) and in vivo (15,38). Morever, glutamatergic antagonists have been found to block this LTP phenomenon (43,45).

These observations suggest that, among the different receptors of the septal region, glutamatergic receptors are good candidates for mediation of the secondary decrease and long-lasting inhibition in hippocampal SDHACU produced by RM testing.

EXPERIMENT 1

As a preliminary step, this experiment was designed to determine the effect of pharmacological stimulation and blockade of septal glutamatergic receptors on hippocampal SDHACU in mice kept under resting conditions (referred to as basal activity of the septo-hippocampal cholinergic system). Our hypothesis was that an increase in glutamatergic hippocampal-septal transmission induced by intraseptal infusion of glutamatergic agonists should reduce septo-hippocampal cholinergic activity. Moreover, an increase in the hippocampal SDHACU as a result of glutamatergic antagonists injections would indicate that glutamatergic receptors mediate a tonic inhibition of the septo-hippocampal cholinergic pathway.

METHOD

Animals

One hundred and fourteen male mice of the C57Bl.6JIco strain obtained from IFFA Credo, Lyon, France were used. They were aged between 6 and 8 weeks on arrival in the laboratory, and were housed in a environment-controlled animal room on a 12 L : 12 D cycle with free access to food and water. After 3 to 5 weeks, the animals were placed in individual cages. Surgery was started a fortnight later.

TABLE 1

SUMMARY OF EXPERIMENTAL DESIGN OF EXPERIMENT 1

Experiment 1A	Experiment 1B	Experiment 1C	Experiment 1D
Bilateral injection into the lateral septum	Unique injection into the medial septum	Bilateral injection into the lateral septum	Bilateral injection into the lateral septum
Vehicle [7]	Vehicle [5]	Vehicle [7]	Vehicle [7]
2.5 ng GLU [7]	2.5 ng GLU [6]	5 ng KYN [8]	10 ng AP5 [8]
25 ng GLU [7]	250 ng GLU [6]	50 ng KYN [7]	100 ng AP5 [7]
250 ng GLU [8]		500 ng KYN [8]	1000 ng AP5 [8]

GLU: glutamic acid; KYN: hynurenic acid; AP5: 2-amino-5-phosphonovaleric acid. Numbers in brackets indicate number of animals.

Surgery

Mice were implanted under general anaesthesia (sodium thiopental 70 mg/kg IP) with either one or two guide cannulae (0.4 mm diam; 8 mm long) aimed vertically towards the medial septum or each of the lateral septal nuclei. Guide cannulae were fixed to the skull bone using dental cement and fine bone screws. Stereotaxic coordinates used were: 0.6 mm anterior to the bregma, 1.9 mm ventral from the skull surface and 0.35 mm laterally for bilateral implantation towards the lateral septum.

Injections

Injections into the septal area were performed in freely moving mice via injection cannulae (0.2 mm diam., 9.3 mm long) attached to a 1 μl Hamilton syringe via polyethylene catheter tubing. The syringe was held in a constant rate infusion pump until a volume of 0.2 μl had been injected (3-min period). In all cases, correct injection flow rates were checked visually. The cannula was left in place for a further 4 min before removal.

Drugs

Glutamic acid was purchased from Sigma. Both the glutamatergic antagonists, kynurenic acid and 2-amino-5-phosphonovaleric acid (AP5) were obtained from Research Biochemicals Incorporated (Natick, MA). Solutions of drugs were prepared freshly before use by dilution in sterile saline.

Neurochemical Analysis

Animals were killed by brief immersion in liquid nitrogen followed by immediate decapitation and dissection of the brain. The hippocampus was rapidly dissected on a glass plate placed over liquid nitrogen vapour. In vivo cholinergic activity was quantified by measuring the rate of sodium-dependent high-affinity choline uptake (SDHACU) in crude synaptosomal (P2) fractions from fresh samples of hippocampus according to Atweh et al. (1), as modified by Durkin et al. (11). Briefly, P2 samples (about 1 mg/ml protein) were incubated in normal Krebs-Ringer buffer and also in sodium-free medium (NaCl was replaced by equimolar LiCl) at pH 7.4 for 4 min at 37°C, in the presence of 0.25 mM external (methyl-^{3}H)choline chloride (Amersham, France). Uptake was terminated by filtration on HA-type 0.45 μm pore size filters (Millipore). Filters were dissolved and counted in a liquid scintillation counter (Beckman). The amount of choline taken up by the sodium-dependent high-affinity mechanism was calculated from the difference between the sodium and sodium-free incubations.

General Procedure

In order to match the different experiments as closely as possible, all mice were subjected to a progressive food-deprivation schedule before being injected and sacrificed for neurochemical analysis. In the memory-testing experiments (see Experiment 2) mice were food deprived as the tasks were food motivated.

Ten days after surgery, animals were gradually and partially food deprived so they weighed around 90% of their ad lib weight by the fourth day. During this period they were handled individually twice a day and restrained by the experimenter. This manipulation was aimed at facilitating the subsequent injection procedure and at reducing the fear associated with the injection protocol.

The injections were carried out on the fourth day of food deprivation in 4 independent experiments as detailed in Table 1. The injections were performed outside the animal room. However, the animals were moved back to their home cage in the animal room as soon as the injection was over and they stayed there for 20 min before being sacrified for neurochemical assays.

RESULTS

Analysis of variance (ANOVA) including the factor treatment (with either three or four levels, depending on the number of doses used) was performed for each experiment. It was followed by Dunnett -tests between individual groups.

Effects of Glutamic Acid (GLU)

In this experiment, GLU was injected bilaterally into the lateral or medial septum. The results from these two experiments (1A and 1B) are presented in Fig. 1.

The effect of treatment was not significant [medial septum: $F(2, 14) = 2.7$, $p = 0.10$; lateral septum: $F(3, 25) = 1.55$, $p = 0.23$]. However, an ANOVA performed on the data from both experiments indicated that there was a significant effect of treatment [vehicle vs. GLU 2.5 ng vs. GLU 250 ng; $F(2, 33) = 4.97$, $p = 0.014$], irrespective of the site of injection (interaction treatment $\times$ site, $F = 0.28$). Compared to the groups treated with either the vehicle or 250 ng of GLU, animals receiving 2.5 ng of GLU displayed a significant decrease in SDHACU (respectively -17.6% and -16.6%; $p < 0.05$ in both cases).

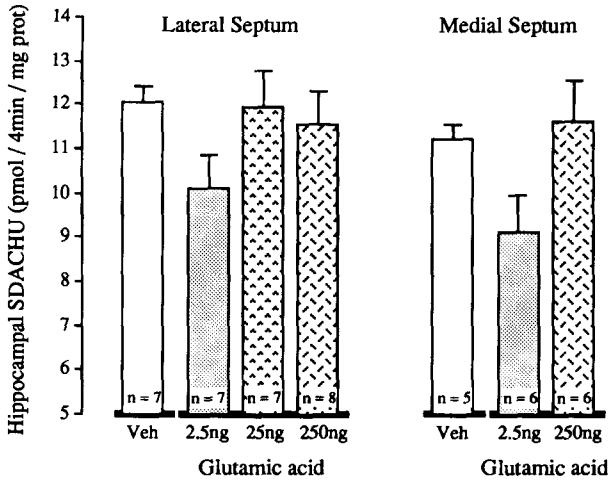

FIG. 1. Dose-response on basal hippocampal SDHACU of glutamic acid (GLU) after injection into the medial septum (left) and bilateral injection into the lateral septum (right).

Effects of Glutamatergic Antagonists

Kynurenic acid (KYN). The results (Experiment 1C) are summarized in Fig. 2.

There was a significant effect of treatment, $F(3, 25) = 4.3$, $p = 0.015$. The bilateral injection of 5 ng KYN resulted in a significant increase in SDHACU as compared to vehicle injection (+16.7%; $p < 0.02$). The two higher doses of KYN (50 ng and 500 ng) had no effect ($p > 0.30$ with respect to vehicle).

2-Amino-5-phosphonovaleric acid (AP5). The results (Experiment 1D) are summarized in Fig. 3.

There was a significant effect of treatment, $F(3, 29) = 11.8$, $p < 0.0001$. With respect to vehicle, the intraseptal injection of 10 ng of AP5 did not produce any significant change in SDHACU (−6.8%, $p > 0.20$), but with respect to vehicle, the injection of 100 ng and 1 μg of AP5 resulted in a significant increase in choline uptake (AP5 100 ng, +19.4%; and AP5 1 μg, +21.0%; $p < .01$ in both cases).

DISCUSSION

In quiet animals under basal conditions, intraseptal injections of glutamic acid (GLU) in lateral or medial septum had no statistically significant effects on hippocampal SDHACU. However, the lowest dose used (2.5 ng), irrespective of the site of injection, consistently induced a significant decrease (−15%) in choline uptake. Conversely, intraseptal injections of either the glutamatergic antagonists kynurenate (KYN) or 2-amino-5-phosphonovaleric acid (AP5) led to a dose-dependent increase in hippocampal SDHACU. Taken together, these findings are consistent with the idea that septal glutamatergic receptors mediate an inhibitory input to the septo-hippocampal cholinergic neurons. This conflicts with the proposal of Costa et al. (6) in which septal glutamatergic receptors provide a phasic excitatory input to cholinergic neurons. Although our present data provide no evidence for such an excitatory function, the observed dose-dependent effects of both the agonist (GLU) and the antagonists (KYN and AP5) may not be in accord with an inhibitory influence.

Our observation that the inhibition of SDHACU resulting from intraseptal injections of GLU at 2.5 ng completely disappeared at higher doses (25 and 250 ng) is somewhat puzzling. This could, perhaps, be accounted for by excitotoxic effects of this extremely powerful excitatory amino acid (41) on septal cells, which could also account for the earlier findings of Costa et al. (6). These authors observed that intraseptal injections of 500 ng of kainic acid did not alter hippocampal turnover of ACh in control mice (our own data are consistent with this observation), whereas it reduced the phenobarbital-induced decrease in ACh turnover in anesthetised animals. Because septal GABAergic neurons have been shown to be

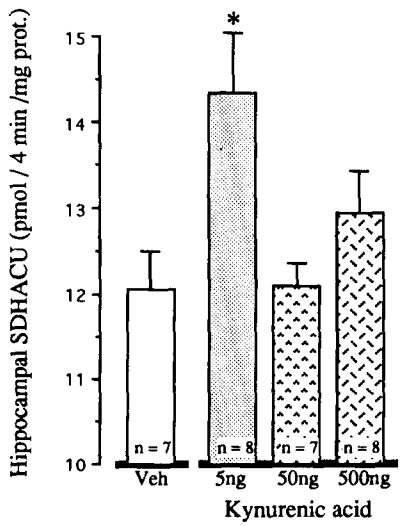

FIG. 2. Effects of bilateral injection into the lateral septum of different doses of kynurenic acid (KYN) on basal hippocampal SDHACU. *Significantly different from vehicle $p < 0.02$.

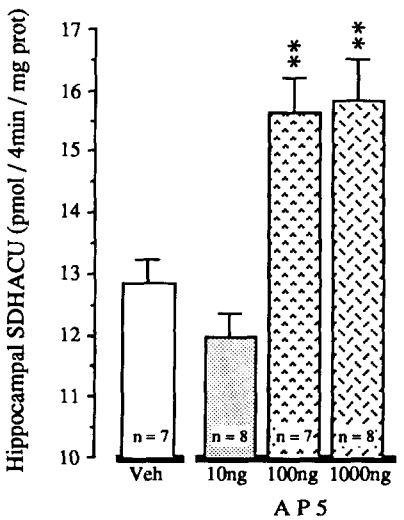

FIG. 3. Dose-response of bilateral injections of AP5 into the lateral septum on basal hippocampal SDHACU. **Significantly different from vehicle $p < 0.01$.

involved in barbiturate-induced inhibition of septo-hippocampal cholinergic neurons, Costa and his collaborators proposed that the disinhibitory effect of kainic acid injection on cholinergic activity was mediated via two serially interposed GABAergic interneurons linking excitatory glutamatergic afferents in the lateral septum with cholinergic neurons of the medial septum-diagonal band [see also (4)]. In this interpretation, the acute activation of the first set of interneurons by kainic acid inhibits the second set, producing a phasic disinhibition of septo-hippocampal cholinergic activity. In common with our data, these findings could, however, be explained by the excitotoxicity of both of these amino acids. Intraseptal injection of either GLU or kainic acid could, as a result of the overexcitation they produce, temporarily reduce the excitability of (a unique set of) GABAergic neurons, thereby producing the disinhibition of septo-hippocampal cholinergic activity observed in the anesthetised mice.

Whatever the case, injections of both the glutamatergic antagonists KYN (at the lowest dose) and AP5 (at the two highest doses) produced a significant increase in hippocampal SDHACU, with no evidence for the opposite pattern of changes at the other doses. These observations are consistent with an inhibitory influence of glutamatergic receptors on septo-hippocampal cholinergic neurons. KYN is reported to have greater affinity towards the glycine site of the NMDA complex than towards the other two glutamatergic receptor types (5), whereas AP5 is considered to be a more universal NMDA antagonist (7). The similarity in the effects of the low dose of KYN and the higher doses of AP5 may be a reflexion of selective blockade of NMDA receptors. This implies that septal NMDA receptors mediate a tonic inhibition on the septo-hippocampal cholinergic pathway. This seems unlikely because it is generally accepted that NMDA receptors contribute little to basal glutamatergic synaptic transmission, but appear to be involved in the extreme cases of intense synaptic activity (43). Other reports, however, have indicated that NMDA receptors do contribute to this basal glutamatergic transmission in both the hippocampus and the laterodorsal septum (45). The lack of effect of the highest doses of KYN we observed is difficult to explain because this antagonist has been shown to block glutamatergic transmission in the lateral septum (42).

In conclusion, the results of this experiment supports the idea that, in resting mice, septal glutamatergic receptors mediate an inhibitory input to septo-hippocampal cholinergic neurons (see the final conclusion for further discussion). However, further experiments will be required to delineate the contribution of each receptor subtype, and assess the apparent tonic-inhibitory influence mediated via NMDA receptors.

EXPERIMENT 2

As pointed out in the introduction, previous experiments (29) showed that spatial discrimination training (reference memory: RM) induced biphasic alterations in hippocampal cholinergic activity. Each training session was found to result in an immediate and short-lived increase followed by a decrease and long-lasting inhibition of hippocampal SDHACU. Moreover, the time course of the secondary posttesting decrease in SDHACU was found to be correlated with both the degree of training and the individual profile of learning. Together with other reports relative to testing-induced changes in cholinergic activity (see introduction), these observations led us to postulate involvement of an active inhibitory mechanism in the observed decrease and long-lasting inhibition of activity of the septo-hippocampal cholinergic neurons following RM training.

Because the results of Experiment 1 suggested that the hippocampal glutamatergic fibers ending in the lateral septum exert an inhibitory influence on the septo-hippocampal cholinergic path, the following experiment was designed to assess the possible mediation of glutamatergic receptors in the post-RM training decrease in hippocampal cholinergic activity. We, therefore, studied the effects of intraseptal injections of the glutamatergic antagonist KYN on hippocampal SDHACU in both resting naive controls and in mice trained on a spatial RM task. The dose of 5 ng of KYN was chosen because the results of experiment one showed that this dose produced significant alterations in hippocampal SDHACU.

METHOD

Methods were the same as in Experiment 1, except that half the mice were submitted to spatial discrimination training.

Animals

One hundred and seven mice of the C57Bl6JIco strain were used. Under general anaesthesia, each animal was implanted with two guide cannulae above the lateral septal nuclei. After recovery, all the animals were subjected to the food-deprivation schedule described in Experiment 1. They were randomly assigned to two groups: a quiet (n = 52) and an active group (n = 55). The animals in the active group were trained in a radial arm maze task for either one (n = 27) or nine daily sessions (n = 28).

Apparatus and Behavioral Training

The apparatus and the behavioral design have been fully described elsewhere (29). Briefly, an elevated, fully automated 8-arm radial maze was used. Each mouse was assigned a constant set of three baited arms. They were chosen such that the three angles joining them were always 90°, 135°, and 135°. At the beginning of each trial, each of these three arms was prebaited with a single food pellet. The mouse was allowed to move freely in the maze until the last reward had been collected. A daily session consisted of six trials with an intertrial interval of 1 min when the animal was confined to the central platform of the maze. In the present experiment, two parameters were recorded: a) reference memory (RM) errors: each RM error was defined as the first entry into any nonbaited arm on any one trial (with a maximum of five per trial). b) Within- and between-sessions improvement in performance. The within-session improvement was assessed by taking the difference between the number of RM errors made in trials 4–6 and trials 1–3 within the same session, whereas the reduction in RM errors over trials 1–3 of the nth session relative to trials 4–6 of the $(n-1)$th session was used to evaluate between-session improvement. In the previous study (29), two subgroups of animals were distinguished on the basis of these scores. Good learners displayed a certain degree of between-session improvement, whereas bad learners only improved within the sessions. The same type of analysis was used in the present experiment.

General Procedure

The active groups received different amounts of training. They were subjected to either one or nine sessions of training with a 24-h gap between sessions.

Animals were bilaterally injected into the lateral septum (as

previously described in Experiment 1) with either 0.2 μl of physiological saline or the same volume containing 5 ng of KYN. Active animals were injected 20 min before either the first or the ninth session of training. In each condition, half of them were sacrificed for neurochemical analysis immediately after the end of the session (30 s posttest group), whereas the other half were killed after an additional interval of 15 min (15 min posttest group). Because the duration of a training session depends on the degree of training (about 30 min for the first session vs. 15 min for the ninth), the interval between injection and sacrifice differed among experimental conditions and ranged from a minimum of 35 min (animals sacrificed 30 s after the ninth session of training) to a maximum of 65 min (mice killed 15 min after the first session). Quiet animals were injected with vehicle or KYN and sacrificed at different intervals after the injection (35, 50, or 65 min) to match the injection–sacrifice interval of each active group. As detailed in Table 2, four experiments were performed, each corresponding to a specified condition of amount of training and posttest interval.

RESULTS

An ANOVA including both the factor treatment (vehicle vs. 5 ng KYN) and the factor behavior (quiet vs. active) was carried out on hippocampal SDHACU measured in each condition of amount of training and posttest interval.

After the First Training Session

The results (Experiments 2A and 2B) are summarized in Fig. 4.

30 s posttest. There was no effect of treatment ($F < 1$), but a main effect of behavior, $F(1, 27) = 42.5$, $p < 0.0001$. Thus, whatever the treatment (interaction treatment × behavior: $F < 1$), the training session resulted in a significant (+25%) increase in hippocampal SDHACU relative to the quiet condition (see fig. 4, on the left).

15 min posttest. There was no effect of treatment, but, here again, a significant effect of behavior, $F(1, 19) = 20.4$, $p < 0.001$. However, a tendency towards a treatment × behavior interaction was observed, $F(1, 19) = 3.4$, $p = 0.08$. Specifically, for the 15 min posttest interval, hippocampal SDHACU did not significantly differ between quiet and active conditions in the vehicle-treated groups, whereas SDHACU was still significantly increased in KYN-treated active mice relative to their matched quiet controls (see Fig. 4, on the right).

As also can be seen in Fig. 4, SDHACU in the trained animals did not differ as a function of treatment at any posttest interval. In the quiet condition, however, the intraseptal injection of KYN that had no effect on SDHACU when measured 50 min postinjection, actually produced a slight decrease in SDHACU 65 min postinjection.

After the Ninth Training Session

The results (Experiments 2C and 2D) are summarized in Fig. 5.

30 s posttest. There was no effect of treatment, but a main effect of behavior, $F(1, 26) = 9.9$, $p < 0.01$. Following the completion of this ninth session, hippocampal SDHACU was, thus, elevated with respect to the resting control condition. However, this increase was only significant in the vehicle-treated animals ($p < 0.05$). As can also be seen in Fig. 5

TABLE 2

SUMMARY OF EXPERIMENTAL DESIGN OF EXPERIMENT 2

Behavior	Amount of Training	Treatment	Delay of Survival Posttest	Delay of Survival Postinjection
Exp. 2A				
Trained [8]	One session	Vehicle	30 s	50 min
Trained [8]	One session	5 ng KYN	30 s	50 min
Naive [6]	–	Vehicle	–	50 min
Naive [8]	–	5 ng KYN	–	50 min
2B				
Trained [6]	One session	Vehicle	15 min	65 min
Trained [5]	One session	5 ng KYN	15 min	65 min
Naive [6]	–	Vehicle	–	65 min
Naive [6]	–	5 ng KYN	–	65 min
2C				
Trained [7]	9 sessions	Vehicle	30 s	35 min
Trained [7]	9 sessions	5 ng KYN	30 s	35 min
Naive [5]	–	Vehicle	–	35 min
Naive [7]	–	5 ng KYN	–	35 min
2D				
Trained [7]	9 sessions	Vehicle	15 min	50 min
Trained [7]	9 sessions	5 ng KYN	15 min	50 min
Naive [6]	–	Vehicle	–	50 min
Naive [8]	–	5 ng KYN	–	50 min

KYN: kyurenic acid. Numbers in brackets indicate number of animals.

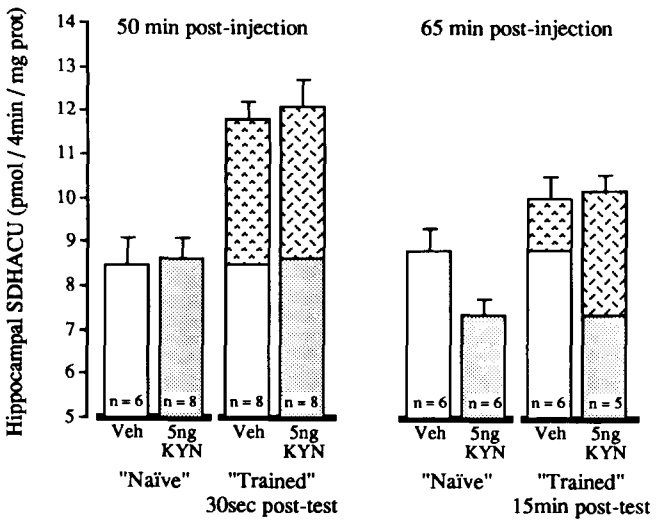

FIG. 4. On the left: hippocampal SDHACU in naive (quiet) mice and in mice trained on one session of spatial discrimination. All animals had received a bilateral injection in the lateral septum of either vehicle or 5 ng KYN 50 min before sacrifice. Trained animals were injected 20 min before the start, and killed immediately at the end of the session (30 s posttest). On the right: the same as on the left except that all animals had received the bilateral injection of either vehicle or 5 ng KYN 65 min before sacrifice, so that the trained animals were injected 20 min before the start and killed 15 min after the end of the session.

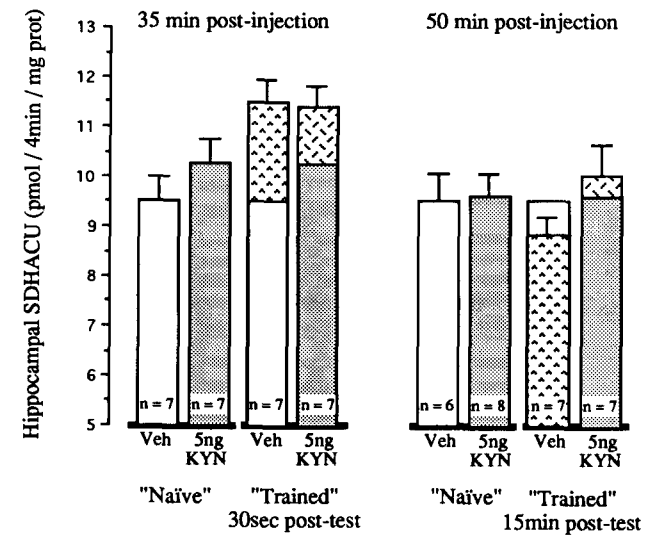

FIG. 5. On the left: hippocampal SDHACU in naive (quiet) mice and following the ninth session in mice trained for 9 days on spatial discrimination. All animals had received a bilateral injection into the lateral septum of either vehicle or 5 ng KYN 35 min before sacrifice. Trained animals were injected 20 min before the start and killed immediately at the end of the session (30 s posttest). On the right: the same as on the left except that all animals had received the bilateral injection of either vehicle or 5 ng KYN 50 min before sacrifice so that the trained animals were injected 20 min before the start, and killed 15 min after the end of the session.

(left), quiet animals treated with KYN 35 min before sacrifice displayed a slight increase (+7.7%; NS) in SDHACU compared with the vehicle group.

15 min posttest. There was no effect of treatment and no effect of behavior. However, as can be seen in Fig. 5 (right), there was a slight but nonsignificant interaction between the two. For the trained animals, hippocampal SDHACU was slightly decreased in the vehicle-treated mice compared to their naive controls, whereas the opposite pattern was observed in the KYN groups.

Additional Analysis

In this analysis the results of these four experiments were integrated to provide a more detailed account of the effects of intraseptal injection of KYN on hippocampal SDHACU. The data were analysed in three ways to assess the effects of KYN a) on basal SDHACU (quiet groups) as a function of interval between injection and sacrifice, b) on the testing-induced changes in this cholinergic marker as a function of both the amount of training and the injection–sacrifice interval, c) as a function of the behavioral profiles (i.e., good learners vs. bad learners), and d) on response accuracy recorded on the ninth session.

Effects of KYN on hippocampal SDHACU as a function of the injection–sacrifice interval. The data from Experiment 1 using the 5 ng dose of KYN (20 min postinjection) were included in this analysis. Figure 6A shows that, following the initial injection-induced increase in SDHACU, there was a progressive and relatively linear [regression analysis: $r = -0.68$; $F(1, 27) = 23.6, p < 0.001$] decrease in this cholinergic marker with increase in time (up to 65 min) between injection and sacrifice, $F(3, 25) = 7.36, p < 0.001$.

Effects of KYN on the testing-induced changes in SDHACU. In view of the rapid changes in the effect of KYN (5 ng) on SDHACU with injection–sacrifice interval, the net effect of this treatment on cholinergic activity as a function of training must be estimated from the absolute difference in SDHACU between the quiet and active conditions for each particular interval. SDHACU, in active KYN-treated animals, was, thus, corrected by subtracting the mean difference in SDHACU between KYN-injected and matched vehicle-injected animals under quiet conditions from each individual value. For each amount of training (1 and 9 sessions), an ANOVA including both factors treatment (vehicle vs. 5 ng KYN) and posttest interval (30 s vs. 15 min) was performed on those corrected measures. The results are summarised in Fig. 6B.

The effect of treatment emerged as an interaction with the posttest interval following the ninth session, $F(1, 24) = 4.3, p = 0.05$, but not following the first session ($p = 0.14$) [for the pooled sessions first + ninth: $F(1, 47) = 6.3$, $p = 0.015$]. This was due to the significant decrease in SDHACU between the 30 s and 15 min posttest intervals observed in the vehicle groups [pooled sessions: $F(1, 24) = 30.3$, $p < 0.001$], whereas SDHACU was not significantly different across the same intervals in the KYN groups, $F(1, 23) = 1.28, p = 0.27$. Thus, the intraseptal injection of KYN slowed the cholinergic deactivation following spatial discrimination training. This was particularly noticeable by the ninth session.

Effects of KYN on the time course of cholinergic deactivation as a function of learning profile (ninth session). In a previous study (29), the time course of cholinergic deactivation following the ninth session of spatial discrimination training was found to be significantly correlated with individual learning profile. In the animals displaying significant between-

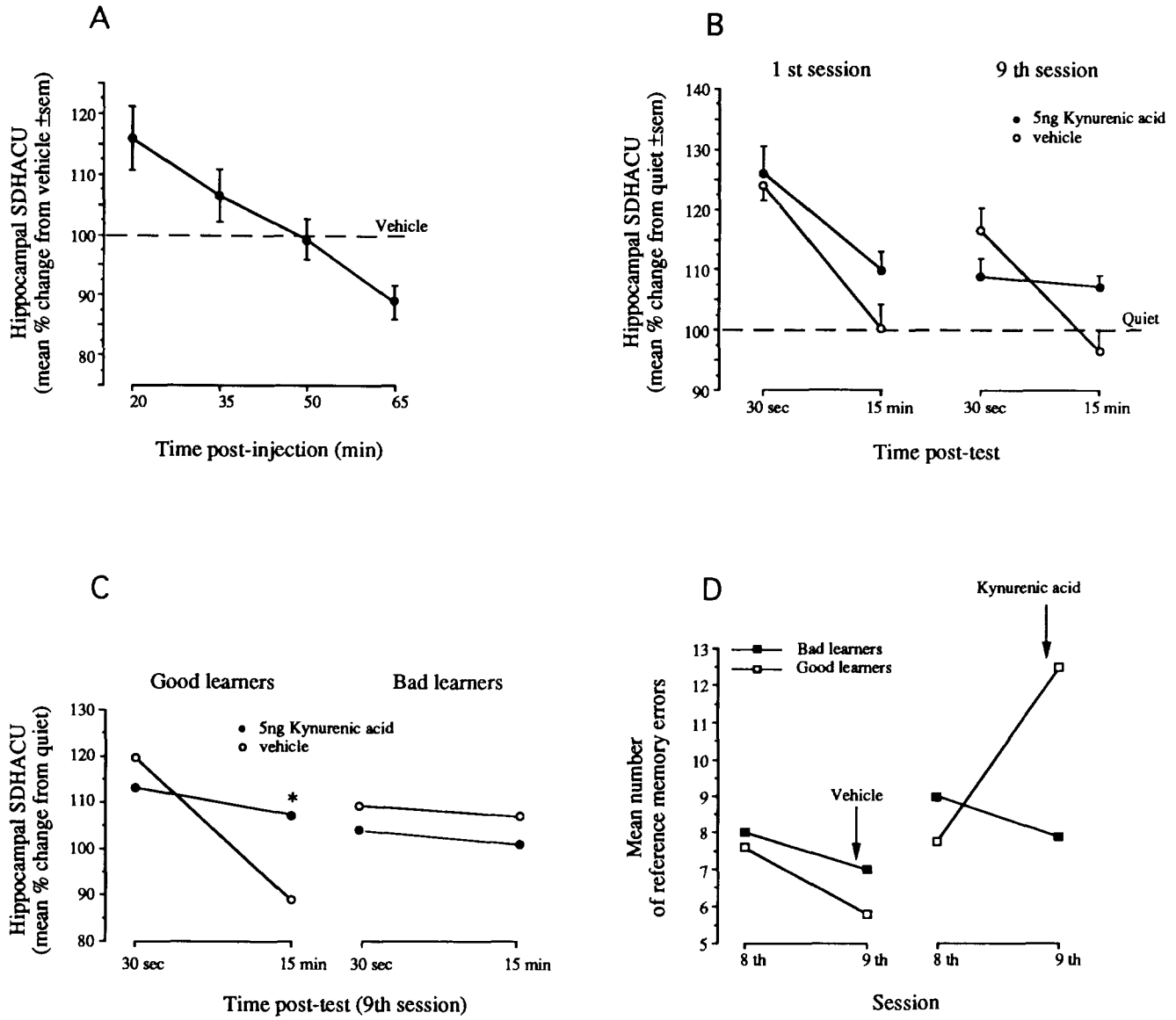

FIG. 6. (A) Hippocampal SDHACU in quiet mice injected with 5 ng KYN at different times after injection (percent changes from vehicle condition). (B) Effects of pretest intraseptal injection of KYN (5 ng, 20 min before the training session) on hippocampal SDHACU 30 s and 15 min posttest on the first (left) and on the ninth (right) sessions. (C) Hippocampal SDHACU in good learners (on the left) and in bad learners (on the right) 30 s and 15 min after the ninth training session in animals receiving intraseptal injections of either vehicle or 5 ng KYN 20 min before this (ninth) session. *Significantly different from controls; $p < 0.05$. (D) Effects of intraseptal injections of KYN on difference in mean number of RM errors between the 8th and the ninth session of spatial discrimination training as a function of the behavioral profile of learning across the preceding (first to eighth) sessions. On the left: effects of vehicle injection (20 min before testing) on the number of RM errors in the good learners ($n = 8$) and the bad learners ($n = 6$). On the right: the same as on the left but with KYN injection (good learners, $n = 6$; bad learners, $n = 8$).

session improvement over the nine learning sessions, there was a significant fall in SDHACU over the 15 min following completion of the ninth training session (cf. 30 s vs. 15 min post-session values). Conversely, in those animals that improved their performance mostly within sessions, hippocampal SDHACU did not change significantly over the 15-min posttest period. These two groups were referred to as good learners and bad learners, respectively, because the between-session improvers had generally better discrimination scores (less RM errors over all sessions) than the within-sessions improvers.

Using the same behavioral analysis, both vehicle- and KYN-injected animals ($n = 28$) from the present study were divided into two equal groups on the basis of their pretreatment between-session vs. within-session improvement (mean improvement over the eight preceding sessions; cf. the Method section). An ANOVA that included this factor (i.e., behavioral profile) together with both factors treatment and posttest interval was then performed on the hippocampal SDHACU, determined after the ninth training session.

The statistical analysis revealed a significant three-way in-

teraction [treatment × behavioral profile × delay: $F(1, 20) = 6.0$, $p = 0.024$]. As can be seen in Fig. 6C, this was because intraseptal injections of KYN only altered the time course of hippocampal SDHACU following the ninth training session in the good learners. Indeed, between 30 s and 15 min posttest, hippocampal SDHACU decreased significantly in the vehicle-treated good learners, but not in the KYN-treated good learners. In the bad learners, KYN did not alter the slight but nonsignificant decrease in SDHACU observed over the same posttest interval.

Effect of intraseptal injections of KYN on behavioral performance (ninth session). Because the pretesting intraseptal injections of KYN seemed to interfere with the posttesting deactivation of cholinergic activity in good learners only, we examined the effects of this treatment on response accuracy (on the ninth session) in relation to the pretreatment behavioral profile. Thus, an ANOVA including the two factors treatment and behavioral profile was performed on the repeated measures (eighth and ninth sessions) of RM errors.

There was a significant three-way interaction, $F(1, 24) = 4.24$, $p < 0.05$. As can be seen in Fig. 6D, this was because intraseptal injections of KYN before the ninth session resulted in a significant increase in RM errors over those recorded in the previous session, this increase being, however, dependent on the behavioral profile. Thus, as compared to vehicle-treated mice, good learners injected with KYN were significantly impaired on the ninth session, whereas bad learners were not ($p > 0.90$).

DISCUSSION

The results from this second experiment showed that intraseptal injections of 5 ng KYN induced changes in hippocampal presynaptic cholinergic activity in both naive mice (basal activity) and in mice trained in a radial arm maze discrimination task. In the naive animals, there was a transient increase in hippocampal SDHACU, which then returned to basal levels about 35–50 min postinjection (Fig. 6A), which is consistent with a rapid elimination of KYN from the brain (44). In the trained animals, the same treatment administered 20 min before testing slowed the cholinergic deactivation over the 15 min posttest (Fig. 6B). These results indicated that the cholinergic deactivation following RM training may be mediated, at least in part, by glutamatergic synapses in the lateral septum. However, the sensitivity to intraseptal injections of KYN as measured by both the neurochemical data and the memory score after treatment, differed between animals displaying different behavioral profiles over the eight preceding sessions of training (Fig. 6C and D). Intraseptal injections of KYN led to a dramatic behavioral impairment, together with a modified time course of the posttesting cholinergic activity in the good learners, but had no effect in the bad learners. These observations are in general agreement with the results of our previous study (29), and will be discussed in terms of the involvement of the septo-hippocampal cholinergic pathway in memory processes.

This experiment was designed to assess the mediation of septal glutamatergic receptors in the posttest inhibition of hippocampal cholinergic activity subsequent to the initial cholinergic activation following RM training (in radial arm maze). The amplitude of the posttest decrease in SDHACU was somewhat less than that found in our previous study [see (29), Fig. 1], where hippocampal SDHACU measured in trained animals, especially in the good learners, was significantly below basal level 15 min after the ninth training session. In the present study, cholinergic activity 15 min posttest was not significantly lower even in the good learners in the vehicle-injected group compared to the naive animals. This discrepancy could, perhaps, be accounted for by the effects of vehicle injection in the lateral septum. Nevertheless, intraseptal injections of KYN did interfere with the posttraining hippocampal cholinergic deactivation (Fig. 6B), thereby suggesting that septal glutamatergic receptors are involved in some way with the regulation of septo-hippocampal cholinergic activity accompanying certain memory tasks. However, this deactivation was never completely blocked in the KYN-treated animals (no significant effect of treatment was found 15 min posttest). The effects of KYN may derive from a variety of mechanisms that are not mutally exclusive. Firstly, as mentioned above, KYN should have short-lived activity after injection into the septum. Secondly, the ninth training session results indicate that any effect of this treatment should only be observed in the good learners. This might explain why KYN injection had little influence on the testing-induced cholinergic changes in a group including both good and bad learners. Finally, apart from the putative active involvement of glutamatergic receptors in posttraining cholinergic deactivation, there may also be some passive deactivation. This could help account for the less than total blockade of the secondary posttraining decrease in cholinergic activity by intraseptal injections of KYN.

Whatever the case, glutamatergic fibers ending in the lateral septal area appear to be involved in the reduction of septo-hippocampal cholinergic activity following radial arm maze discrimination training. This secondary deactivation resulted in a long-lasting inhibition (from 15 min to 9 days posttest) of the cholinergic pathway (29). This is indicative of an LTP-like mechanism sustaining the posttraining alterations in septo-hippocampal cholinergic activity. Because glutamatergic synapses in the lateral septum have been shown to display a long-lasting enhancement in efficiency after high frequency stimulation of fimbria fibers [e.g., (15,38)], septal glutamatergic receptors could mediate the long-lasting inhibition of hippocampal cholinergic neurons following RM training. This implies that RM training, like high-frequency fimbrial stimulation, would result in a form of LTP of these glutamatergic synapses. Interestingly, in a recent study, Garcia et al. (16), using the same task as ours (29), observed a training-induced enhancement of synaptic transmission in the lateral septum. They found that as training progressed, the mice displayed a progressive and persistent increase in the amplitude of field potentials evoked by fimbrial stimulation in the lateral septum. Moreover, the magnitude of this behavioral LTP was found to be related to the individual discrimination performance. These findings are consistent with our observation of relationships between level of training and the behavioral performance in the same task, and the amplitude and the time course of the testing-induced changes in hippocampal SDHACU. Here, the sensitivity to intraseptal injections of a glutamatergic antagonist was found to differ between good learners and bad learners. Good but not bad learners displayed behavioral impairment together with a noticeable change in the posttraining cholinergic deactivation following intraseptal injections of KYN. Taken together, the correlations between behavioral parameters and both cholinergic (29) and electrophysiological (16) markers of the activity of the septo-hippocampal system and the results of the present pharmacological study support the idea that septal glutamatergic receptors mediate, via an LTP-like mechanism, a long-lasting inhibition of the septo-hippocampal cholinergic pathway after spatial RM training. This inhibition was thought to be in-

volved in consolidation processes. This possibility is supported [see (29) for a detailed discussion, see also (3)] by the relationships we and others have found between memory testing and hippocampal SDHACU. Firstly, the time course of the cholinergic deactivation was related to the behavioral retention score. Secondly, posttest cholinergic inhibition has been observed after RM training but not after WM testing. The functional significance of this posttraining decrease in cholinergic activity will have to await the results of further behavioral and pharmacological studies aimed at selectively interfering with it. However, the notion that it underlies consolidation of some, but not all types of information is supported by Naghara et al.'s finding (34) that a pharmacologically induced posttraining inhibition of the septo-hippocampal cholinergic path resulted in a facilitation of spatial learning but not of passive avoidance. These considerations are of relevance for the development of procholinergic drugs to treat certain memory disturbances, as enhancing overall cholinergic transmission in the CNS may not necessarily improve memory. Precise adjustment of the activity of the cholinergic cells as a function of both the type and the phase of memory could be crucial to certain cognitive processes (19). This idea is supported in the present study by the massive behavioral impairment observed in the good learners after intraseptal injections of KYN accompanied by only slight interference with septo-hippocampal cholinergic activity. Finally, the difference in sensitivity between the good learner and bad learner groups to intraseptal injections of KYN may reflect the differential contribution of septo-hippocampal cholinergic neurons to the memory performance displayed by each of these two groups. It is generally assumed that most behavioral paradigms can be solved by a variety of strategies relying to different extents on any given memory system. The present results, along with those of our previous study, suggest that this radial maze task can probably be solved by both cholinergic-dependent and cholinergic-independent strategies.

CONCLUSION

The results presented here point to an indirect inhibitory role for glutamatergic receptors localized in the lateral septum on septo-hippocampal cholinergic cells in the medial area (see Discussion section of Experiment 1). Although the nature of the link between glutamatergic receptors and cholinergic neurons remains to be elucidated, it should be borne in mind that it may not be direct because there are relatively few projections from the lateral septum to the medial area (27). Although the interactions between acetylcholinergic and glutamatergic systems in the septum have yet to be well characterized, the results from the second experiment strongly suggest that septal glutamatergic synapses influence the activity of septo-hippocampal cholinergic neurons contingent on radial arm RM training. This effect appears to be involved in some (but not all) forms of learning. Our results are consistent with an LTP-like mechanism in the septum sustaining the post-RM training inhibition of the septo-hippocampal cholinergic path. It is tempting to suggest that a mechanism of this sort is involved in memory consolidation.

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REFERENCES

1. Atweh, S. F.; Simon, J. R.; Kuhar, M. J. Utilization of sodium-dependent high affinity choline uptake in vitro as measure of the activity of cholinergic neurons in vivo. Life Sci. 17:1535–1544; 1975.
2. Blaker, W. D.; Cheney, D. L.; Gandolfi, O.; Costa, E. Simultaneous modulation of hippocampal cholinergic activity and extinction by intraseptal muscimol. J. Pharmacol. Exp. Ther. 225:361–365; 1983.
3. Buzsaki, G. Two-stage model of memory trace formation: A role for "noisy" brain states. Neuroscience 31:551–570; 1989.
4. Cheney, D. L. Drug effects on transmitter dynamics: An overview. In: Hanin, I., ed. Dynamics of neurotransmitter function. New York: Raven Press; 1984:63–80.
5. Collingridge, G. L.; Lester, R. A. J. Excitatory amino acid receptors in the vertebrate central nervous system. Pharmacol. Rev. 40:143–210; 1989.
6. Costa, E.; Panula, P.; Thompson, H. K.; Cheney, D. L. The transsynaptic regulation of the septal hippocampal cholinergic neurons. Life Sci. 32:165–179; 1983.
7. Davies, J.; Francis, A.; Jones, A. W.; Watkins, J. C. 2-Amino-5-phosphonovalerate (2 APV), a potent and selective antagonist of amino acid-induced synaptic excitation. Neurosci. Lett. 21:77–81; 1981.
8. De France, J. F.; Yoshimara, H.; McCrea, R. A.; Kitai, S. T. Pharmacology of the inhibition in the lateral septal region. Exp. Neurol. 48:502–523; 1975.
9. Decker, M. W.; Pelleymounter, M. A.; Gallagher, M. Effects of training on a spatial memory task on high affinity choline uptake in hippocampus and cortex in young adult and aged rats. J. Neurosci. 8:90–99; 1988.
10. Dunnett, S. B. Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria-fornix on delayed matching in rats. Psychopharmacology (Berlin) 87:357–363; 1985.
11. Durkin, T. P.;, Hashem-Zadeh, H.; Mandel, P.; Ebel, A. A comparative study of the acute effects of ethanol on the cholinergic system in hippocampus and striatum of inbred mouse strains. J. Pharmacol. Exp. Ther. 220:203–208; 1982.
12. Dutar, P.; Lamour, Y.; Jobert, A. Septohippocampal neurons in the rat: An in vivo intracellular study. Brain Res. 340:135–142; 1986.
13. Fibiger, H. C. Central cholinergic systems and memory. In: Squire, L. R.; Lindenlaub, E., eds. The biology of memory. Stuttgart: F. K. Schattauer Verlag; 1990:381–394.
14. Gallagher, M.; Pelleymounter, M. A. An age-related spatial learning deficit: Choline uptake distinguishes "impaired" and "unimpaired" rats. Neurobiol. Aging 9:363–369; 1988.
15. Garcia, R.; Jaffard, R. The hippocampo-septal projection in mice: Long-term-potentiation in the lateral septum. Neuroreport 3:193–196; 1992.
16. Garcia, R.; Vouimba, M. R.; Jaffard, R. Spatial discrimination training induces LTP-like changes in the lateral septum of mice. Neuroreport 5:329–332; 1993.
17. Givens, B. S.; Olton, D. S. Cholinergic and GABAergic modulation of medial septal area: Effect on working memory. Behav. Neurosci. 104:849–855; 1990.
18. Hagan, J. J.; Salamone, J. D.; Simpson, J.; Iversen, S. D.; Morris, R. G. M. Place navigation in rats is impaired by lesions of medial septum and diagonal band but not nucleus basalis magnocellularis. Behav. Brain Res. 27:9–20; 1988.
19. Jaffard, R.; Micheau, J. Central cholinergic systems, learning and memory. In:Delacour, J., ed. The memory system of the brain. Advanced Series in Neuroscience, vol. 4. World Scientific Publishing Co.; 1994:359–430.

20. Jakab, R. L.; Leranth, C. Catecholaminergic, GABAergic, and hippocamposeptal innervation of GABAergic "somatospiny" neurons in the rat lateral septal area. J. Comp. Neurol. 302:305–321; 1990.
21. Jakab, R. L.; Leranth, C. Somatospiny neurons in the rat lateral septal area are synaptic targets of hippocamposeptal fibers. A combined EM/Golgi and degeneration study. Synapse 6:10–22; 1990.
22. Joels, M.; Urban, I. J. A. Amino acid neurotransmission between fimbria-fornix fibers and neurons in the lateral septum of the rat: A microiontophoretic study. Exp. Neurol. 84:126–139; 1984.
23. Joels, M.; Urban, I. J. A. Electrophysiological and pharmacological evidence in favor of aminoacid neurotransmission in fimbria-fornix fibers innervating the lateral septal complex of rats. Exp. Brain Res. 54:455–462; 1984.
24. Kelsey, J. E.; Landry, B. A. Medial septal lesions disrupt spatial mapping ability in rats. Behav. Neurosci. 102:289–293; 1988.
25. Kesner, R. P.; Crutcher, K. A.; Meason, M. O. Medial septal and nucleus basalis magnocellularis lesions produce order memory deficits in rats which mimic symptomatology of Alzheimer's disease. Neurobiol. Aging 7:287–295; 1986.
26. Kuhar, M. J.; Murrin, L. C. Sodium-dependent, high affinity choline uptake. J. Neurochem. 30:15–21; 1978.
27. Leranth, C.; Deller, T.; Buzsaki, G. Intraseptal connections redefined: Lack of a lateral septum to medial septum path. Brain Res. 583:1–11; 1992.
28. Leranth, C.; Frotscher, M. Organization of the septal region in the rat brain: Cholinergic-GABAergic interconnections and the termination of hippocampo-septal fibers. J. Comp. Neurol. 289: 304–314; 1989.
29. Marighetto, A.; Micheau, J.; Jaffard, R. Relationships between testing-induced alterations of hippocampal cholinergic activity and memory performance on two spatial tasks in mice. Behav. Brain Res. 56:133–144; 1993.
30. M'Harzi, M.; Palacios, A.; Monmaur, P.; Willig, F.; Houcine, O.; Delacour, J. Effect of selective lesions of fimbria-fornix on learning set in the rat. Physiol. Behav. 40:181–188; 1987.
31. McLennan, H.; Miller, J. J. The hippocampal control of neuronal discharges in the septum of the rat. J. Physiol. (Lond.) 237: 607–624; 1974.
32. Meck, W. H.; Church, R. M.; Wenk, G. L.; Olton, D. S. Nucleus basalis magnocellularis and medial septal area lesions differentially impair temporal memory. J. Neurosci. 7:3505–3511; 1987.
33. Miyamoto, M.; Kato, J.; Narumi, S.; Nagaoka, A. Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Res. 419:19–31; 1987.
34. Nagahara, A. H.; Brioni, J. D.; McGaugh, J. L. Effects of intraseptal infusion of muscimol on inhibitory avoidance and spatial learning: Differential effects of pretraining and posttraining administration. Psychobiology 20:198–204; 1992.
35. Olton, D. S.; Givens, B. S.; Markowska, A. L; Shapiro, M; Golski, S. Mnemonic functions of the cholinergic septo-hippocampal system. In: Squire, L. R.; Weinberger, N. M.; Lynch, G.; McGaugh, J. L., eds. Memory: Organisation and locus of change. New York: Oxford University Press; 1991:250–272.
36. Poucet, B. Object exploration, habituation and response to a spatial change in rats following septal or medial frontal cortical damage. Behav. Neurosci. 103:1009–1016; 1989.
37. Raaijmakers, W. G. M. High affinity choline uptake in hippocampal synaptosomes and learning in the rat. In: Ajmone-Marsan, C.; Matthies, H., eds. Neuronal plasticity and memory formation. New York: Raven Press; 1982:373–385.
38. Racine, R. J.; Milgram, N. W.; Hafner, S. Long-term potentiation in the rat limbic forebrain. Brain Res. 260:217–231; 1983.
39. Rauca, C.; Kammerer, R.; Matthies, H. Choline uptake and permanent memory storage. Pharmacol. Biochem. Behav. 13:21–25; 1980.
40. Richter, J.; Gormley, J. M. Inhibition of high-affinity choline uptake in the rat hippocampus by in vivo injection of phenobarbital in the medial septum. J. Pharmacol. Exp. Ther. 237:563–568; 1986.
41. Rothman, S. M.; Olney, J. W. Excitotoxicity and the NMDA receptor. Trends Neurosci. 10:299–302; 1987.
42. Stevens, D. R.; Cotman, C. Excitatory amino-acid antagonists depress transmission in hippocampal projection to the lateral septum. Brain Res. 382:437–440; 1986.
43. Stevens, D. R.; Cotman, C. W. Long-term potentiation of excitatory and inhibitory synaptic potentials in the hippocampal-septal projection of the rat. Brain Res. 558:120–122; 1991.
44. Turski, W. A.; Schwarcz, R. On the disposition of intrahippocampally injected kynurenic acid in the rat. Exp. Brain Res. 71: 563–567; 1988.
45. Van Den Hooff, P.; Urban, I. J. A.; De Wied, D. Vasopressin maintains long-term potentiation in rat lateral septum slices. Brain Res. 505:181–186; 1989.
46. Wenk, G.; Hepler, D.; Olton, D. Behavior alters the uptake of (^{3}H) choline into acetylcholinergic neurons of the nucleus basalis magnocellularis and medial septal area. Behav. Brain Res. 13: 129–138; 1984.
47. Zucker, J.; Calkins, D.; Zabawska, J.; Lai, H.; Horita, A. Effects of intraseptal drug administration on pentobarbital-induced narcosis and hippocampal choline uptake. Pharmacol. Biochem. Behav. 28:433–436; 1987.