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Pharmacology Biochemistry and Behavior, Vol. 53, No. 3, pp. 541-547, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)02048-E

Consequences of Selective Blockade of Septal Noradrenergic Afferents on Anxiety and Spatial Working Memory Performance in Mice

MURIEL BELOTTI[1] AND DANIEL GALEY

Laboratoire de Neurosciences Comportementales et Cognitives, CNRS URA 339, Université de Bordeaux I, Avenue des Facultés, 33405 Talence Cedex, France

Received 25 January 1995; Revised 24 July 1995; Accepted 25 July 1995

BELOTTI, M. AND D. GALEY. *Consequences of selective blockade of septal noradrenergic afferents on anxiety and spatial working memory performance in mice.* PHARMACOL BIOCHEM BEHAV 53(3) 541-547, 1996. – This experiment was designed to investigate the role of septal noradrenergic (NA) afferents in the control of anxiety and spatial working memory. To this end, C57Bl/6 mice were infused bilaterally into the lateral septal nuclei with 500 ng/0.2 μl of BE 2254, a selective α1 postsynaptic adrenoceptor antagonist. The consequences of this reversible treatment were evaluated 20 min later on the anxiety level measured in an elevated plus-maze and on spatial working memory, evaluated under four different conditions via the learning of a delayed nonmatching to place (DNMTP) rule achieved in an eight-arm radial maze. In these conditions, the BE 2254, as well as the saline-injected control group, showed an elevation of the anxiety level that may be the indirect expression of a nonspecific septal dysfunction induced by the vehicle injection rather than the normal behavioral response produced by the decrease of septal NA activity. This septal dysfunction also impaired spatial working memory but only when mnesic difficulty of the task is increased, suggesting that this impairment expresses a general memory deficit rather than a working memory deficit per se. A lack of spatial working memory deficits in BE 2254 or saline-injected animals was also observed in two other conditions of the behavioral protocol. However, when treatments were applied before the first exposure of animals to the radial maze (exploration session), only the group which received BE 2254 was impaired during the acquisition session for the rule performed 24 h later. This delayed perturbation seems to be linked, at this stage of the learning procedure, to the lack of NA-dependent processes taking place during the exploration session. Taken together, these data suggest that septal NA mechanisms are more essential at initial stage of this learning, when animals process new features of the situation, than during the expression of spatial working memory per se.

Intracerebral injection Septal area Noradrenaline Anxiety Spatial working memory

NUMEROUS studies have implicated the hippocampus and the septo-hippocampal cholinergic (ACh) system in distinct functions, such as the encoding and use of spatial information (29,35), working (14,15,30,39), and/or spatial working memory (2,22,23) and the production of internal inhibition (3,10,11). On the other hand, based on an extensive review of litterature and experimental data, it has been postulated that septo-hippocampal ACh activation underlies anxiety (18,19). The activity of septal ACh neurons is transsynaptically modulated, directly or indirectly, by numerous transmitter systems. These afferents, which originate from hypothalamic and brain stem structures, participate in the regulation of emotional, motivational, and attentional processes that are important factors for learning and memory functions (9,21). For example, noradrenaline (NA), via septal α1 receptors, may exert a phasically active control on ACh neurons (5,24).

Many theories have implicated brain NA, especially in locus coeruleus neurons, as being important in learning or in processes such as attention or emotion that contribute to it (6,17,26,31). This conception is consistent with a number of reports suggesting that suppression of NA function impairs the ability of mammalian organisms to respond effectively to challenging situations (16,40).

In this context, studies conducted in our laboratory have attempted to investigate the role of septal NA operations in spatial working memory, which is characterized by the pro-

[1] To whom requests for reprints should be addressed.

cessing of trial dependent information by experimental animals (1,14,20,30,33). Moreover, there is also evidence that NA and ACh interact in influencing learning and memory (8). Consequently, it seems reasonable to postulate that spatial working memory is dependent on septal NA/ACh interactions (25).

As part of a continuing program designed to investigate the role of this interaction in the relationship between anxiety and memory, we have infused, into the septal region of mice, a selective $\alpha 1$ postsynaptic adrenoceptor antagonist (BE 2254), to block the effects of NA activation, during behavioral testing. The consequences of this reversible treatment were evaluated on the anxiety level and on spatial working memory performance, investigated in four different conditions via the learning of a delayed nonmatching to place (DNMTP) rule carried out in an eight-arm radial maze.

METHOD

Animals

Male mice of the inbred strain C57Bl/6 Jico obtained from IFFA-CREDO, Lyon, France, were used. Upon arrival in the laboratory, at the age of 8 weeks, they were housed collectively with ad lib food and water access, in a constant temperature room (22°C), maintained on a 12 L : 12 D cycle. At the age of 12–16 weeks they were individually housed in the same conditions for 1 week before operation. All experiences were carried out during the light cycle. At this stage, animals were 14–18 weeks old and weighed about 28–30 g.

Surgery

One group of animals ($n = 50$) was allocated to the intact control condition, whereas the others ($n = 113$) received, under anaesthesia (sodium thiopental 70 mg/kg IP), the implantation of two guide-cannulae (8 mm long, o.d. 0.46 mm, i.d. 0.255 mm). The tips of the guide-cannulae were vertically positioned 1.30 mm from each lateral septal (LS) nuclei to minimize damage to this area and to structures located above (cf. histological data, Fig. 7). The stereotaxic coordinates used were the following: anteroposterior refering to biegma: AP = +0.90 mm, lateral refering to sagittal line: L = ±0.40 mm and vertically below the skull surface: V = +2.10 mm. The two guides were fixed to the skull bone by three screws covered with dental cement. Following operation the animals were replaced in the animal room for a recovery period of ten days before the experiments began.

Intraseptal Injection Procedure

The $\alpha 1$ adrenoceptor antagonist BE 2254 (Beiersdorf-Lilly GmbH, Hambourg, Germany) was dissolved in a 0.9% physiological saline solution and injected at the dose of 500 ng in 0.2 μl ($n = 62$). This dose of BE 2254 was choosen on the basis of its inhibitory effect on the hippocampal cholinergic activation observed when animals were introduced in an eight-arm radiai maze (24). Bilateral intraseptal injections were carried out 20 min before each testing situation in freely moving mice through two injection needles (9.3 mm long, o.d. 0.23 mm), which were inserted into each guide-cannulae and which protruded 1.30 mm beyond the tip of the guides. Each injection (0.2 μl) was delivered simultaneously and in a constant manner over a 3-min period, via a 2 μl Hamilton syringe connected by polyethylene tubing. The needles were retained in the guides for an additional 3-min period before removal, to ensure the diffusion. Animals of the saline group ($n = 51$) received, under identical conditions, injections of the drug vehicle only.

Behavioral studies

Anxiety. The anxiety level was measured by the comparison of exploratory activities measured in two tasks with different anxiogenic potential.

The elevated-plus maze constitutes a strong anxiogenic environment. This test of anxiety is based on the strength of antagonism between exploratory tendencies and the avoidance of novel open spaces by animals.

The maze, situated in a quiet room with many extramaze cues, is a gray plus-shaped Plexiglas maze. This apparatus is elevated to a height of 55 cm and consists of two open arms (30 × 7 cm) and two enclosed arms (30 × 7 × 17 cm). Each arm extends from a central platform (7 × 7 cm), which is illuminated by a light bulb providing a 100 lx intensity. Behavioral data were collected by an observer who sat quietly behind a folding screen.

After a 10-day postoperative recovery, the experimental phase began. The mice of the three groups (control, saline, and BE 2254) were handled every day for 4 consecutive days. On the fifth day, the test involved placing each mouse into a mobile cylinder located on the platform of the maze for 10 s, and then, following withdrawal of the cylinder, allowing it to explore freely the apparatus for a period of 8 min. The following parameters were considered as constituting the anxiety index: a) the number of entries into the open arms divided by the total number of arm entries (activity ratio); b) the time spent on the open arms divided by the time spent on both the open and closed arms (time ratio). A mouse was considered to have entered an arm when all four paws had crossed into the arm. The maze was cleaned after each mouse was tested.

Immediately after the plus-maze test, each mouse was placed onto the four-hole board. This partially enclosed and weakly illuminated (15 lx) apparatus, situated in a separate quiet room, constitutes a less anxiogenic condition. The floor (40 × 40 × 30 cm) of this black Plexiglas box has four holes, 3 cm in diameter, and equally spaced. Infrared photocells, directly beneath each hole, provide automatic measures of the number of head dips and time spent head dipping. In addition, four pairs of photocells mounted in the walls of the box provide automated measures of the level of locomotor activity.

Animals were allowed to freely explore the four-hole board during a 6-min period. Three parameters were recorded: a) the number of head dips through the holes, b) the total time of head dipping, and c) the number of exchanges between the four holes.

This test enabled to verify that the drug did not alter the normal exploratory ability of animals. The protocol used for the anxiety measure is summarized in Fig. 1.

Spatial working memory investigation. Spatial working memory performance was measured in an eight-arm radial maze in different conditions, via the acquisition and retention of a DNMTP rule.

The automated eight-arm radial maze, constructed of gray Plexiglas, 130 cm in diameter, is located in a room (3 × 3 m) decorated with various pictures and objects to facilitate spatial orientation. The arms (50 × 11 cm) radiate in a symmetrical manner from a circular platform (30 cm in diameter). A food tray is located at the end of each arm, at the entrance of which are situated automatically operated doors. Photobeam cells enabled the recording of behavioral parameters. The computer program controls the sequence of door opening and

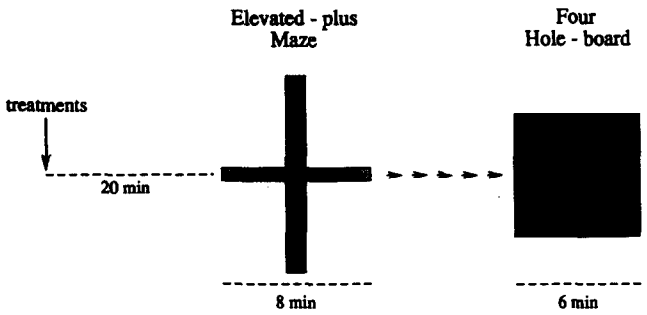


FIG. 1. Protocol used for the anxiety evaluation. The treatments were applied 20 min before the test. Exploratory capacities were evaluated immediatly following the anxiety measure proper.

closing, according to the specifications of each particular test. A closed-circuit video system allows the experimenter to visually control the test in a neighboring room.

The DNMTP test assesses the animals' 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 eight arms of the maze (presentation trial). They are then presented with a choice between this previously visited arm and a new arm (choice trial). A correct choice allows the mouse to obtain a food pellet reward only in the arm that was not previously presented. Good choice thus presupposes that animals have remembered the arm previously visited. When this procedure is applied as 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 may be increased by simply increasing the delay between presentation and choice. In this case, the time-dependent decay of the memory leads to a subsequent reduction in choice accuracy. Alternatively, the time period between presentation and choice may be occupied by a variable number of others interpolated forced visits to arms. In this variant, choice accuracy at the discrimination test cumulates both time-dependent decay of the memory trace and interference effects.

Before learning, mice were progressively food-deprived during 3 days (they received successively 3, 2, and 1 g of daily food) to reduce their body weights to 85% of normal and to maintain this level throughout the entire ensuing experimental period.

Exploration session. On the fourth day, mice were submitted to an exploration session that allowed familiarizing themselves with the apparatus and the environmental context. At the beginning of the session, the eight doors were automatically opened 5 s after the animal had been placed, by the experimenter, on the platform of the maze. Mice could freely enter each arm and find one food pellet in each tray. During this stage, repetition errors, as measured by the choices of arms already visited (20), and number of trials achieved before making the first repetition error were recorded as an index of working memory abilities. The exploration session was terminated when all eight pellets had been collected.

Acquisition of the DNMTP rule. The day following the exploration session each mouse was presented successively with two forced visits arms (e.g., arm 1, then arm 4). Immediately following this, arm 1 and either arm 8 or 2 (one of the two adjacent arms), were opened simultaneously. A correct response and a food pellet reward was obtained for the choice of the previously nonvisited arm. The same procedure was immediately used for a choice between arm 4 and either arm 5 or 3. To avoid any locomotor or position strategy, the place samples were automatically determined in a quasirandom manner, with each problem counterbalanced by an opposite one. Eight trials (16 choices) per day were used with an intertrial interval of 1 min. When mice attained the criterion of 80% correct choices over two consecutive days, they were submitted to the DNMTP testing procedure.

DNMTP testing procedure. In our protocol, mice were tested with problems of two levels of difficulty corresponding to one (DNMTP rule) or three intercalated forced arm visits between presentation and the choice. Each daily session was composed of a total of 12 different trials (4 for the rule and 8 for 3 intercalated visits). DNMTP testing was continued until the mice achieved a minimum criterion of 75% correct choices for the 3 interposed visits condition during two consecutive days.

Thus, these three different stages of the experimental procedure allowed the animals to express their spatial working memory in four different conditions: a) a free condition during the exploration session, when animals collected the eight pellets, by avoiding to return into an arm already visited (repetition error); b) during acquisition phase of the DNMTP rule in which forced arm choices constrain the animals not to employ strategy other than one based on the use of extramaze spatial cues for solving the task; c) during the DNMTP testing procedure also using forced conditions, in which the protocol enabled an evaluation of spatial working memory during the application phase for the rule; and d) during the introduction of trials using three intercalated arms that enabled an evaluation of the consequences of increasing the mnesic difficulty of the test.

Consequently, the effects of NA blockade were evaluated on these separate forms of working memory expressions. For this reason, treatments were administered in three different groups of mice, either the first day of DNMTP testing procedure (protocol A) for the first experiment realized, or the first day of rule learning (protocol B) for the second experiment, or the day of exploration session (protocol C) for the third one. In all cases, treatments were applied 20 min before the test. The general protocol used for the working memory study is presented in Fig. 2.

Histology

At the conclusion of the behavioral testing, animals received an overdose of chloroform. They were sacrified by decapitation and heads with guide-cannulae were placed in a formaldehyde solution (10%) during 1 week. Thereafter, the

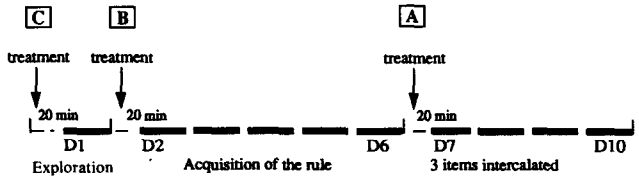


FIG. 2. Protocol used for the acquisition and retention of the DNMTP rule. The treatments were applied at three levels of the procedure (protocols A, B, C) corresponding to four distinct conditions of expression of the spatial working memory (see the Method section for further details).

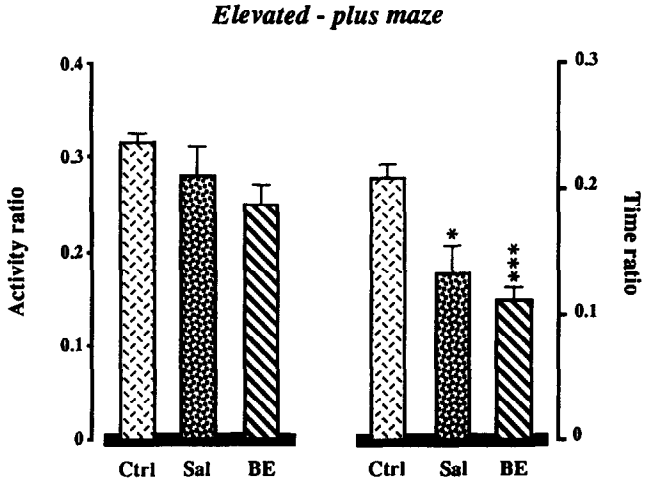
brains were removed from the skull and placed in a formol-saccharose solution (30%) for 24 h. Then, brains were frozen and sectionned frontally using a cryostat. The 80 μm sections were stained with thionine and examined microscopically to determine the good placement of the cannulae.

RESULTS

Behavioral data from each of the groups of animals were analyzed initially using a global one-way ANOVA, followed by post hoc pair-wise comparisons using the Fisher's protected least significant difference test.

Anxiety Measure

Elevated plus-maze. In this task, the less elevated are the ratios (activity and time ratio), the more anxious are the subjects. The mean activity and time ratios for control, saline, and BE 2254 animal groups are shown in Fig. 3. A one-way analysis of variance showed a significant effect of treatments on the time ratio, $F(2, 72) = 5.18$, $p = 0.0078$. Post hoc analysis revealed that both saline- and BE 2254-injected mice displayed a significant decrease in this measure, as compared to controls (respectively, $p < 0.05$, and $p < 0.01$) without differing significantly from each other. On the other hand, there were no significant differences between groups for the activity ratio, $F(2, 72) = 2.03$, NS.



Four hole - board

	Head-dips number	Head-dips time (s)	Crosses number
Control	32.5 ± 2.3	21.2 ± 2.6	18.8 ± 1.3
Saline	29.5 ± 3.1	15.1 ± 1.5	14.3 ± 1.7
BE 2254	31.9 ± 2.8	19.9 ± 2.2	14.3 ± 1.1

FIG. 3. Top: consequences of intraseptal application of BE 2254 (2 × 500 ng/0.2 μl) and saline on mean (± SEM) activity ratios (number of entries in open arms divided by the total number of arms entries) and time ratios (time spent in open arms divided by the time spent on open and closed arms). The less elevated are the ratios the more anxious are the subjects. Bottom: effects of the same treatments on distincts parameters of exploratory behavior (± SEM) evaluated in the four-hole board (*$p < 0.05$; ***$p < 0.01$ as compared to control group).

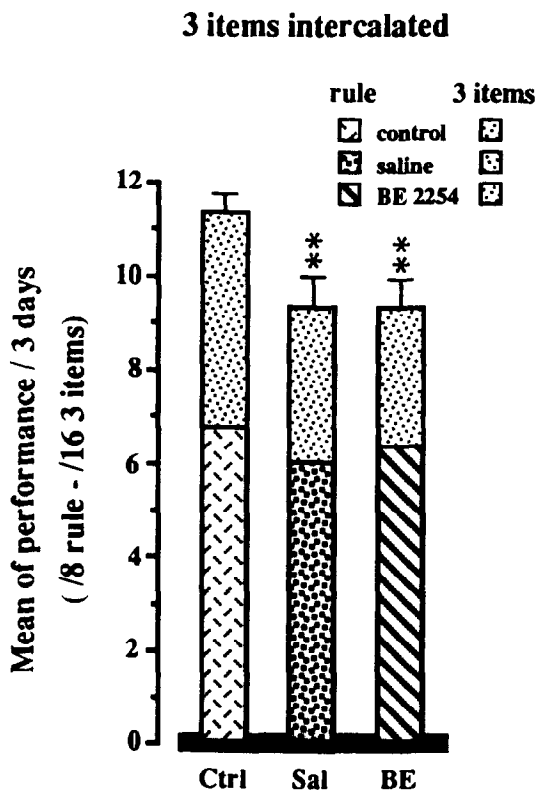


FIG. 4. Effects of intraseptal injection of BE 2254 (2 × 500 ng/0.2 μl) and saline on mean number of correct choices (± SEM) performed at two different levels of mnesic difficulty: one arm (rule) and three arms intercalated between presentation and choice trials (**$p < 0.02$ as compared to control group).

Four-hole board. There were no significant differences between the three groups for the number of head dips, $F(2, 72) = 0.26$, NS, the time of head dips, $F(2, 72) = 1.84$, NS, or the number of crossings, $F(2, 72) = 3.04$, NS, confirming that the decreased time ratio observed in the elevated plus-maze is not the result of nonspecific decrease in exploration abilities, but might traduce an anxiogenic-like effect.

Spatial Working Memory Investigation

For each protocol the results are expressed as the means of the performance scores observed over the first three daily sessions of training following treatments.

Protocol A. The results of the DNMTP testing procedure (Fig. 4) differ, depending on whether we consider rule performance or performance when three arms were intercalated between initial presentation and choice. In the first case, no effect of either both saline or BE 2254 treatments is observed, $F(2, 25) = 1.59$, NS. However, on trials including three intercalated arms, we observed a memory deficit, which, once again, concerns both BE 2254 as well as saline-injected animals, $F(2, 25) = 5.17$, $p = 0.013$, but without any statistically significant between-group difference ($p > 0.05$).

Protocol B. Figure 5 summarizes the results. Injection of BE 2254 or saline on the first day of rule acquisition did not produce any significant modification of performance in comparison with that of the control subjects, $F(2, 34) = 0.54$, NS.

Thus, the overall results of these two experiments do not

support the concept that activation of septal α1 NA receptors modulates spatial working memory stricto sensu.

Protocol C. Results concerning the exploration session, when treatments were carried out 20 min before the first entry into the novel environment of the radial maze, are shown in Table 1. In this situation, no significant difference appeared between groups whatever the parameter considered, whether this was the number of trials before making the first repetition error or the total number of repetition errors, $F(2, 20) = 0.59$, NS; $F(2, 20) = 2.27$, NS, successively. These data suggest that there was no deficit in spatial working memory in this situation when animals were allowed to freely collect pellets in the radial maze.

However, when animals were subsequently submitted to the DNMTP acquisition, a significant group effect was observed, $F(2, 20) = 29.87$, $p = 0.0001$). Post hoc analysis revealed that the performance scores of BE 2254 animals were significantly lower than those of both control and saline groups ($p < 0.01$).

These results (Fig. 6) suggest that intraseptal blockade of α1 NA receptors during the exploration session produces a delayed effect that appears only when a modification of some features of the experimental procedure was introduced.

Histological Data (Fig. 7)

This photomicrograph shows the placement of the two cannulae into the lateral septal nuclei in a vehicle-injected mouse. In this selected example, which represents the case of the most observable alteration induced by the vehicle injection in saline group animals, an attenuation of staining in the close vicinity of the cannulae tips can be noted. This alteration restricted to the injection site attests to a degree of functional perturbation induced by the vehicle injection.

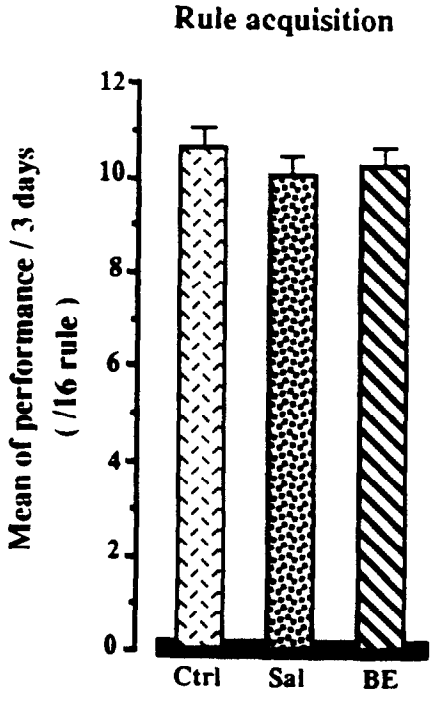


FIG. 5. Mean number of correct choices (± SEM) of the three groups (control, saline, and BE 2254) during the subsequent acquisition of the rule.

TABLE 1

EXPLORATION

	Number of Trials/ 1°Rep. Error	Number of Rep. Error
Control	4.2 ± 0.5	5.5 ± 1.2
Saline	3.3 ± 0.4	11.8 ± 2.9
BE 2254	3.8 ± 0.7	13 ± 3.6

Mean number of arms visited (± SEM) for the three groups (control, saline, BE 2254) before making the first repetition error and on number of repetition errors (± SEM) achieved in the eight-arm radial maze during the exploration session.

DISCUSSION

The aim of this study was to investigate, by using a reversible pharmacological intracerebral approach, the role of NA mechanisms in the septal region on anxiety and spatial working memory. In summary, our results show that bilateral infusion of a selective α1 antagonist, BE 2254 (13) into each LS nucleus induces an increase in the level of anxiety in comparison with the control group, when animals were exposed to an anxiogenic situation (elevated plus-maze). However, this phenomenon is also observable in the saline group animals, thus suggesting the existence of a nonspecific effect induced by the drug vehicle injection. This interpretation is supported by histological analysis showing alterations in the close vicinity of the cannulae tips in both BE 2254- and saline-injected mice.

These results are, nevertheless in accordance with previous reports attributing an anxiolytic function to the LS (37,41). Because GABAergic neurons participate in the regulation of the septal cholinergic activity (5,12), it is tempting to conclude, according to Gray's hypotheses (18,19), that the increase of anxiety scores we observed, in mice of the BE 2254 and saline groups, could be related to cholinergic activation induced by the functional perturbation of these GABAergic neurons by the vehicle injection. However, this interpretation is not completely supported by neurochemical data that demonstrate an inhibiting influence of BE 2254 on septohippocampal cholinergic activation (24).

Another explanation could be that the increase of anxiety scores was an indirect expression of septal dysfunction. Indeed, it is known that LS connect directly or indirectly hypothalamic and amygdaloid areas (36), which are both structures involved in the control and expression of emotion. For example, it was demonstrated that septal stimulation inhibited emotional responses elicited by hypothalamic stimulation (38). In these conditions, the increased anxiety observed in BE 2254 and saline groups could be due to an impairment of septal control normally exerted on some structures. Whatever the appropriate interpretation may be, we suggest that this elevated anxiety could be related in a secondary manner to a dysfunction of the septal region when animals are exposed to the plus-maze context.

We can compare the effects of both treatments on anxiety with those obtained using the protocol A during the spatial working memory investigation. In this situation, where three arms were intercalated between presentation and choice, a similar deficit appeared in both saline and BE 2254 groups. These results suggest that unavoidable neural dysfunction pro-

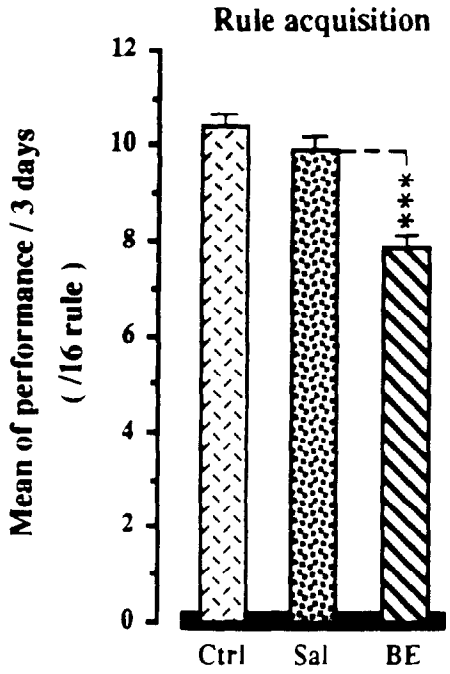


FIG. 6. Effect of intraseptal application of BE 2254 (2 × 500 ng/0.2 μl) administered 20 min before the exploration session on mean number of correct choices (± SEM) performed 24 h later during the acquisition of the rule (***$p < 0.01$ as compared to saline group).

duced by vehicle injection induces both an exaggerated anxious response when animals were exploring a new situation, and an impairment of spatial working memory performance when the mnesic difficulty of the task is increased. It is possible that these two phenomena are related to a dysfunction of the same neural processes. This cognitive impairment could, thus, explain their increased tendency to avoid open arms in the elevated plus-maze, which, in this case, is an indication of an exagerated anxiety level. However, this spatial working memory deficit does not affect strictly the working memory component because the subjects are not impaired for the expression of the DNMTP rule. It, thus, probably reflects a more general memory disturbance, for example, an increase in sensitivity to proactive interference or an accelerated rate of forgetting of spatial information. Likewise, in this situation, the absence of any difference between saline and BE 2254 animals does not support the view that NA is responsible for this effect. This interpretation does not agree with previous reports using the same procedure after intraseptal application of phenoxybenzamine (25), which conclude that NA is responsible of the spatial working memory deficit observed in these conditions.

In protocol B, intraseptal injection of BE 2254 did not perturb the acquisition of the rule. This result, compared with those of protocol A, seems to suggest that septal NA processes are not essential for spatial working memory. This conclusion is in agreement with reports that have shown that NA depletion does not alter radial arm maze performance (4,7,34). Moreover, animals were able to perform correctly in the situation of protocol B in spite of the probable septal dysfunction induced by the vehicle injection.

However, when the treatments were applied before the exploration session (protocol C), two kinds of results were observed. First, the spatial working memory of saline, and BE 2254-treated animals was not significantly affected during the exploration session when the animals were under the influence of the treatments. This result is also consistent with data from protocol A and B. Thus, it appears that a septal dysfunction, induced by vehicle injection, becomes manifest only when the mnesic difficulty of the task is increased. Second, the BE 2254 group exhibited a subsequent impairment in the acquisition of the DNMTP rule. An explanation of this delayed action of BE 2254 is offered by numerous reports that have implicated NA, especially in locus coeruleus neurons, as being important in learning, through processes such as attention or emotion (6,17,26,31). Moreover, it has been argued that the septohippocampal system and its NA afferents, belong to a behavioral inhibition system, which mediates increasing attention to environmental features depending on the degree of novelty inherent to the situation (19). In this condition, one can suppose that the blockade of septal NA operations provokes an impairment of attentional processes. This attentional function, which involves the participation of the NA system, is important in the course of the exploration session. Indeed, at this moment, animals express their working memory through the free exploration of the radial maze (30). During this time, normal animals process extramaze cues (27-29), whereas BE 2254-treated animals, because of their supposed attentional impairment, have probably some difficulty for this operation.

According to O'Keefe and Nadel's theory (1978), the result of hippocampal processing cues of the environment is to form a cognitive map of the experimental context. Consequently, the deficit of BE 2254 animals during the learning of the DNMTP rule might express a lack of this prior environment-based knowledge. Because the deficit appeared only during a complex learning task, the lack of a cognitive-contextual map might manifest itself only when the subject was required to perform under cognitive increased load.

Finally, our results seem to suggest that NA/ACh interac-

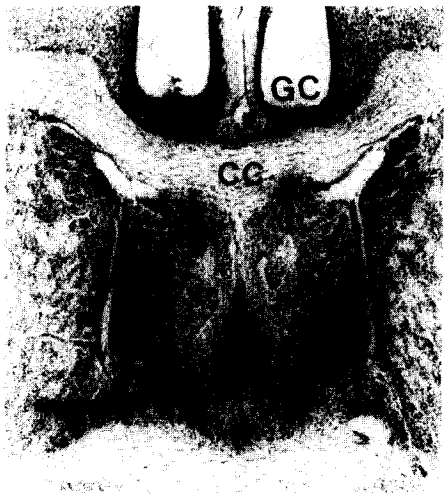


FIG. 7. Photomicrograph of thionin stained section of the brain showing a typical cannulae placement in lateral septal nuclei in a vehicle-injected mice. Note, in this selected example, the attenuation of staining in a limited area surrounding the cannulae tips. Abbreviations: CC, corpus callosum; GC, guide-cannulae track; LS, lateral septal nucleus.

tions taking place in the septal region (5,32) do not have the same functional importance at all stages of the DNMTP task. Because septohippocampal cholinergic activity plays a crucial role in the spatial working memory (2,22,23), this also suggest a successive involvement of these different transmitter systems throughout the learning procedure we have used. In conclusion, the nonspecific increase of anxiety level, induced by vehicle injection, leads us to suggest that this phenomenon would be an indirect expression of abnormal septal functioning. Our results also suggest that septal NA activation is more essential at the initial stages of this learning, when animals process new features of the environment according to O'Keefe and Nadel's cognitive mapping theory.

ACKNOWLEDGEMENTS

This study was supported by the CNRS URA 339. We are grateful to Doctor Eva Hofferber of Beiersdorf-Lilly GmbH, Hamburg, Germany, for generously supplying BE 2254. We are also grateful to the anonymous referees for their helpful criticism and comments.

REFERENCES

1. Baddeley, A. Working-memory. Oxford: Clarendon; 1986.
2. Brito, G. O.; Davis, B. O.; Stopp, L. C.; Stanton, M. E. Memory and the septo-hippocampal cholinergic system in the rat. Psychopharmacology (Berlin) 81:315–320; 1983.
3. Carlton, P. L. Cholinergic mechanisms in the control of behavior by the brain. Psychol. Rev. 70:19–39; 1963.
4. Chrobak, J. C.; DeHaven, D. L.; Walsh, T. J. Depletion of brain norepinephrine with DSP-4 fails to alter acquisition or performance of a radial arm maze task. Behav. Neural Biol. 44:144–150; 1985.
5. Costa, E.; Panula, P.; Thompson, H. K.; Cheney, D. L. The transsynaptic regulation of the septal-hippocampal cholinergic neurons. Life Sci. 32:165–180; 1983.
6. Crow, T. J.; Arbuthnott, G. W. Function of catecholamine-containing neurones in mammalian central nervous system. Nature 238:245–246; 1972.
7. Decker, M. W.; Gallagher, M. Scopolamine-disruption of radial arm maze performance: Modification by noradrenergic depletion. Brain Res. 417:59–69; 1987.
8. Decker, M. W.; McGaugh, J. L. The role of interactions between the cholinergic system and other neuromodulatory systems in learning and memory. Synapse 7:151–168; 1991.
9. DeFrance, J. F., ed. The septal nuclei. New York: Plenum; 1976.
10. Douglas, R. Pavlovian conditioning and the brain. In: Boakes, R.; Halliday, M., eds. Inhibition and learning. New York: Academic Press; 1972:529.
11. Douglas, R. The development of hippocampal function: Implications for theory and therapy. In: Isaacson, R.; Pribram, K., eds. The hippocampus, vol. 2. New York: Plenum Press; 1975:327.
12. Durkin, T. P. GABA-ergic mediation of indirect trans-synaptic control of septo-hippocampal cholinergic activity in resting mice and mice submitted to spatial working memory testing. Behav. Brain Res. 50:155–165; 1992.
13. Engel, G.; Hoyer, D. BE 2254, a new high affinity radioligand for α1 adrenoceptors. Eur. J. Pharmacol. 73:221–224; 1981.
14. Friedman, H. R.; Goldman-Rakic, P. S. Activation of the hippocampus and dentate gyrus by working-memory: A 2-deoxyglucose study of behaving Rhesus monkeys. J. Neurosci. 8:4693–4706; 1988.
15. Givens, B. S.; Olton, D. S. Cholinergic and GABAergic modulation of medial septal area: Effect on working-memory. Behav. Neurosci. 104:849–855; 1990.
16. Glavin, G. B. Stress and brain noradrenaline: A review. Neurosci. Biobehav. Rev. 9:233–243; 1985.
17. Grant, S. J.; Redmond, D. E., Jr. Neuronal activity of the locus coeruleus in awake *Macaca arctoides*. Exp. Neurol. 84:701–708; 1984.
18. Gray, J. A. The neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system. Oxford: Oxford University Press; 1982.
19. Gray, J. A. The neuropsychology of emotion and personality. In: Stahl, S. M.; Iversen, S. D.; Goodman, E. C., eds. Cognitive neurochemistry. Oxford: Oxford University Press; 1987.
20. Honig, W. K. Studies of working memory in the pigeon. In: Hulse, S. H.; Fowler, H.; Honig, W. K., eds. Cognitive processes in animal behavior. Hillsdale, NJ: Erlbaum; 1978:211–218.
21. Isaacsson, R. L. The limbic system. New York: Plenum; 1982.
22. 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 Press; 1985:167–181.
23. Kelsey, J. E.; Vargas, H. Medial septal lesions disrupt spatial, but not non spatial, working memory in rats. Behav. Neurosci. 107: 565–574; 1993.
24. Marighetto, A.; Jaffard, R.; Micheau, J. Effects of intraseptally injected noradrenergic drugs on hippocampal sodium-dependent-high-affinity-choline-uptake in "resting" and "trained" mice. Brain Res. 252:120–128; 1994.
25. Marighetto, A.; Durkin, T.; Toumane, A.; Lebrun, C.; Jaffard, R. 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. 34:553–558; 1989.
26. Mason, S. T. Catecholamines and behavior. Cambridge: Cambridge University Press; 1984.
27. O'Keefe, J. Place units in the hippocampus of the freely moving rat. Exp. Neurol. 51:78–109; 1976.
28. O'Keefe, J.; Conway, D. H. Hippocampal place units in the freely moving rat: Why they fire where they fire. Exp. Brain Res. 31: 573–590; 1978.
29. O'Keefe, J.; Nadel, L. The hippocampus as a cognitive map. Oxford: Oxford University Press; 1978.
30. Olton, D. S.; Becker, J. T.; Handelmann, G. E. Hippocampus: Space and memory. Behav. Brain Sci. 2:313–365; 1979.
31. Roberts, D. C. S. An evaluation of the role of noradrenaline in learning. In: Cooper, S. J., ed. Theory in psychopharmacology. New York: Academic; 1981:123–148.
32. Robinson, S.; Malthe-Sorenssen, D.; Wood, P.; Commissiong, J. Dopaminergic control of the septal-hippocampal cholinergic pathway. J. Pharmacol. Exp. Ther. 208:476–479; 1979.
33. Roitblat, H. L. Introduction to comparative cognition. New York: Freeman; 1987.
34. Sara, S. J. Noradrenergic-cholinergic interaction: Its possible role in memory dysfunction associated with senile dementia. Arch. Gerontol. Geriatr. 1:99–108; 1989.
35. Sutherland, R.; Whishaw, I.; Regehr, J. Cholinergic receptor blockade impairs spatial localisation by use of distal cues in the rat. J. Comp. Physiol. Psychol. 96:563–573; 1982.
36. Swanson, L. W.; Kölher, C.; Björklund, A. The septo-hippocampal system. In: Björklund, A.; Hökfelt, T.; Swanson, L. W., eds. Handbook of chemical neuroanatomy, vol. 5. Amsterdam: Elsevier; 1987.
37. Thomas, E. Forebrain mechanisms in the relief of fear: The role of the lateral septum. Psychobiology 16:36–44; 1988.
38. Thomas, E.; Evans, G. J. Septal inhibition of aversive emotional states. Physiol. Behav. 31:673–678; 1983.
39. Watts, J.; Stevens, R.; Robinson, C. Effects of scopolamine on radial maze performance in rats. Physiol. Behav. 26:845–851; 1981.
40. Weiss, J. M.; Simson, P. G. Neurochemical basis of stress-induced depression. Psychopharmacol. Bull. 21:447–457; 1985.
41. Yadin, E.; Thomas, E.; Grishkat, H. L.; Srickland, C. E. The role of the lateral septum in anxiolysis. Physiol. Behav. 53:1077–1083; 1993.