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Involvement of Septal Muscarinic Receptors in Cholinergically Mediated Changes in Rat Rearing Activity

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MONMAUR, P., A. SHARIF AND M. M'HARZI. *Involvement of septal muscarinic receptors in cholinergically mediated changes in rat rearing activity.* PHARMACOL BIOCHEM BEHAV **58**(2) 577–582, 1997—We tested the hypothesis that septal muscarinic receptors of the rat are involved in exploratory behavior control, at least as reflected in the measure of rearing activity. At a dose capable of inducing hippocampal theta rhythm, carbachol injection into the septum significantly increased the number of rearings from the 2nd to the 5th min postinjection. The increase was maximal in the 3rd min and gradually declined until the 9th min postinjection, when it was near the control level. This behavioral effect was blocked by prior injection of atropine. These data, when considered with other findings showing a similar motor response caused by the hippocampal injection of the cholinergic agonist, strongly suggest that the cholinoceptive cholinergic components of the septo-hippocampal system play a role in the release of exploratory behavior. This role might be mediated via muscarinic receptors at the septal level. Moreover, the time course of behavioral action of carbachol injected into the septum is of particular importance for studies on the effect of the drug on performance of the rat in learning and memory tests requiring contribution of exploratory behavior. © 1997 Elsevier Science Inc.

Carbachol Rearing Muscarinic receptors Septum Hippocampus Theta rhythm

SEVERAL observations have suggested that central cholinergic systems may be involved in the control of the locomotor activity of the rat (3,4,10). However, locomotor cholinergic influences differ according to the brain area considered. For example, injection of carbachol (CAR), a powerful cholinergic agent, the action of which is mediated via muscarinic receptors into the medial anterior hypothalamic/preoptic area or the tegmental pedunculopontine nucleus, produced a decrease in locomotor activity and rearing. This behavioral CAR effect was reversed by a low dose of atropine (ATR). These data were interpreted to mean that the cells of both the tegmental pedunculopontine nucleus and the anterior hypothalamic/preoptic cholinoceptive area are involved in decrease of locomotor and rearing activities (3,4). Conversely, injection of CAR into the hippocampus of the rat increased locomotor activity and rearing. This behavioral effect was blocked by coinjection of atropine, suggesting that the hippocampal muscarinic receptors play a role in the control of locomotor activity (10).

The medial septal nucleus and the vertical limb of the diagonal band of Broca may be the only source of hippocampal cholinergic terminals and acetylcholine (ACh) outflow (1,8,9,24). Moreover, binding and autoradiographic studies have shown the presence of muscarinic receptors in the septum (17,34,40). These studies have provided neurochemical support for the cholinoceptive nature of at least some septal cells, the activity of which is directed by ascending cholinergic influences (2,15, 32,33).

Rearing is an important component of motor manifestation of spontaneously occurring exploratory behavior in rodents. New evidence for this includes the recent finding by Clément

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et al. (6) that rearing activity is strongly correlated with locomotor activity in the open field. Because rearing is a clearly indentifiable and quantifiable behavioral parameter as opposed to other manifestations of rat motor exploratory activity, it often serves as an index of exploratory behavior.

With these facts in mind, we tested the hypothesis that septal muscarinic receptors of the rat are involved in exploratory behavior control, at least as reflected in the measure of rearing activity. The time course of this locomotor response to local injection of the drugs was also determined. Because hippocampal theta (θ) rhythm critically depends on the activity of cholinergic septal neurons providing terminals on the hippocampal formation (20), we also examined the contributions made by septal muscarinic receptors on the hippocampal electroencephalogram (EEG).

MATERIALS AND METHODS

Forty-two male Wistar rats weighing 280–320 g at the start of the experiment were obtained from Janvier (France). The rats were housed in single cages under constant temperature (24 ± 2°C) and a 12-h/12-h light/dark cycle, with ad libitum access to food and water.

The experiment reported in this paper was carried out in accordance with the European Communities Council directive of 24 November 1986 (86/609/EEC) for the care and use of laboratory animals as adopted by French legislation. Qualified experimentators should be certified and authorised by the Ministry of Agriculture (Animal Health and Welfare Unit). A certificate of authorisation was issued by the Ministry Department to P. Monmaur.

The general surgical, intraseptal injection and recording techniques were similar to those previously described (23). Briefly, θ rhythm was recorded from both CA1 and dentate θ generators of the right dorsal hippocampus. The two generators were reached under ether (diethyl ether, Prolabo) anesthesia by continuously recording EEG with two monopolar electrodes of stainless steel wire (120 μm in diameter) insulated except at the cut end. This procedure allowed well-developed CA1 and dentate θs, which were approximately phase reversed, to be obtained in most animals. A guide cannula (23-gauge hypodermic needle) was aimed at the right lateral septal nucleus. The stereotaxic coordinates were A 8.6 mm from the lambda, L 0.5 mm from the midline and P 4.6 mm from the bregma-skull surface. A septal injection cannula (30-gauge dental needle), which protrudes 3 mm below the guide cannula, was filled with drugs and connected to glass microsyringe prior to insertion into the guide cannula. Between injections, the guide cannula was occluded with a stainless steel wire pin. The rats were allowed at least 8 days to recover from surgery before behavioral testing and subsequent EEG recording. Microinjections were given not more than three times in a single site, and a minimum of 8 days separated the injections. Drugs were dissolved in 0.9% saline (SAL; sodium chloride, Chaix and Du Marais) and injected in a volume of 0.2 μl. In our previous work, we reported that intraseptal injection of 1 μg of CAR (carbamylcholine chloride, Sigma) resulted in a clear-cut elicitation of both hippocampal θ rhythm and exploratory behavior in freely moving rats (22). These EEG and behavioral effects of CAR were blocked by local injection of 20 μg of ATR (atropine sulfate, Sigma). These data prompted us to use 1 μg (5.45 nmol) of CAR and 20 μg (29.55 nmol) of ATR in the present investigation. Animals were divided into four groups: 11 rats received SAL (SAL group = control group), 14 rats received CAR (CAR group), 11 rats received ATR (ATR group) and 6 animals received ATR plus CAR (ATR+CAR group) into the septum.

Rearing was assessed in a cage made of a transparent Plexiglass cylinder (50 cm high and 20 cm in diameter). The animals were placed in the cylinder with the injection cannula inserted into the guide cannula, and the rats were left for 1 min. Then, 0.2 μl of the drug solution or saline was injected in the unrestrained animal over 1 min. The injection cannula was left in place for 9 min after completion of the injection, except when two successive injections of drugs (ATR + CAR) were given. In the latter pharmacological paradigm, injection of CAR was started immediately after the end of ATR application. After completion of the injection, the number of rearings was counted by two independent experimenters in 1-min intervals for 9 min. The onset of rearing was recorded when the animal's forepaws left the floor and ended when the forepaws returned in contact with it.

At the end of the 9-min session, the rats were placed in an EEG recording box, and the hippocampal electroencephalographic activity was recorded for 10 min. Recordings included EEG associated with both spontaneous movements and awake immobility. Behavior was observed and coded on the ongoing EEG with a pen, thus allowing the relationship between behavior and concomitant EEG to be analyzed.

At the completion of the experiment, each animal was infused with 0.2 μl of methyl blue. Ten minutes later, under deep ether anesthesia, rats were decapitated, and the septo-hippocampal system was exposed and cut. Resulting slices of fresh brain tissue were checked under a binocular microscope for injection site placements.

Data were analyzed by analysis of variance (ANOVA) for repeated measures. Whenever a significant effect was obtained, the post hoc Student–Newman–Keuls test was used for pairwise comparisons. A level of $p < 0.05$ was considered significant.

RESULTS

Histology

Methyl blue was concentrated within the right lateral septal region in 35 of 42 animals. In the 7 remaining rats, the dye was seen clearly in both the right lateral septal region and in the lateral aspect of the adjacent medial septal nucleus. In most rats, however, a clear-cut blue track was also detected in both the corpus callosum and cortex overlying the right lateral septum, probably as a result of the spread of the solution along the external side of the cannula. These histological data are in agreement with previous studies (21–23).

General Behavior

In agreement with our previous observations (22), CAR induced the rat to intensively explore the novel environment. A clear-cut increase in walking, sniffing, rearing, head displacements and vibrissae movements were observed following CAR injection. This period of behavioral activation lasted for about 6–9 min. Intraseptal injection of ATR generally produced behavioral hyperactivity resembling general agitation. Although it was still detectable 9 min postatropine injection, this behavior was significantly reduced at this time.

Rearing Activity

Figure 1 illustrates the time course of rearing over the postdrug testing period. There was significant difference among groups [$F(3, 38) = 6.23$, $p < 0.002$]. Subsequent com-

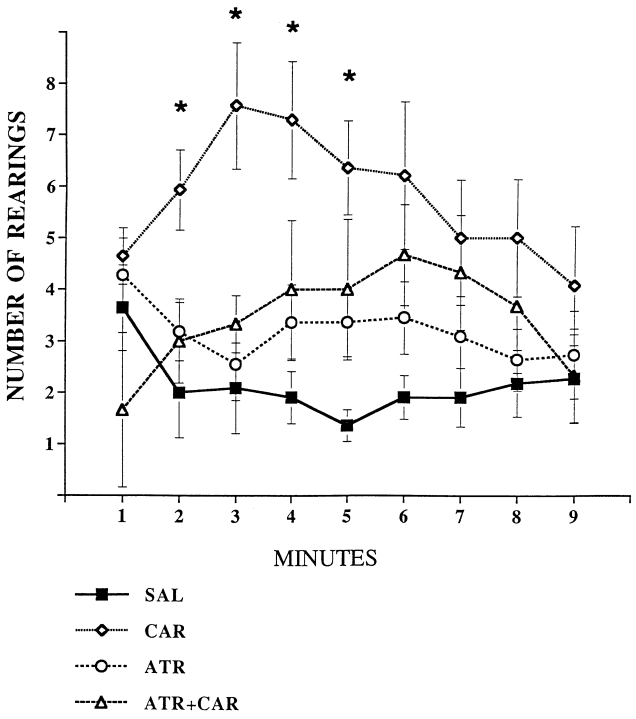

FIG. 1. Graph summarizing the effects of intraseptal injection of carbachol (CAR) and atropine (ATR) on rearing activity in the rat. Each point represents the mean ± SEM. Carbachol alone (5.45 nmol) significantly increased rearing from the 2nd to the 5th min postinjection. *$p < 0.05$ (carbachol vs. other drugs). Atropine (29.55 nmol) alone or in combination with carbachol did not significantly change rearing activity. SAL = saline control.

parisons revealed that the total cumulated score in the CAR group was significantly higher than that of the SAL group ($p < 0.05$), whereas the other groups did not differ statistically from each other or from the SAL group ($ps > 0.1$). There was also a significant group × time interaction [$F(3, 304) = 1.78$, $p < 0.025$], indicating that rearing activity changed over the testing period among groups. Pairwise comparison revealed that CAR significantly increased rearing from the 2nd to the 5th min postinjection. The increase in this activity was maximal in the 3rd min and gradually declined until the 9th min postinjection, when it was near the control level. ATR alone also showed a tendency to increase rearing as compared with SAL, but this effect was nonsignificant. The antimuscarinic drug, when infused in combination with CAR, clearly antagonized the CAR effect. The average rearing score (mean ± SEM) calculated over the testing period in the ATR + CAR group (3.44 ± 0.34) was significantly lower than that of the CAR group (5.78 ± 0.36; $p < 0.05$), but nonsignificant vs. that of control group (2.14 ± 0.22; $p > 0.1$).

Hippocampal EEG

Figure 2 depicts the typical effect on the hippocampal EEG of injection of drugs into the septum. Following injection of saline into the septum, the hippocampal EEG did not differ from that usually seen in the undrugged conditions: exploration (walking, running, rearing and sniffing) was always accompanied by θ, whereas consumatory behavior (eating, drinking) and relaxed immobility (eyes wide open) were associated with large irregular activity. Injection of ATR alone did not result in significant change in the correlation between hippocampal EEG and behavior. In contrast, θ was associated with behavioral immobility, resembling a behavioral alert state, in addition to voluntary movements in rats injected with CAR. This EEG effect of CAR was blocked by ATR, although blockage was incomplete in some animals. These EEG results are essentially in agreement with results collected in previous investigations (19,22).

DISCUSSION

The present results confirm previous observations (19,22) that, at the dose which facilitates hippocampal θ, injection of CAR into the septal complex results in an increase in rearing activity of freely moving animals. As CAR significantly increased and ATR blocked rearing in the present study, the increased rearing seen after intraseptal CAR injection appears to be the result of activation of septal cholinergic receptors. Previous physiological studies have shown that hippocampal cholinergic θ and ACh outflow are significantly increased following injection of CAR into the septum (7,19,22), which may be the only source of cholinergic input into the hippocampus (1,8,9) and the pace maker of hippocampal θ (30). These results and those of Flicker and Geyer (10), in which CAR manipulation of the hippocampus resulted in a considerable increase in locomotion and rearing, strongly support the hypothesis that activation of the cholinoceptive component of the cholinergic septohippocampal pathway may be crucial for exploratory behavior control, at least as reflected in measure of rearing activity. This hypothesis is in agreement with the view that the septohippocampal system, at least when functioning in θ mode, is involved in the programming and the execution of so-called voluntary movements such as walking, running, rearing, etc. (25,38), which are parts of spontaneously occurring exploratory behavior. Also, more recent behavioral results are relevant to this hypothesis: intraseptal injection of CAR increases rats' mobility in a T-maze task (12) and reverses the behavioral effects of intraperitoneal administration of scopolamine, a powerful muscarinic antagonist, in animals performing the same task (11). However, the possibility that increased rearing seen following septal CAR injection may, at least in part, result from nonspecific central excitation caused by cholinoceptive septal inputs to brain areas other than the hippocampal formation (35,37) cannot be completely ruled out. Further investigation is needed to test this possibility.

The precise intraseptal neurophysiological mechanism by which CAR exerts its behavioral effect is not clear. Binding and autoradiographic studies have suggested that M2 muscarinic receptors are concentrated in the septum, whereas there are no or few M1 subtypes (5,27,28). There is evidence that, in the brain, M1 receptors are located postsynaptically, whereas M2 receptors are located in presynaptic terminals, acting as autoreceptors to reduce acetylcholine release and, in postsynaptic neurons, receive cholinergic innervation (28,29,39). Interestingly, electrophysiological work has revealed that an identified septohippocampal neuron is strongly excited by intraseptal application of CAR, suggesting an important role for the postsynaptic M1 muscarinic receptors in cholinergically mediated septohippocampal cell activity (18). In this context, the increased rearing seen in the present study may be the result of activation of presumed septal postsynaptic M1 muscarinic receptors and of presynaptic M2 muscarinic recep-

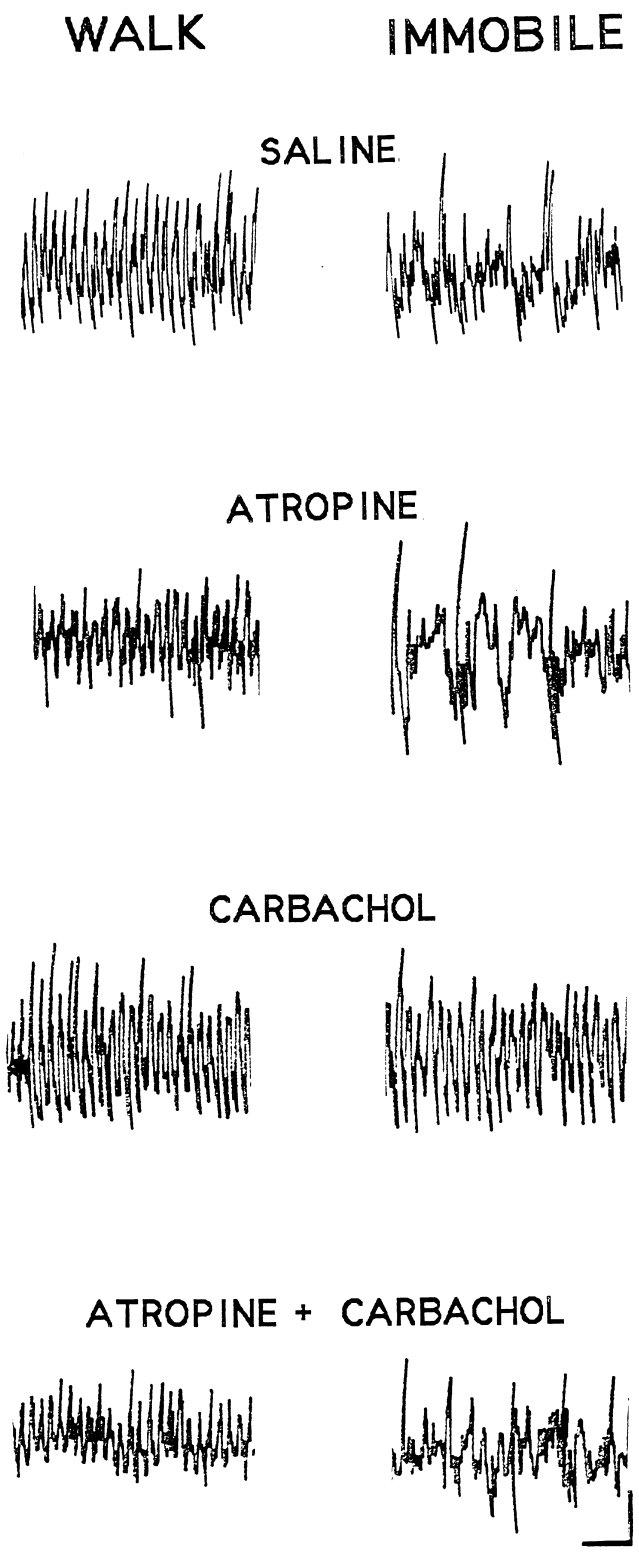

FIG. 2. The qualitative effect of intraseptal injection of drugs on hippocampal EEG during walking (WALK) and behavioral immobility with eyes wide open (IMMOBILE). Saline and atropine alone (29.55 nmol) did not change the relation between EEG and behavior: θ rhythm accompanied walking, whereas large irregular activity (no θ) was associated with behavioral immobility. Carbachol (5.45 nmol) alone elicited θ during behavioral immobility, an effect that was blocked by prior injection of atropine (ATROPINE + CARBACHOL). Recordings were taken 10 min postdrug injection. Calibrations = 1 s and 500 μV.

tor subtypes. In the present study, hippocampal θ was associated with behavioral immobility, resembling a behavioral alert state and exploratory activity in rats injected with CAR. This EEG effect of CAR was blocked by ATR. In our previous study using urethane-anesthetized rats (23), we found that intraseptal injection of ATR, a nonspecific muscarinic agent, abolished θ elicited by local application of CAR, whereas gallamine, a specific M2 antagonist that displays high affinity for the septal region (27) failed to suppress completely this rhythm. Mecamylamine, a nicotinic antagonist, also failed to alter CAR-induced θ. On the basis of these data, we have suggested that the latter result may have resulted from coactivation of both M2 and non-M2 muscarinic receptors at the septal level (23). When taken collectively, these data strongly support the idea that cholinoceptive cholinergic septal cells involved in the production of hippocampal θ rhythm play an important role in the control of behavioral states involving exploration and/or alertness.

The activity of the septum is regulated by ascending cholinergic inputs originating more probably in the pedunculopontine (ch5) and laterodorsal tegmental (ch6) nuclei (2,15, 32,33). In this context, the ascending brainstem cholinergic influences on the septum may play a role in initiating and maintaining exploratory behavior, at least as reflected in the measure of rearing activity. If this hypothesis is confirmed, then cholinoceptive cholinergic septal cells may be an important relay station for exploratory behavior control between cholinergical brainstem neurons and the cholinoceptive cells of the hippocampus. Thus, we suggest that the present pharmacological analysis supports the view that a relatively organized cholinergic mesopontine-hippocampal di- or polysynaptic pathway plays a role in initiating and maintaining exploratory behavior. Although such a view is parsimonious, it does not explain previous findings showing that CAR application to different brain sites involving the nucleus accumbens, the dorsal perifornical area, hypothalamic regions and reticular formation (14,16,31) also results in increased locomotor activity and thus indicating that cholinergic receptors diffusely distributed across the brain are involved in exploratory behavior. In fact, this gives rise to the question as to whether the cholinoceptive cholinergic septohippocampal cells are the direct and/or indirect target of cholinoceptive brain sites, the activation of which elicits exploratory activity. The electrophysiological findings, that an increasing population of septal units changes their activity with increased level of diffuse ascending influences (2,13,26,36) may support this notion.

The present study revealed that CAR significantly increases rearing from the 2nd to the 5th min postinjection and that the increase is maximal in the 3rd min and gradually declines until the 9th min postinjection, when it is near the control level. These data provide information about the time course of CAR-elicited rearing activity and indicates that the behavioral action of the drug has a short latency and a limited but not negligible duration. This result might be of particular interest for investigations concerned with the effect of intraseptal injection of cholinergic agonists and antagonists on the behavioral performance of rats in learning and memory tasks (spatial or nonspatial) requiring contribution of exploratory behavior (11,12). Indeed, because intraseptal CAR af-

fects behavior, conclusions about effects of the drug on learning and memory processes can only be drawn if the effects of the drug on behavior can be excluded or experimentally dissociated from learning and memory processes. In this testing procedure, one can make the prediction that results and, therefore, their interpretations might differ significantly depending on whether behavioral testing is carried out immediately or at different time points after intraseptal CAR injection.

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REFERENCES

1. Amaral, D. G.; Kurj, J.: An analysis of the origins of the cholinergic and non-cholinergic septal projections to the hippocampus formation. J. Comp. Neurol. 204:37–59; 1985.
2. Brazhnik, E. S.; Vinogradova, O. S.: Control of the neuronal rhythmic bursts in the pacemaker of theta-rhythm: effects of anaesthetic and anticholinergic drugs. Brain Res. 380:94–106; 1986.
3. Brudzynski, S. M.; Wu, M.; Mongenson, G. J.: Decrease of locomotor activity by injections of carbachol into the anterior hypothalamic/preoptic area of the rat. Brain Res. 373:38–46; 1986.
4. Brudzynski, S. M.; Wu, M.; Mongenson, G. J.: Modulation of locomotor activity induced by injections of carbachol into the tegmental pendoculopontine nucleus and adjacent areas in the rat. Brain Res. 451:119–125; 1988.
5. Buckley, N. J.; Bonner, T. I.; Brann, M. R.: Localization of a family of muscarinic receptors mRNAs in rat brain. J. Neurosci. 8:4646–4652; 1988.
6. Clément, Y.; Martin, B.; Venault, P.; Chapouthier, G.: Involvement of regions of the 4th and 7th chromosomes in the open field activity of mices. Behav. Brain Res. 70:51–57; 1995.
7. De Boer, P.; Moor, E.; Zaagsma, J.; Westerink, B. H. C.: Consequences of septal manipulation on the release of acethylcholine from the ventral hippocampus: an in vivo microdialysis study. In: Rollema, H.; Westerink, B. H. C.; Drijhout, W. J., eds. Monitoring molecules in neuroscience. Proceedings of the 5th international conference on in vivo methods. Groningen; 1991:279–282.
8. Dudar, J. D.: The role of the septal nuclei in the release of acetylcholine from the rabbit cerebral cortex and dorsal hippocampus and the effect of atropine. Brain Res. 129:237–246; 1977.
9. Dudar, J. D.; Whishaw, I. Q.; Szerb, J. C.: Release of acetylcholine from the hippocampus of freely moving rats during sensory stimulation and running. Neuropharmacology 18:673–678; 1979.
10. Flicker, C.; Geyer, M. A.: Behavior during hippocampal microinfusions. II. Muscarinic locomotor activation. Brain Res. Rev. 4:105–127; 1982.
11. Givens, B.; Olton, D.: Bidirectional modulation of scopolamine-induced working memory impairements by muscarinic activation of the medial septal area. Neurobiol. Learn. Mem. 63:269–276; 1995.
12. Givens, B.; Olton, D.: Cholinergic and GABAergic modulation of medial septal area: effect on working memory. Behav. Neurosci. 104:849–855; 1990.
13. Gogolak, G.; Petsche, H.; Sterc, J.; Stumpf, C.: Septum cell activity in the rabbit under reticular stimulation. Brain Res. 4:508–510; 1967.
14. Hernandez-Peon, R.; Havez-Ibarra, P. J.; Morgane, P. J.; Timo-Iaria, C.: Limbic cholinergic pathways involved in sleep and emotional behavior. Exp. Neurol. 8:93–111; 1963.
15. Jones, B. E.; Cuello, A. C.: Afferents to the basal forebrain cholinergic cell area from pontomesecephalic-catecholamine, serotonin, and acetylcholine-neurons. Neuroscience 31:37–61; 1989.
16. Jones, D. L.; Mogenson, G. J.; Wu, M.: Injections of dopaminergic, cholinergic, serotoninergic and GABAergic drugs into the nucleus accumbens: effects on locomotor activity in the rat. Neuropharmacology 20:29–37; 1981.
17. Kobayashi, R. M.; Palkovits, M.; Hruska, R.; Rothchild, R.; Yamamura, H. I.: Regional distribution of mucarinic cholinergic receptors in rat brain. Brain Res. 154:13–23; 1978.
18. Lamour, Y.; Dutar, P.; Jobert, A.: Septo-hippocampal and other medial septum-diagonal band neurons: electrophysiological and pharmacological properties. Brain Res. 309:227–239; 1984.
19. Lawson, V. M.; Bland, B. H.: The role of the septohippocampal pathway in the regulation of hippocampal field activity and behavior—analysis by the intraseptal microinfusion of carbachol, atropine and procaine. Exp. Neurol. 120:132–144; 1993.
20. Lee, M. G.; Chrobak, J. J.; Sik, A.; Wiley, R. G.; Buzsaki, G.: Hippocampal theta activity following selective lesion of the septal cholinergic system. Neuroscience 62:1033–1047; 1994.
21. Monmaur, P.; Ayadi, K.; Breton, P.: Hippocampal EEG responses induced by carbachol and atropine infusions into the septum and the hippocampus in the urethane-anesthetized rat. Brain Res. 631:317–324; 1993.
22. Monmaur, P.; Breton, P.: Elicitation of hippocampal theta by intraseptal carbachol injection in freely moving rats. Brain Res. 544:150–155; 1991.
23. Monmaur, P.; Puma, C.; Sharif, A.: Involvement of M2-muscarinic and non-M2 muscarinic receptors in hippocampal theta-rhythm induced by carbachol infusion into the septum of the rat. Brain Res. 656:199–204; 1994.
24. Moor, E.; de Boer, P.; Beldhuis, H. J. A.; Westerink, B. H. C.: A novel approach for studying septo-hippocampal cholinergic neurons in freely moving rats: a microdialysis study with dual-probe design. Brain Res. 648:32–38; 1994.
25. Oddie, S. D.; Stefanek, W.; Kirk, I. J.; Bland, B. H.: Intraseptal procaine abolishes hypothalamic stimulation-induced wheel-running and hippocampal theta field activity in rats. J. Neurosci. 16:1948–1956; 1996.
26. Petsche, H.; Gogolak, G.; Van Zwieten, P. A.: Rhythmicity of septal cell discharges at various levels of reticular excitation. Electroencephalogr. Clin. Neurophysiol. 14:202–211; 1965.
27. Price, M.; Messer, W. S., Jr.; Hoss, W.: Regional distribution of muscarinic receptors preferring gallamine in the rat brain. Biochem. Pharmacol. 35:4171–4176; 1986.
28. Quirion, R.; Araujo, D.; Regenold, W.; Boksa, P.: Characterization and quantitative autoradiographic distribution of {3H}acetylcholine muscarinic receptors in mammalian brain. Apparent labelling of an M2-like receptors sub-type. Neuroscience 29:271–299; 1989.
29. Raiteri, M.; Leardi, R.; Marchi, M.: Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in rat brain. J. Exp. Ther. 228:209–214; 1984.
30. Rawlins, J. N. P.; Feldon, J.; Gray, J. A.: Septo-hippocampal connections and the hippocampal theta rhythm. Exp. Brain Res. 37:49–63; 1979.
31. Romaniuk, A.; Brudzynski, S.; Gronska, J.: Rage reaction evoked by intrahypothalamic injection of carbachol in cats. Acta Physiol. Pol. 24:623–630; 1973.
32. Sato, K.; Fibiger, H. C.: Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. J. Comp. Neurol. 253:277–302; 1986.
33. Semba, K.; Reiner, B. P.; Mcgeer, E. G.; Fibiger, H. C.: Brainstem afferents to the magnocellular basal forebrain studies by axonal transport, immunohistochemistry, and electrophysiology in the rat. J. Comp. Neurol. 267:4433–4453; 1988.
34. Spencer, G., Jr.; Horvath, E.; Traber, J.: Direct autoradiographic determination of M1 and M2 muscarinic acetylcholine receptor distribution in the rat brain: relation to cholinergic nuclei and projections. Brain Res. 380:59–68; 1986.
35. Staiger, J. F.; Nürnberger, F.: The efferents connections of the lateral septal nucleus in the guinea pig: intrinsic connectivity of the septum and projections to other telencephalic areas. Cell Tissue Res. 264:415–426; 1991.
36. Stumpf, C.; Petsche, H.; Gogolak, G.: The differential influence of drugs upon both the septal cell firing pattern and the hippocampus theta activity. Electroencephalogr. Clin. Neurophysiol. 14:407–415; 1982.
37. Swanson, L. W.; Cowan, W. M.: The connections of the septal region in the rat. J. Comp. Neurol. 186:621–656; 1979.

38. Vanderwolf, C. H.; Kramis, R.; Gillespie, L. A.; Bland, B. H.: Hippocampal rhythmic slow activity and neocortical low voltage fast activity: relations to behavior. In: Issacson, R. L.; Pribram, K. H., eds. The hippocampus, vol. 2. New York: Plenum Press; 1975:101–128.
39. Vilaro, M. T.; Wiederhold, K. H.; Palacios, J. M.; Mengod, G.: Muscarinic M2 receptor mRNA expression and receptor binding in cholinergic and non-cholinergic cells in the rat brain: a correlative study using an in situ hybridization histochemistry and receptor autoradiography. Neuroscience 47:367–393; 1992.
40. Wamsley, J. K.; Zarbin, M. A.; Birdsall, J. M.; Kuhar, M. J.: Muscarinic cholinergic receptors: autoradiographic localization of high and low affinity agonist binding sites. Brain Res. 200:1–12; 1980.