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Septal α-Noradrenergic Antagonism In Vivo Blocks the Testing-Induced Activation of Septo-Hippocampal Cholinergic Neurones and Produces a Concomitant Deficit in Working Memory Performance of Mice

ALINE MARIGHETTO, THOMAS DURKIN, ABDOULAYE TOUMANE, CÉCILE LEBRUN AND ROBERT JAFFARD

Laboratoire de Psychophysiologie URA CNRS 339, Université de Bordeaux I Avenue des Facultés, 33405 Talence Cedex France

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MARIGHETTO, A., T. DURKIN, A. TOUMANE, C. LEBRUN AND R. JAFFARD. *Septal α-noradrenergic antagonism in vivo blocks the testing-induced activation of septo-hippocampal cholinergic neurones and produces a concomitant deficit in working memory performance of mice.* PHARMACOL BIOCHEM BEHAV **34**(3) 553–558, 1989.—In order to test the hypothesis that α-noradrenergic receptors in the septum 1) play an important functional role in the mediation of trans-synaptic control of the neurones of the cholinergic septo-hippocampal pathway and 2) produce resultant modulation of working memory performance, we have investigated the effects in vivo of the acute intraseptal injection of an α-antagonist, phenoxybenzamine, in mice. Neurochemical analysis was performed using measures of the kinetics of sodium-dependent high-affinity choline uptake in samples of hippocampus from injected mice and their relevant controls in both quiet conditions and immediately following selective working memory testing in an 8-arm radial maze. Results show that whereas the injection of phenoxybenzamine produces no significant alteration of the activity of the cholinergic septo-hippocampal neurones in quiet conditions, the pretrial (20 min) administration of this drug almost totally abolished the usually observed increase in hippocampal cholinergic activity induced by testing. This inhibition of cholinergic activation was associated with a parallel working memory deficit. The results provide further direct support for the hypothesis that septal noradrenergic afferents via α-receptors mediate a phasic and net excitatory trans-synaptic influence on the cholinergic septo-hippocampal pathway during working memory testing and thereby significantly contribute to the modulation of the level of working memory performance.

α-Noradrenergic antagonist Phenoxybenzamine Septum High-affinity choline uptake Hippocampus Working memory Mouse

THE hypothesis of a significant functional intervention of central cholinergic systems in learning and memory processes is presently supported by a wide and long-established data base. However, to a large degree, the evidence is still mostly circumstantial and has been derived mainly from studies into the effects on memory performance subsequent to the systemic injection of cholinergic agonists or antagonists, experimental lesions to the neurones of origin of ascending cholinergic pathways, neuropathological investigations into the aetiology of dementing disorders or from investigations into memory deficits in both humans and animals which occur during the course of natural aging (4, 5, 7, 9, 38).

Far less studies have been carried out into more direct experimental investigations of the cholinergic hypothesis via approaches aimed at quantifying temporal changes in the activity of central cholinergic neurones during learning or subsequent to memory testing procedures designed to specifically segregate different forms of memory. Such studies have shown that following learning there are measurable changes in choline acetyltransferase activity (17), acetylcholine levels (24) and especially increases in sodium-dependent high-affinity choline uptake in the hippocampus (18, 19, 30, 37). The use of analysis of high-affinity choline uptake kinetics, which is a specific presynaptic marker of cholinergic neurones and represents the rate-limiting step in acetylcholine synthesis (19), has been of central importance in more recent investigations of central cholinergic involvement of learning and memory processes (10,37), since this marker provides a dynamic

and sensitive index of the state of activity of cholinergic neurones at the time of sacrifice in individual subjects. Using this technique, we recently showed (14,35) that either spatial discrimination testing or specific working memory protocols in an 8-arm radial maze (delayed nonmatching) induced a significant (approximately 30%) increase in the activity of cholinergic neurones in both the hippocampus and frontal cortex (at 30 sec posttest) in comparison to nontested (quiet control) mice. Furthermore, from a comparison of the amplitude and time course of the cholinergic activation induced in both brain regions following repeated (15 days) and selective testing for working and reference memory, it was observed that cholinergic activity varied both as a function of the type of memory tested and also the daily repetition of tests (18, 35, 36). These experiments, which included a group of active control mice, showed that a large and significant fraction of the cholinergic activation in both structures was specifically linked to the use of memory processes. Apart from the fundamental problem of interpreting the eventual functional significance of these changes in cholinergic activity in information transfer and memory processes, these results also pose the question as to the identification of the synaptic mechanisms which provoke these testing-induced increases of activity in cholinergic neurones.

Immunocytochemical studies of the distribution of cholinergic perikarya in the rodent brain using monoclonal antibodies to choline acetyltransferase (25) have identified the neurones of the cholinergic innervation to the hippocampus as having their cell bodies of origin in the medial septal nucleus and the diagonal band of Broca (groups Ch1 and Ch2). The cell bodies of origin of the cortical cholinergic afferents are situated more caudally in the nucleus basalis magnocellularis (NBM) in the vicinity of the ventral pallidum and substantia innominata (group Ch4). However, both groups are situated along a single extended continuum of cholinergic cell bodies which exhibits a marked topographic organisation of its efferents. Furthermore, these cell bodies do not form part of the primary pathways transmitting sensory information. The changes in the activity of the cholinergic neurones of the septo-hippocampal and NBM-cortical pathways during testing must, therefore, be necessarily secondary to changes in the trans-synaptic input from other afferents (15). A number of studies have investigated this trans-synaptic control of the cholinergic neurones at the septal level [reviewed in (8)]. Using intraseptal injection of agonists and antagonists of transmitters identified as endogenous to the septal complex, combined with measures of the turnover rate of ACh in the hippocampus, in rats, these studies were not only able to identify the net excitatory or inhibitory action of a number of neurotransmitters on cholinergic activity, but further to describe whether this action was mediated tonically or phasically. For example, dopaminergic terminals from neurones originating in the A10 mesencephalic group (21,26) were identified as mediating a tonic inhibitory control on cholinergic septo-hippocampal neurones. However, on the basis of results which showed that intraseptal injection of haloperidol or chronic destruction of dopaminergic afferents using 6-hydroxydopamine (12–14) did not suppress the activation of the septo-hippocampal cholinergic neurones induced by radial maze testing of mice, we postulated that the regulation of the activity of the cholinergic neurones of the septo-hippocampal pathway (and probably that of the NBM-cortical pathway also) involves at least two major trans-synaptic components. The first, mediated by the summation of the tonically active afferents (e.g., dopamine), governs the level of activity (basal state) in awake, but otherwise quiet subjects. A second independent set of phasically active afferents mediates the acute increase in cholinergic activity observed during memory testing. Among the phasically active excitatory afferents to the septum identified by Costa *et al.* (8) noradrenaline via septal α-receptors appears as one major candidate for this role, especially in view of its previous implications in learning (16), memory processing (28,33) senescent memory dysfunction (34,39) and selective attention (22,32).

In the present study, we report on the effects of intraseptal injection of a nonselective α-noradrenergic antagonist (phenoxybenzamine) on the activity of the cholinergic neurones of the septo-hippocampal pathway in both quiet control mice and in mice submitted to working memory testing in an 8-arm radial maze.

METHOD

Animals

Subjects were male mice of the BALB/c strain obtained at the age of 5–6 weeks from IFFA-CREDO, Lyon, France. They were housed in a climatized animal room on a 12-hour light-dark schedule with ad lib access to food and water. At the age of 8–12 weeks they were individually housed and divided randomly into a total of 6 experimental groups. One-half of the animals was allocated to the "quiet control" condition, whereas the other was assigned for "working memory testing" in the radial maze. Within each of these two major subgroups three different treatment groups were constituted: drug-injected, vehicle-injected and intact controls.

Operation

Mice were implanted under general anaesthesia (sodium thiopental 70 mg/kg IP) with two guide cannulae (0.4 mm dia.; 8 mm long) aimed vertically towards 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 bregma, ±0.35 mm each side of the sagittal suture and 1.9 mm ventral from the skull surface. Following operation the mice were replaced in the animal house for a recovery period of 10 days before the start of the experimental phase.

Injection Protocol

A series of preliminary experiments was conducted in order to compare the effects of bilateral intraseptal injection of doses of 250, 500 and 1500 ng/0.2 μl of phenoxybenzamine on the activation of hippocampal high-affinity choline uptake produced by working memory testing. The results obtained, which showed that the inhibitory effects obtained with the two higher doses were very similar, led us to choose the dose of 500 ng for our further experimental analyses.

Bilateral intraseptal injections of phenoxybenzamine (500 ng/0.2 μl) or vehicle (pyrogen-free 0.9% physiological saline) were performed in freely moving mice via injection cannulae (0.2 mm dia) attached to 1 μl Hamilton syringes via polyethylene catheter tubing. The syringes were held in a constant rate infusion pump and injection was conducted over a 3-min period. In all cases, correct injection flow rates were visually controlled. The cannulae were left in place for a further 2 min before removal. Following sacrifice for neurochemical analysis, all cannula placements were verified histologically as being correctly located above the lateral septal nuclei.

Behavioural Analysis

Apparatus. Specific working memory tests were conducted using an automated and elevated 8-arm radial maze based on that described by Olton *et al.* (27) and scaled-down in size to be adapted for use with mice. The maze is constructed of grey Plexiglas and comprises a circular central platform (30 cm dia) from which radiate in a symmetrical fashion, 8 arms (50 cm long

× 11 cm wide). At the end of each arm, a circular food pellet tray is situated. The maze is equipped with doors at the entrance of each arm and with photoelectric cells which detect the position of the animal. This information is constantly transmitted to a microcomputer allowing automatic recording of arm choices, latencies and running speeds. The microcomputer program also controls the sequence of door opening and closing according to the specifications of each particular test. A closed circuit video system allowed the experimenter to visually control the test in a neighbouring room.

The working memory test. The specific working memory test is based on the delayed nonmatching to sample (DNMTP) procedure. This test assesses the animal's ability to distinguish a novel stimulus from one made familiar on the basis of a single presentation. In our experimental conditions, mice are presented, by a forced visit, to one of the arms of the 8-arm radial maze. They are then presented with a choice between this previously visited arm and an adjacent new arm. A correct choice allows the mouse to obtain a food pellet reward only in the arm which had not been previously visited. When this procedure is presented as a series of successive trials using different arms of the maze, the protocol selectively tests working memory performance on the basis of this acquired nonmatching rule. The difficulty of the test can be increased by increasing the delay between presentation and choice, delay which is occupied in our experimental protocol by interposed forced visits. Thus, this test cumulates both time-dependent decay of the memory trace and interference effects.

Behavioral protocol. The experimental phase began when operated mice had recovered for at least 10 days. Throughout the entire ensuing experimental period the mice were food-deprived to maintain their body weights at 87% of their free-feeding weight.
Habituation. In order to familiarise mice with the radial maze and its environment, animals were first allowed free exploration sessions on two successive days. During this stage all the doors of the maze were open so that mice could freely enter each arm and find a food pellet reward. Each daily session was terminated when all eight food pellets had been collected.
Acquisition of the delayed nonmatching rule. In this stage, mice were submitted to 4 daily training sessions in order to acquire the delayed nonmatching to sample rule. Each mouse was presented successively with forced visits to two arms (e.g., arm 1 then arm 4). Immediately following this, arm 1 and either arm 8 or 2 were opened simultaneously. A correct response and a food pellet reward was obtained for a choice of the previously unvisited arm. The same procedure then was used for choice between arm 4 and arm 3 or 5. In order to avoid any locomotor or position strategy, the place samples were automatically determined in a quasi-random manner with each problem counterbalanced by an opposite one. For example, for a given problem, if the correct arm is located to the left of the previously visited arm, the mouse will be next confronted with a problem for which the correct arm choice will be located on the adjacent right side of the initial sample. Consequently, if a mouse adopts a systematic left or right choice strategy its performance will be only at chance level (50%). Eight trials (16 choices) per day were used with an intertrial interval of 1 min.
Working memory test procedure. When mice attained the minimum criterion of 12 correct choices out of 16 on two consecutive days on the delayed nonmatching rule, they were submitted to the DNMTP testing procedure. In our protocol, mice were tested with problems of 2 levels of difficulty corresponding to either 1 or 5, interpolated forced arm visits between presentation and choice as described above. Each daily test session was composed of a total of 6 different problems (3 for each level of difficulty) and which were randomly distributed throughout each session. The interval between successive problems was 1 min and DNMTP testing was continued over 3 successive days until mice had been submitted a minimum of 9 problems for each level of difficulty.

Having completed this series of working memory tests the effects of intraseptal drug or vehicle injection on memory performance were tested 24 hours later as compared to intact controls. The injections were carried out for both quiet and active subjects, 40 min before sacrifice for neurochemical analysis. Mice of the quiet control groups remained in their home cages during this period. Injected animals of the active groups were submitted during the second (20 min) half of this period to a shortened session (6 trials equally divided into the 2 levels of difficulty) of working memory testing. Active mice were sacrificed 30 sec following the end of this session. A supplementary group of active subjects (control, vehicle and drug) underwent a longer session of working memory testing (4 or 5 trials for each level of difficulty) in order to provide a more complete behavioural analysis.

Neurochemical Analysis

Cholinergic activity in vivo in the septo-hippocampal pathway was quantified by measuring the velocity of the sodium-dependent high-affinity choline uptake mechanism in crude synaptosomal (P2) fractions from fresh samples of hippocampus using the technique of Atweh *et al.* (3) as modified by Durkin *et al.* (11).

RESULTS

Neurochemistry

Results are summarised in Fig. 1. Analysis of variance with "treatment" (intact, vehicle and phenoxybenzamine) and "behavior" (quiet vs. active) as between groups factors showed that, globally, working memory testing induced a significant increase in high-affinity choline uptake, $F(1,39)=28.5$, $p<0.001$. The increase in hippocampal cholinergic activity induced by memory testing, however, depended on the treatment [interaction treatment × behavior: $F(2,39)=3.96$, $p=0.026$]. Thus, while in quiet conditions there was no significant effect of treatment on high-affinity choline uptake, $F(2,22)=0.21$, n.s., the testing-induced

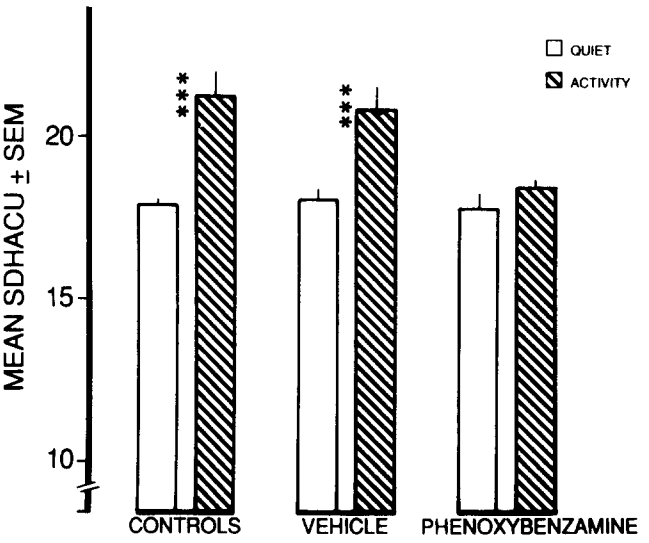

FIG. 1. Bar diagram showing the mean velocity of sodium-dependent high-affinity choline uptake in hippocampal P2 fractions (pmoles choline/4 min/mg protein ± S.E.M.) measured in mice of the intact control, vehicle-injected and phenoxybenzamine-injected (500 ng/0.2 μl bilaterally in the lateral septum) groups 40 min after injection in quiet control conditions (open bars) and in mice 30 sec following working memory testing in the radial maze (shaded bars). ***$p<0.001$ in comparison to respective quiet control group.

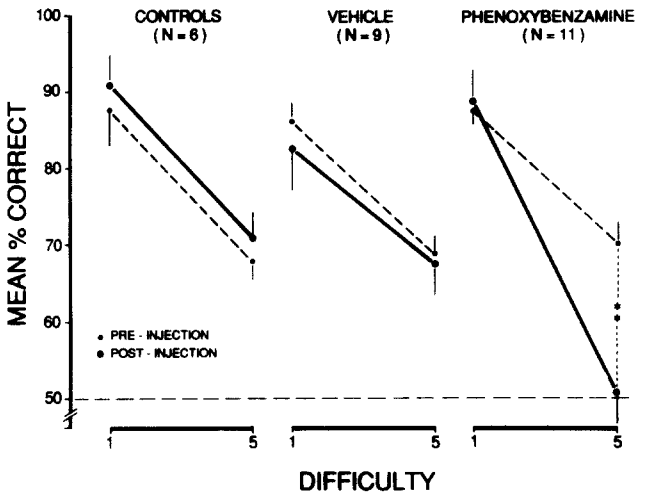

FIG. 2. Graph showing the level of working memory performance (mean percentage of correct choices ± S.E.M.) of mice of the intact control (left), vehicle-injected (centre) and phenoxybenzamine-injected groups for the two levels of testing difficulty (1 or 5 interposed forced visits) both before and following injection (pre or post). $^{**}p<0.01$.

increase in hippocampal cholinergic activity observed in intact (+18.4%) and vehicle-injected (+15.6%) mice ($p<0.001$ in both cases) was almost totally suppressed in phenoxybenzamine-injected subjects (+3.9%; n.s.). Finally, following testing, phenoxybenzamine-treated mice exhibited significantly lower hippocampal cholinergic activity than either vehicle-injected, $F(1,13)=5.98$, $p=0.028$, or intact subjects, $F(1,8)=14.42$, $p=0.005$.

Behavior

In the preinjection testing phase, an analysis of variance showed that the working memory performance [between groups factor; $F(2,23)=0.12$] did not differ among the three groups which exhibited a similar and significant decrease of response accuracy as a function of task difficulty [difficulty, $F(1,23)=64.7$, $p<0.001$; interaction group × difficulty, $F(2,23)=0.12$].

Results are summarized in Fig. 2. Analysis of variance showed that neither controls nor vehicle-injected mice exhibited significant changes in their own global performance between the preinjection and postinjection testing phase [respectively, $F(1,5)=1.88$, $p=0.23$; $F(1,8)=0.25$]. In contrast, phenoxybenzamine-injected animals were significantly impaired as compared to their preinjection performance, $F(1,10)=8.87$, $p=0.013$, notably because they exhibited a significantly larger decline in performance as a function of task difficulty than they did in the preinjection phase [interaction treatment × difficulty: $F(1,10)=9.2$, $p=0.012$]: although no differences were observed for one interposed visit (88.6 vs. 87.5%), they were markedly impaired in the 5 forced-visit conditions (50.6 vs. 69.9%); $F(1,10)=22.4$, $p<0.001$. The same conclusion can be drawn from a direct comparison between phenoxybenzamine- and vehicle-injected animals. Thus, in the postinjection testing phase, experimental subjects exhibited an accelerated rate of decay of response accuracy as a function of task difficulty, $F(1,18)=7.64$, $p=0.012$, while exhibiting normal (in fact slightly higher) performance for one interposed visit (88.6 vs. 82.6%; n.s.) and impaired choice accuracy for the 5 forced visit conditions [50.6 vs. 67.4; $F(1,18)=8.41$, $p=0.01$].

DISCUSSION

The major results of this study show that whereas the intraseptal injection in vivo of an α-noradrenergic antagonist produced no significant alteration to the level of septo-hippocampal cholinergic activity measured in quiet conditions (basal state), the same injection produced a near-total suppression of the activation (activated state) of these neurones which is usually induced during working memory testing. Thus, as previously outlined (cf., Introduction), septal α-noradrenergic receptors appear to belong to a phasically active excitatory system engaged during memory testing and which is relatively independent of the systems which trans-synaptically regulate the basal level of activity of the cholinergic septo-hippocampal pathway. These results are in agreement with others (8) which also showed that the septal α-noradrenergic input is a phasically active excitatory system towards cholinergic septo-hippocampal neurones. These studies showed that intraseptal injection of phenoxybenzamine could block the increase in the hippoampal turnover rate of ACh induced by injection of amphetamine, while having no effect on the basal level of cholinergic activity. Additional experiments using the intraseptal injection of β-antagonists are needed to explore the possibility that the β-receptor-coupled adenylate cyclase system might intervene in the regulation of basal cholinergic septo-hippocampal activity.

In parallel with this pharmacological blockade of hippocampal cholinergic activation, we observed a concomitant memory deficit in the phenoxybenzamine-injected mice, thus providing further evidence for the implication of septo-hippocampal cholinergic mechanisms in working memory processing [6, 7, 14]. In tested mice where septo-hippocampal cholinergic activity did not increase significantly over the basal level, we observe a deficit in working memory which does not seem to be attributable to a general performance deficit since the subjects are selectively impaired only in the difficult level. In our experimental conditions, the memory test cumulates both time-dependent decay of the memory trace and interference effects. From complementary experiments using memory tests in which retention delay is not occupied by forced visits, we may be able to conclude as to whether the main effect of α-noradrenergic blockade of septo-hippocampal cholinergic activation is, more probably, related to an increase in sensitivity to retroactive interference or simply reflects a passive and accelerated rate of forgetting of spatial information. Whatever the case may be, we observe that increased cholinergic activity appears to be more necessary when the mnesic effort demanded is increased. This is in general agreement with the hypothesis of Rawlins (31) in which he proposes that the hippocampus acts as an intermediate-term high-capacity memory buffer which acts in parallel with a limited capacity short-term memory system. The hippocampal system appears to be critical only when the memory task includes either rather long retention intervals or situations of increased interference. Complementary investigations examining the mnesic consequences of reduction in the basal level of septo-hippocampal cholinergic activity are currently in progress in order to strengthen the eventual correlation between the level of septo-hippocampal cholinergic activity and working memory performance.

The cholinergic hypothesis has been frequently invoked in studies on the effect of aging on memory performance (4,9). In this context, experiments from our laboratory using the same protocol (18) have compared the working memory performances of young (2 months) and old (24–26 months) mice combined with parallel measures of basal and activated cholinergic activity in both the hippocampus and frontal cortex following testing. The results showed that whereas basal levels of cholinergic activity varied less than 10% in both structures as a function of age in quiet conditions, the cholinergic activation normally observed immediately following memory testing in young mice was absent in the hippocampus of old mice, whereas still present, although

somewhat attenuated in the cortex of these subjects [cf., (20)]. This absence of test-induced hippocampal cholinergic activation was also correlated with a selective interference-related working memory deficit in the old mice. It is, thus, tempting to suggest that the blockade of septal α-noradrenergic transmission in the present study has mimicked the effects of the aging process concerning working memory performance. This would be in agreement with the hypothesis of Zornetzer (39) that memory impairment during aging could result primarily from hypofunction of the noradrenergic afferents and their interactions with central cholinergic neurones. We may, therefore, suggest that noradrenergic interaction with the cholinergic neurones of the medial septal nucleus provide a primary neurophysiological substrate for this hypothesis. In this context it may be noted that some studies have previously reported positive effects on delayed response and delayed non-matching performance in certain situations subsequent to the systemic administration of α_2-noradrenergic agonists in both lesioned rats and aged monkeys (1, 2, 34). Furthermore, improvement of memory performance in Korsakoff patients has also been observed following chronic administration of relatively high doses of the α_2 agonist clonidine (23). The possibility exists, therefore, that current attempts to improve central cholinergic transmission in neuropathological states such as Alzheimer's disease or even possibly during natural aging might find alternative strategies to precursor loading or cholinomimetics by using such trans-synaptically based treatments aimed either at chronically increasing the basal firing rate of cholinergic neurones or more optimally by acutely increasing their capacity to increase firing rate when stimulated.

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REFERENCES

1. Arnsten, A. F.; Goldman-Rakic, P. S. α_2-Adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged non-human primates. Science 230:1273–1276; 1985.
2. Arnsten, A. F.; Cai, J. X.; Goldman-Rakic, P. S. The α_2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: evidence for α_2-receptor subtypes. J. Neurosci. 8:4287–4298; 1988.
3. Atweh, S.; Simon, J. R.; Kuhar, M. Utilization of sodium-dependent high affinity choline uptake *in vitro* as a measure of the activity of cholinergic neurons *in vivo*. Life Sci. 17:1535–1544; 1975.
4. Bartus, R. T.; Dean, R. L.; Pontecorvo, M. J.; Flicker, C. The cholinergic hypothesis: a historical overview, current perspective and future directions. Ann. NY Acad. Sci. 44:332–358; 1985.
5. Bartus, R. T.; Flicker, C.; Dean, R. L.; Fisher, S.; Pontecorvo, M.; Figueiredo, J. Behavioural and biochemical effects of nucleus basalis magnocellularis lesions: implications and possible relevance to understanding or treating Alzheimer's disease. Prog. Brain Res. 70: 345–361; 1986.
6. Brito, G. N. O.; Davis, B. J.; Stopp, L. C.; Stanton, M. E. Memory and the septo-hippocampal cholinergic system in the rat. Psychopharmacology (Berlin) 81:315–320; 1983.
7. Collerton, D. Cholinergic function and intellectual decline in Alzheimer's disease. Neuroscience 19:1–28; 1986.
8. 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.
9. Decker, M. W. The effects of aging on hippocampal and cortical projections of the forebrain cholinergic system. Brain Res. Rev. 12:423–438; 1987.
10. 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.
11. Durkin, T.; 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. Durkin, T.; Galey, D.; Micheau, J.; Beslon, H.; Jaffard, R. The effects of acute intraseptal injection of haloperidol *in vivo* on hippocampal cholinergic function in the mouse. Brain Res. 376:420–424; 1986.
13. Galey, D.; Durkin, T.; Sifakis, G.; Kempf, E.; Jaffard, R. Facilitation of spontaneous and learned spatial behaviours following 6-OHDA lesions of the lateral septum: a cholinergic hypothesis. Brain Res. 340:171–174; 1985.
14. Galey, D.; Toumane, A.; Durkin, T.; Jaffard, R. In vivo modulation of septo-hippocampal cholinergic activity in mice: relationships with spatial reference and working memory performance. Behav. Brain Res., in press; 1989.
15. Gray, J. The neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system. Oxford: Oxford University Press; 1982.
16. Harley, C. W. A role for norepinephrine in arousal, emotion and learning?: limbic modulation by norepinephrine and the Kety hypothesis. Prog. Neuropsychopharmacol. Biol. Psychiatry 11:419–458; 1987.
17. Jaffard, R.; Galey, D.; Micheau, J.; Durkin, T. The cholinergic septo-hippocampal pathway: learning and memory. In: Will, B. E.; Schmitt, P.; Dalrymple-Alford, J. C., eds. Brain plasticity, learning and memory. New York: Plenum; 1985:167–181.
18. Jaffard, R.; Durkin, T.; Toumane, A.; Marighetto, A.; Lebrun, C. Experimental dissociation of memory systems in mice: behavioural and neurochemical aspects. Arch. Gerontol. Geriatr. Suppl. 1:55–70; 1989.
19. Kuhar, M.; Murrin, L. Sodium-dependent high affinity choline uptake. J. Neurochem. 30:15–21; 1978.
20. Lamour, Y.; Dutar, P. Central cholinergic systems in the rat: an electrophysiological approach in young and aged animals. In: Bes, A., *et al.* Senile dementia: Early detection. Paris: John Libbey Eurotext; 1986.
21. Lindvall, O.; Stenevi, U. Dopamine and noradrenaline neurons projecting to the septal area in the rat. Cell Tissue Res. 190:383–405; 1978.
22. Mason, S. T. Noradrenaline in the brain: Progress in theories of behavioural function. Prog. Neurobiol. 16:263–303; 1981.
23. McEntee, W. J.; Mair, R. G. Memory enhancement in Korsakoff's psychosis by clonidine: further evidence for a noradrenergic deficit. Ann. Neurol. 7:466–470; 1980.
24. Matthies, H.; Rauca, C.; Liebmann, H. Changes in the acetylcholine content of different brain regions of the rat during a learning experiment. J. Neurochem. 23:1109–1113; 1974.
25. Mesulam, M. M.; Mufson, E. J.; Wainer, B. H.; Levey, A. I. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature. Neuroscience 10:1185–1201; 1983.
26. Moore, R. Y. Catecholamine innervation of the basal forebrain. 1. Septal area. J. Comp. Neurol. 177:665–684; 1978.
27. Olton, D. S.; Becker, J. T.; Handelmann, G. E. Hippocampus, space and memory. Behav. Brain Sci. 2:313–365; 1979.
28. Quatermain, D. The role of catecholamines in memory processing. In: Deutsch, J. A., ed. Physiological basis of memory. New York: Academic Press; 1983:387–423.
29. Raaijmakers, W. 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.
30. Rauca, C.; Kammerer, E.; Matthies, H. Choline uptake and permanent memory storage. Pharmacol. Biochem. Behav. 13:21–25; 1980.
31. Rawlins, J. N. P. Some neurophysiological properties of the septo-hippocampal system. In: Trimble, M. R.; Zarifian, E., eds. Psychopharmacology of the limbic system. Oxford: Oxford University Press;

1985:17–50.
32. Robbins, T. W.; Everitt, H. B. Noradrenaline and selective attention. In: Will, B.; Schmitt, P.; Dalrymple-Alford, J. C., eds. Brain plasticity, learning and memory. New York: Plenum Press; 1985.
33. Sara, S. J. Noradrenergic modulation of selective attention: Its role in memory retrieval. In: Olton, D.; Gamzu, E.; Corkin, S., eds. Memory dysfunctions: an integration of animal and human research from clinical and preclinical perspectives. New York: New York Academy of Sciences; 1985:178–193. (Ann. NY Acad. Sci., vol. 444.)
34. Sara, S. J. Noradrenergic-cholinergic interaction: its possible role in memory dysfunction associated with senile dementia. Arch. Gerontol. Geriatr. Suppl. 1:99–108; 1989.
35. Toumane, A.; Durkin, T.; Marighetto, A.; Galey, D.; Jaffard, R. Differential hippocampal and cortical cholinergic activation during the acquisition, retention, reversal and extinction of a spatial discrimination in an 8-arm radial maze by mice. Behav. Brain Res. 30:225–234; 1988.
36. Toumane, A.; Durkin, T.; Marighetto, A.; Jaffard, R. The durations of hippocampal and cortical cholinergic activation induced by spatial discrimination testing of mice in an eight-arm radial maze decrease as a function of acquisition. Behav. Neural Biol. 52:279–286; 1989.
37. 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.
38. Wenk, G. L.; Hughey, D. J.; Olton, D. S. Basal forebrain cholinergic neurons and Alzheimer's disease. In: Coyle, J. T., ed. Animal models of dementia: a synaptic neurochemical perspective. New York: Alan R. Liss, Inc.; 1987:187–216.
39. Zornetzer, S. The noradrenergic locus coeruleus and senescent memory dysfunction. In: Crook, T.; Bartus, R. T.; Ferris, S.; Gershon, S., ed. Treatment development strategies for Alzheimer's disease. Madison, CT: Mark Powley Assoc., Inc.; 1986:337–359.