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Cholinergic Activity in the Rat Hippocampus, Cortex and Striatum Correlates With Locomotor Activity: An In Vivo Microdialysis Study

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DAY, J., G. DAMSMA AND H. C. FIBIGER. *Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: An in vivo microdialysis study.* PHARMACOL BIOCHEM BEHAV **38**(4) 723–729, 1991.—The possible relationship between behavioral arousal and acetylcholine release in the striatum, hippocampus and frontal cortex was investigated in rats. In vivo microdialysate concentrations of acetylcholine and choline from these brain structures, and photocell beam interruptions (as a measure of behavioral arousal), were measured simultaneously under three conditions: after injections of 1) vehicle or 2) scopolamine (0.4 mg/kg), and 3) before and after the beginning of the rats' night cycle. Dialysate concentrations of ACh in all 3 brain structures and locomotor activity were increased after scopolamine and the onset of the lights out condition. Vehicle injections transiently increased ACh in the hippocampus and cortex and caused short-lasting increases in locomotor activity. Under all conditions, the release of ACh from each of the 3 brain structures correlated with the level of locomotor activity.

Acetylcholine Choline Microdialysis Frontal cortex Hippocampus Striatum Locomotor activity Arousal Scopolamine

NEURONS releasing acetylcholine (ACh) are widely distributed in the brain (47) and have been implicated in a variety of behavioral functions. For example, memory and learning (4), circadian rhythms (37, 56, 59), antinociception (25), locomotion (8, 17, 18, 38, 45), sleep-wake cycles (3, 20, 27), and electrocortical arousal (5, 36, 50) have all been reported to have partial cholinergic substrates.

Arousal can be operationally defined as either EEG desynchronization or as behavioral activity, the latter often being measured as gross motor activity as recorded by photocell beam interruptions. Central cholinergic neurotransmission has been associated with both EEG and behavioral measures of arousal. Using the cortical cup technique, increased concentrations of ACh were recovered during EEG desynchronization in cats (29) and during increased behavioral activity in rabbits (9). More recently, significant correlations have been reported to exist between cortical, hippocampal and striatal dialysate ACh concentrations (or whole brain tissue concentrations) and motor activity counts after anticholinergic treatment (19, 52, 54). In preliminary studies in this laboratory, it appeared that under basal conditions, ACh release in the dorsal hippocampus, frontal cortex, and striatum tended to vary with the animals' activity levels. The experiments reported here were undertaken to further examine the possible relationship between ACh release in these brain regions and arousal, as measured by locomotor activity. Photocell beam interruptions and in vivo microdialysate concentrations of ACh and choline (Ch) in the dorsal hippocampus, frontal cortex and dorsal striatum were measured simultaneously under three conditions: 1) after an injection of vehicle; 2) after administration of the muscarinic receptor antagonist, scopolamine; and 3) before and after the beginning of the rats' night cycle.

METHOD

Experimental Protocol and Drugs

Experiments were performed on male Wistar rats (250–330 g) two days after the implantation of a microdialysis probe. Following surgery, rats were housed individually in Plexiglas cages (35 × 35 × 25 cm), were maintained on a 12:12-h light:dark schedule, and had food and water available ad lib. During the light phase of the rats' daily cycle, subjects were first injected with

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water (1 ml/kg, SC), and then 3 h later with the muscarinic antagonist, scopolamine hydrobromide (0.4 mg/kg, Sigma). Experiments continued into the rats' dark phase (when the room lights were turned off) approximately 4 h later. During each of these conditions, locomotor activity and hippocampal, cortical or striatal interstitial concentrations of ACh and Ch were measured simultaneously.

Surgery and Microdialysis

Brain microdialysis was performed as previously described (10,57). Briefly, rats were stereotaxically implanted with a transverse dialysis probe (13,24) under pentobarbital anesthesia (50–60 mg/kg, IP). The probe was placed in one of the three following sites according to the atlas of Paxinos and Watson (40), measured from bregma: striatum A: +1.7, V: −4.75; hippocampus A: −4.3, V: −3.3; frontal cortex A: +2.7, V: −2.5. The probes were made of saponified cellulose ester dialysis fibre (i.d. = 0.22 mm, o.d. = 0.27 mm, molecular weight cut off >10,000 dalton; Cordis Dow Medical), having an active surface length of 6.4, 6.8, or 10.9 mm for the striatum, hippocampus, or frontal cortex, respectively. On completion of each experiment, the probe location was verified using standard histological procedures.

During microdialysis experiments the dialysis fibre was perfused at 5 μl/min, controlled by a syringe pump (Carnegie Medicin). The syringe was connected to the probe inlet by polyethylene tubing (800 × 0.28 mm); the probe outlet was connected to the sample loop (100 μl) of the analytical system by fused silica tubing (800 × 0.1 mm). The sample valve was controlled by an adjustable timer (Valco), and samples (50 μl) were collected and injected at ten-minute intervals.

The composition of the perfusion solution was selected to estimate the ionic composition of the interstitial fluid in the brain (23) and contained NaCl (125 mM), KCl (3 mM), $CaCl_2$ (1.3 mM), $MgCl_2$ (1.0 mM), $NaHCO_3$ (23 mM) in aqueous solution (pH 7.3). To recover detectable dialysate concentrations of ACh, a reversible cholinesterase inhibitory (neostigmine bromide, 0.1 μM; Sigma) was included in the perfusion solution. Thirty minutes of perfusion preceded sample collection to allow for equilibration of the brain with the perfusion solution.

Assay of ACh

ACh was assayed by HPLC-ECD in conjunction with an enzyme reactor (11). ACh and Ch were separated on a reverse phase column (75 × 2.1 mm) pretreated with lauryl sulphate. The eluent from this analytical column then passed through an enzyme reactor (10 × 2.1 mm) containing acetylcholinesterase (EC 3.1.1.7; Sigma, type VI-S) and choline oxidase (1.1.3.17; Sigma), covalently bound to glutaraldehyde-activated Lichrosorb NH_2 (10 μm; Merck). The separated ACh and Ch reacted to give a stoichiometric yield of hydrogen peroxide, which was electrochemically detected at a platinum electrode at a potential of +500 mV versus an Ag/AgCl reference electrode (BAS-LC4B). The mobile phase, 0.2 M aqueous potassium phosphate buffer pH 8.0, was delivered by a pump (LKB-2150) at 0.4 ml/min. The detection limit of the assay was approximately 50 fmol/injection. The time required to complete a chromatogram was 4–5 min. Standards of ACh/Ch were injected hourly.

Motor Activity

During the microdialysis experiments, a Digiscan Animal Activity Monitor [model RXYZCM(16); Omnitech Electronics, Inc.] was used to measure locomotor activity in 10-min blocks corresponding to the 10-min dialysate samples. Stereotypy counts were subtracted from the total horizontal counts to yield a measure of ambulation.

TABLE 1

PROBE CHARACTERISTICS AND BASELINE OUTPUT OF ACh AND Ch IN THE STRIATUM, HIPPOCAMPUS AND FRONTAL CORTEX OF RATS

Brain Region	Baseline Dialysate Output (fmol/min/mm) ACh	Ch	Probe Surface Length (mm)	In Vitro Probe Recovery (%)
Striatum	9.04 ± 0.67	588 ± 130	6.4	21.4 ± 6.2
Hippocampus	2.10 ± 0.48	368 ± 82	6.8	22.5 ± 5.4
Frontal cortex	2.30 ± 0.56	294 ± 82	10.9	33.3 ± 7.6

Dialysate outputs are expressed as the mean (±S.E.M.) of the last 3 prescopolamine values from 4–6 rats. Outputs are corrected for the active surface length of the probes, as shown. Neostigmine (100 nM) was included in the perfusion solution.

Statistical Analyses

Biochemical data were calculated as a percent of baseline concentrations, 100% baseline being defined as the average of the last three prescopolamine values. A univariate analysis of variance (ANOVA) with repeated measures was used to compare the effects of scopolamine and vehicle on ACh and Ch output. ANOVAs were also conducted to evaluate the effects of the vehicle injection and exposure to dark on ACh and Ch. These analyses included the three samples prior to and the three samples after the initiation of these two treatments. Comparisons of the biochemical responses to the three experimental variables between brain regions were also made using ANOVAs. Greenhouse-Geisser adjustments of *df* were made to account for the use of time as a repeated measure. All reported values refer to the interaction effect of time with experimental treatment. Pearson's correlation coefficients between ACh concentrations (in fmol/min) and motor counts were determined for the 3 treatment conditions in each individual rat. Group correlations were carried out as well, in which the data of all animals having probes in the same brain region were combined for analysis. In this case, the biochemical measures were expressed as percentage of baseline. Because motor counts were not normally distributed, for all the correlation analyses these values were normalized using logarithmic transformation.

RESULTS

The average baseline outputs of ACh and Ch in the three brain regions are shown in Table 1 and have been corrected for the differences in probe surface length.

The neurochemical and motor effects of vehicle and scopolamine injections, and exposure to dark, are shown in Figs. 1, 2 and 3 for the striatum, hippocampus and cortex, respectively. Vehicle injections transiently increased extracellular ACh by 54% in the hippocampus ($p<0.05$) and by 161% in the frontal cortex (n.s.), while no change was evident in the striatum. No significant effect of this treatment on dialysate concentrations of Ch was observed in any brain region. The responses of ACh and Ch in the three brain regions to vehicle treatment were not significantly

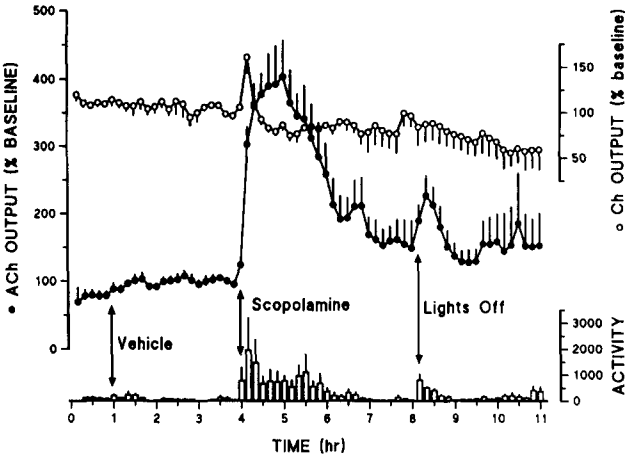

FIG. 1. Striatal dialysate values of ACh (●) and Ch (○) after injections of vehicle and scopolamine and exposure to dark. Activity counts (bars) were measured concurrently. Vehicle (1 ml/kg) and scopolamine (0.4 mg/kg) were injected subcutaneously, and lights off occurred at the appropriate time in the rats' daily cycle. Data points represent group means (n = 4–6) ± S.E.M.

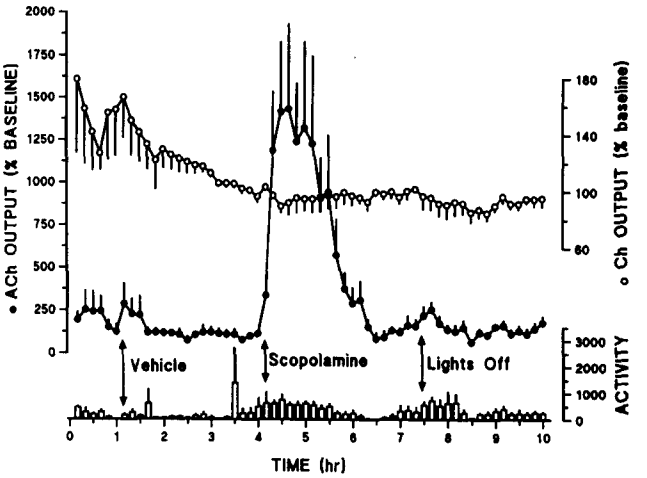

FIG. 3. Frontal dialysate values of ACh (●) and Ch (○) after injections of vehicle and scopolamine and exposure to dark. Activity counts (bars) were measured concurrently. Data points represent group means (n = 4–5) ± S.E.M.

different. Injection of vehicle caused short-lasting increases in motor activity.

Scopolamine (0.4 mg/kg, SC) caused large increases in interstitial ACh and in motor activity. Peak ACh increases of 400, 1200 and 1400% in the striatum, hippocampus and cortex, respectively, occurred within 20 minutes and persisted for approximately one hour. Compared to vehicle values, scopolamine had a significant effect on ACh output (striatum $p<0.001$, hippocampus and cortex $p<0.05$). The drug significantly affected Ch only in the striatum ($p<0.01$) where there was an increase in the second postinjection sample (159% of baseline). The effect of scopolamine on ACh and Ch was significantly smaller in the striatum than in either the cortex or hippocampus ($p<0.05$). The drug responses in the hippocampus and cortex did not differ significantly from each other. Motor activity was greatly increased by scopolamine with approximately the same time course as was observed for the increase in ACh.

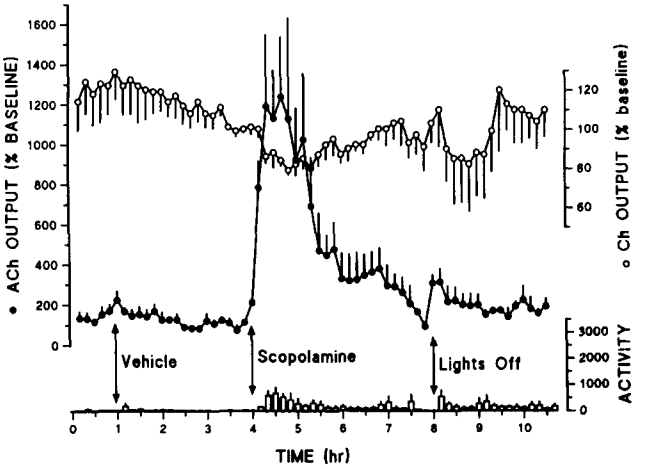

FIG. 2. Hippocampal dialysate values of ACh (●) and Ch (○) after injections of vehicle and scopolamine and exposure to dark. Activity counts (bars) were measured concurrently. Data points represent group means (n = 4) ± S.E.M.

At the onset of the dark phase of the rats' day-night cycle, motor activity increases coincided with ACh increases of 58% in the striatum, 169% in the hippocampus, and 77% in the cortex. In the striatum, ACh showed a significant illumination by time interaction effect ($p<0.05$); the increases in the other areas failed to reach significance. The three brain regions did not differ significantly with respect to the ACh nor Ch response to dark.

To illustrate the nature of the relationship between behavioral activity and ACh measured in either the striatum, hippocampus or cortex, Fig. 4 shows the motor and dialysate profiles of 3 individual animals. Most of the animals tested exhibited significant correlations between locomotor activity and ACh release under all three experimental conditions. After vehicle injection, significant correlations ($p<0.05$) were found in 2 of 4 animals with striatal probes (r = .572–.643), 3 of 4 animals with hippocampal probes (r = .611–.808) and in 3 of 5 animals with frontal cortex probes (r = .665–.704). Scopolamine treatment yielded significant values in all 6 animals with striatal probes (r = .532–.867), in all 4 with hippocampal probes (r = .517–.736) and in 4 of 5 animals with frontal cortex probes (r = .741–.877). During lights-off, motor counts correlated significantly with dialysate ACh concentrations in the striatum (4 of 5 animals, r = .677–.844), in the hippocampus (all 4 animals, r = .493–.926) and in the cortex (3 of 4 animals, r = .696–.899).

As evident in Table 2, when the data were grouped across animals, the correlation values became less robust. This grouping facilitates comparisons between the brain regions across the experimental conditions. The highest correlation between motor activity and ACh output occurred in the hippocampus under the scopolamine condition. The highest value for the striatum was also after scopolamine, while in the cortex ACh concentrations correlated best with motor activity during the dark phase. The lowest correlations for each group were seen after vehicle treatment.

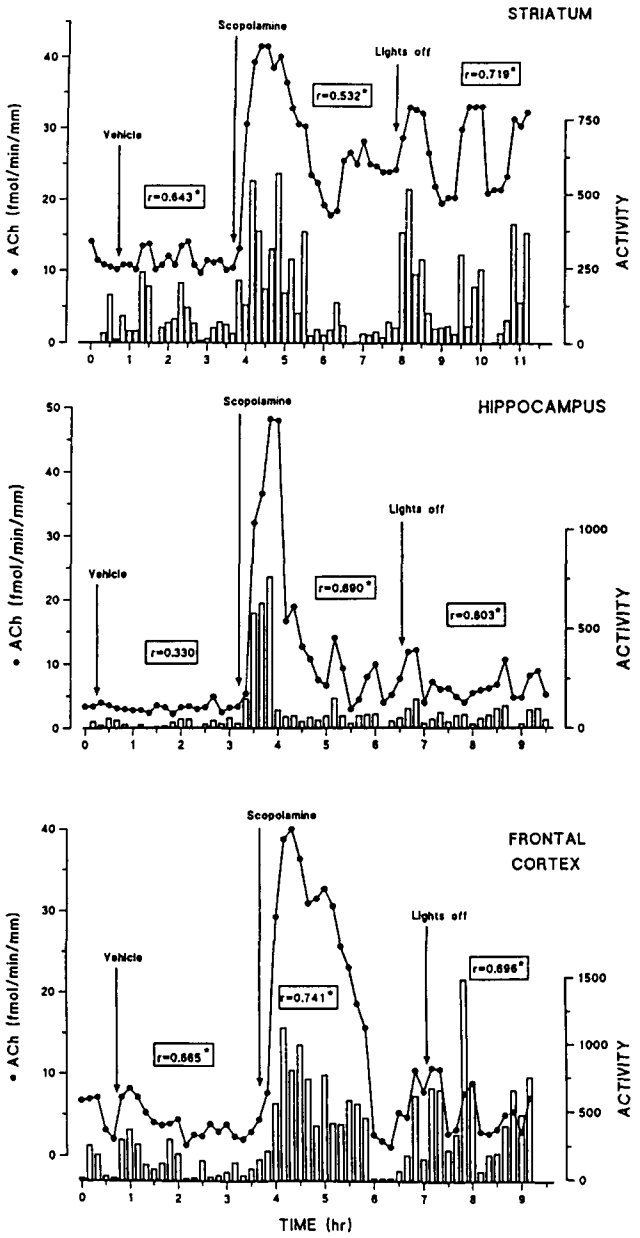

FIG. 4. Dialysate ACh concentrations (●) and activity counts (bars) from three individual rats after injections of vehicle (1 ml/kg) and scopolamine (0.4 mg/kg), and exposure to dark. Insets show Pearson's correlation coefficients of the two measures for the time points within each experimental treatment. ★$p<0.05$.

DISCUSSION

Previous microdialysis studies have reported baseline outputs of ACh in the frontal cortex, hippocampus and striatum that are an order of magnitude higher than those reported here (1, 53, 58). These discrepancies are likely due to differences in probe type and brain placement, postoperative recovery times, type and concentration of the cholinesterase inhibitor, and composition and pH of the perfusate. For example, recent studies have indicated

TABLE 2

CORRELATION OF REGIONAL ACh OUTPUTS AND ACTIVITY COUNTS DURING EXPERIMENTAL TREATMENTS

Brain Region	Treatment		
	Vehicle	Scopolamine	Lights Off
Striatum	.256	.599*	.300*
Hippocampus	.472*	.630*	.595*
Frontal cortex	.151	.468*	.515*

Pearson's correlation coefficients of ACh outputs (as % baseline) in the striatum, hippocampus or cortex (4–6 animals/group) and locomotor activity are shown. Analyses included the two measures for all time points (10–26) within each experimental treatment. *$p<0.05$.

that 24 hours after probe implantation, tissue trauma destabilizes dialysate concentrations of neurotransmitters and their metabolites, and that second or third day measurements may be more physiologically relevant (43,60). The lower concentration of cholinesterase inhibitor used in this study, and the more physiological pH and calcium concentration of the perfusion solution are the most likely sources of the differences between the present and previous results.

The rank order of baseline concentrations measured in the three brain regions compares well with those found previously in dialysates and tissue homogenates: for ACh, striatum >> frontal cortex ≃ hippocampus (1, 28, 53, 55, 58); for Ch, striatum > hippocampus > frontal cortex (28,53). The source of ACh in these regions is anatomically distinct. The striatum contains intrinsic cholinergic interneurons (21, 41, 48), while the hippocampus and cortex receive cholinergic projections mainly from the anterior and posterior regions of the cholinergic basal nuclear complex respectively (46).

Only recently have dialysate concentrations of Ch been reported (10, 52, 53), and the source and significance of this Ch are not well understood. Ch is both a precursor of ACh and a product of its breakdown. Further complicating any interpretation of changes in extracellular Ch is the fact that at least 80% of the Ch turnover in the brain is thought to be involved in phospholipid metabolism (7). Additional investigations of the source of extracellular Ch are required before the meaning of the experimentally manipulated concentrations of dialysate Ch can be determined.

Subcutaneous injection of vehicle tended to increase locomotion and dialysate ACh concentrations in the hippocampus and frontal cortex, although the effect in the latter structure failed to reach significance due to high intersubject variability. This finding illustrates the necessity of including control vehicle injections when studying the effects of drugs on hippocampal or cortical ACh; it also suggests that it may be possible to study cholinergic correlates of behavior in one or both of these structures.

It is well documented that muscarinic antagonists, including scopolamine, increase ACh release in the striatum (16,30), hippocampus (39,51), and cortex (2,44). Recently, this phenomenon has been confirmed using microdialysis (15, 33, 52–54). However, increases in dialysate ACh concentrations in these studies, although produced by larger doses of scopolamine, were not as robust as those reported here. This discrepancy may be explained by some of the methodological considerations discussed above. Specifically, the calcium concentration in the perfusate used in this study more closely approximates the interstitial fluid of the brain than do the concentrations used in earlier studies. Varia-

tions in the calcium concentration of the perfusate can profoundly influence cholinergic (10,14) and, to a lesser extent, dopaminergic (34) responses measured by microdialysis.

The rank order of scopolamine's effect on ACh (cortex ≃ hippocampus >> striatum) may reflect regional differences in muscarinic autoreceptor location and/or function. In the cortex and hippocampus, the effect of the antagonist on ACh release is thought to be mediated by muscarinic autoreceptors located on cholinergic terminals (32, 35, 49). On the other hand, evoked ACh release in the striatum is apparently not controlled by muscarinic terminal autoreceptors (26, 31, 42). The necessary components for the effects of muscarinic antagonists on striatal ACh are known to be intrinsic to this region because local application of antimuscarinics can increase dialysate ACh concentrations (15,33). The increased release of ACh measured in the striatum after scopolamine [this study, (53,54)] may, therefore, be mediated by multi-neuron circuits within the striatum, or possibly by dendritic or somal receptors located on the cholinergic interneurons.

Scopolamine transiently increased dialysate Ch concentrations only in the striatum. This result differs from Toide and Arima (53) who reported that Ch was decreased by approximately 40% in all three brain regions for two hours after scopolamine. As discussed above, there are numerous methodological differences between the two studies that may account for these discrepancies. The present data indicate that Ch, like ACh, can be affected in a regionally selective manner by scopolamine.

Exposure to dark at the appropriate time in the rats' day-night cycle tended to transiently increase ACh concentrations in all three areas with no apparent effect on Ch. However, only the increase in the striatum was statistically significant, and this was due to the high variability of the responses in the other two brain areas. These data suggest that environmental stimuli can influence cholinergic neurotransmission in these brain regions. Whole brain ACh concentrations in rats have been reported to exhibit diurnal oscillations (22). The lowest concentrations of ACh in tissue extracts, thought to reflect high cholinergic activity, were found 6 hours after the onset of the dark period.

The present results indicate that dialysate concentrations of ACh in the striatum, hippocampus and frontal cortex generally correlate with locomotor activity, a measure of behavioral arousal. This extends previous findings that muscarinic receptor antagonist-induced increases in motor activity are associated with increases in frontal and hippocampal dialysate ACh (52), and with decreases in whole brain tissue levels of ACh (19). In addition, the present study identified significant correlations between ACh and activity counts in two drug-free situations. More detailed behavioral monitoring might further characterize these relationships, but the currently available temporal resolution of ACh microdialysis (5–10 min) is a limiting factor in elucidating such relationships.

The concentration of ACh in dialysate is thought to reflect synaptic overflow of neuronally released ACh (12,13). The data reported here thus suggest that ACh release in all three experimental conditions correlates positively with motor activity. In both drug-free conditions, increased release would, therefore, be associated with increased transmission at cholinergic synapses. After injection of scopolamine, however, the increase in ACh release occurred as a result of, and during, muscarinic receptor blockade. The hyperactivity caused by this anticholinergic agent would thus occur in the presence of decreased muscarinic cholinergic transmission. Therefore, while cholinergic transmission correlated with behavioral arousal in two drug-free conditions, it would not appear to be associated with hyperactivity in scopolamine-treated rats. Several factors could account for this apparent inconsistency: 1) Pre- and postsynaptic muscarinic receptors may differ such that the dose of scopolamine used here might preferentially block the presynaptic autoreceptors, resulting in increased ACh release, while the postsynaptic receptors remain unblocked. This would serve to increase ACh transmission which would be consistent with increased locomotor behavior. This seems unlikely, however, as Szerb et al. (51) have reported that scopolamine has a 10-fold lower efficacy at presynaptic than at postsynaptic muscarinic receptors. 2) While scopolamine blocks muscarinic transmission, it would at the same time increase nicotinic cholinergic transmission by increasing ACh release. The results obtained here could be explained if the action of ACh on nicotinic receptors was responsible for the correlation between ACh release and behavioral arousal.

In summary, the experiments presented here illustrate the feasibility of carrying out ACh microdialysis experiments of long duration, coupled to behavioral monitoring, in different brain regions. These data also indicate that various manipulations can affect cholinergic transmission in a regionally selective manner.

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REFERENCES

1. Ajima, A.; Kato, T. Brain dialysis: Detection of acetylcholine release in the striatum, hippocampus and frontal cortex of freely moving rats. Biogenic Amines 5:461–464; 1988.
2. Aquilonius, S.-M.; Lundholm, B.; Winbladh, B. Effects of some anticholinergic drugs on cortical acetylcholine release and motor activity in rats. Eur. J. Pharmacol. 20:224–230; 1972.
3. Baghdoyan, H. A.; Monaco, A. P.; Rodrigo-Angulo, M. L.; Assens, F.; McCarley, R. W.; Hobson, J. A. Microinjection of neostigmine into the pontine reticular formation of cats enhances desynchronized sleep signs. J. Pharmacol. Exp. Ther. 231:173–180; 1984.
4. Bartus, R. T.; Dean, R. L.; Flicker, C. Cholinergic psychopharmacology: An integration of human and animal research on memory. In: Meltzer, H. Y., ed. Psychopharmacology: The third generation of progress. New York: Raven Press; 1987:219–232.
5. Beani, L.; Bianchi, C.; Santinoceto, L.; Marchetti, P. The cerebral acetylcholine release in conscious rabbits with semi-permanently implanted epidural cups. Int. J. Neuropharmacol. 7:469–481; 1968.
6. Borison, R. L.; Diamond, B. I. Neuropharmacology of the extrapyramidal system. J. Clin. Psychiatry (Suppl.) 48:7–12; 1987.
7. Choi, R. L.; Freeman, J. J.; Jenden, D. J. Kinetics of plasma choline in relation to turnover of brain choline and formation of acetylcholine. J. Neurochem. 24:735–741; 1975.
8. Clarke, P. B. S.; Kumar, R. Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br. J. Pharmacol. 80:587–594; 1983.
9. Collier, B.; Mitchell, J. F. The central release of acetylcholine during consciousness and after brain lesions. J. Physiol. 188:83–98; 1967.
10. Damsma, G.; de Boer, P.; Westerink, B. H. C.; Fibiger, H. C. Dopaminergic regulation of striatal cholinergic interneurons: An in vivo microdialysis study. Naunyn Schmiedebergs Arch. Pharmacol.; in press.
11. Damsma, G.; Lammerts van Bueren, D.; Westerink, B. H. C.; Horn, A. S. Determination of acetylcholine and choline in the femtomole range by means of HPLC, a postcolumn enzyme reactor, and electrochemical detection. Chromatographia 24:827–831; 1987.

12. Damsma, G.; Westerink, B. H. C.; de Boer, P.; de Vries, J. B.; Horn, A. S. Basal acetylcholine release in freely moving rats detected by on-line trans-striatal dialysis: pharmacological aspects. Life Sci. 43:1161–1168; 1988.
13. Damsma, G.; Westerink, B. H. C.; de Vries, J. B.; Van den Berg, C. J.; Horn, A. S. Measurement of acetylcholine release in freely moving rats by means of automated intracerebral dialysis. J. Neurochem. 48:1523–1528; 1987.
14. de Boer, P.; Damsma, G.; Fibiger, H. C.; Timmerman, W.; de Vries, J. B.; Westerink, B. H. C. Dopaminergic-cholinergic interactions in the striatum: The critical significance of calcium concentrations in brain microdialysis. Naunyn Schmiedebergs Arch. Pharmacol.; in press.
15. de Boer, P.; Westerink, B. H. C.; Rollema, H.; Zaagsma, J.; Horn, A. S. An M_3-like muscarinic autoreceptor regulates the in vivo release of acetylcholine in rat striatum. Eur. J. Pharmacol. 179:167–172; 1990.
16. Dolezal, V.; Wecker, L. Muscarinic receptor blockade increases basal acetylcholine release from striatal slices. J. Pharmacol. Exp. Ther. 252:739–743; 1990.
17. Fibiger, H. C.; Lynch, G. S.; Cooper, H. P. A biphasic action of central cholinergic stimulation on behavioral arousal in the rat. Psychopharmacologia 20:366–382; 1971.
18. Flicker, C.; Geyer, M. A. Behavior during hippocampal microinfusions. II. Muscarinic locomotor activation. Brain Res. Rev. 4:105–127; 1982.
19. Frances, H.; Jacob, J. Comparaison des effets de substances cholinergiques et anticholinergiques sur les taux cerebraux d'acetylcholine et sur la motilite chez la souris. Psychopharmacologia 21:338–352; 1971.
20. Gadea-Ciria, M.; Stadler, H.; Lloyd, K. G.; Bartholini, G. Acetylcholine release within the cat striatum during the sleep-wakefulness cycle. Nature 243:518–519; 1973.
21. Groves, P. M. A theory of the functional organization of the neostriatum and the neostriatal control of voluntary movement. Brain Res. Rev. 5:109–132; 1983.
22. Hanin, I.; Massarelli, R.; Costa, E. Acetylcholine concentrations in rat brain: diurnal oscillation. Science 170:341–342; 1970.
23. Hansen, A. J. Effect of anoxia on ion distribution in the brain. Physiol. Rev. 65:101–148; 1985.
24. Imperato, A.; Di Chiara, G. Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: A new method for the study of the in vivo release of endogenous dopamine and metabolites. J. Neurosci. 4:966–977; 1984.
25. Iwamoto, E. T. Antinociception after nicotine administration into the mesopontine tegmentum of rats: Evidence for muscarinic actions. J. Pharmacol. Exp. Ther. 251:412–421; 1989.
26. James, M. K.; Cubeddu, L. X. Pharmacologic characterization and functional role of muscarinic autoreceptors in the rabbit striatum. J. Pharmacol. Exp. Ther. 240:203–215; 1987.
27. Jasper, H. H.; Tessier, J. Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. Science 172:601–602; 1971.
28. Jope, R. S. Effects of phosphatidylcholine administration to rats on choline in blood and choline and acetylcholine in brain. J. Pharmacol. Exp. Ther. 220:322–328; 1982.
29. Kanai, T.; Szerb, J. C. Mesencephalic reticular activating system and cortical acetylcholine output. Nature 205:80–82; 1965.
30. Lefresne, P.; Rospars, J. P.; Beaujouan, J. C.; Westfall, T. C.; Glowinski, J. Effects of acetylcholine and atropine on the release of ^{14}C-acetylcholine formed from U-^{14}C-glucose in rat brain cortical and striatal prisms. Naunyn Schmiedebergs Arch. Pharmacol. 303:279–285; 1978.
31. Marchi, M.; Paudice, P.; Caviglia, A.; Raiteri, M. Is acetylcholine release from striatal nerve endings regulated by muscarinic autoreceptors? Eur. J. Pharmacol. 91:63–68; 1983.
32. Marchi, M.; Paudice, P.; Raiteri, M. Autoregulation of acetylcholine release in isolated hippocampal nerve endings. Eur. J. Pharmacol. 73:75–79; 1981.
33. Marien, M. R.; Richard, J. W. Drug effects on the release of endogenous acetylcholine in vivo: measurement by intracerebral dialysis and gas chromatography-mass spectrometry. J. Neurochem. 54:2016–2023; 1990.
34. Moghaddam, B.; Bunney, B. S. Ionic composition of microdialysis perfusing solution alters the pharmacological responsiveness and basal outflow of striatal dopamine. J. Neurochem. 53:652–654; 1989.
35. Molenaar, P. C.; Polak, R. L. Inhibition of acetylcholine release by activation of acetylcholine receptors. Prog. Pharmacol. 3/4:39–44; 1980.
36. Montplaisir, J. Y. Cholinergic mechanisms involved in cortical activation during arousal. Electroencephalogr. Clin. Neurophysiol. 38: 263–272; 1975.
37. Morley, B. J.; Murrin, L. C. AF64 depletes hypothalamic high-affinity choline uptake and disrupts the circadian rhythm of locomotor activity without altering the density of nicotinic acetylcholine receptors. Brain Res. 504:238–246; 1989.
38. Museo, E.; Wise, R. A. Locomotion induced by ventral tegmental microinjections of a nicotinic agonist. Pharmacol. Biochem. Behav. 35:735–737; 1990.
39. Nordström, O.; Bartfai, T. Muscarinic autoreceptor regulates acetylcholine release in rat hippocampus: in vitro evidence. Acta Physiol. Scand. 108:347–353; 1980.
40. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates, 2nd ed. London: Academic Press; 1986.
41. Phelps, P. E.; Houser, C. R.; Vaughn, J. E. Immunocytochemical localization of choline acetyltransferase within the rat neostriatum: A correlated light and electron microscopic study of cholinergic neurons and synapses. J. Comp. Neurol. 238:286–307; 1985.
42. Raiteri, M.; Leardi, R.; Marchi, M. Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. J. Pharmacol. Exp. Ther. 228:209–214; 1984.
43. Reiriz, J.; Mena, M. A.; Bazan, E.; Muradas, V.; Lerma, J.; Delgado, J. M. R.; De Yebenes, J. G. Temporal profile of levels of monoamines and their metabolites in striata of rats implanted with dialysis tubes. J. Neurochem. 53:789–792; 1989.
44. Rospars, J. P.; Lefresne, P.; Beaujouan, J. C.; Glowinski, J. Effect of external ACh and of atropine on ^{14}C-ACh synthesis and release in rat cortical slices. Naunyn Schmiedebergs Arch. Pharmacol. 300:153–161; 1977.
45. Schallert, T.; Whishaw, I. Q.; Ramirez, V. D.; Teitelbaum, P. Compulsive, abnormal walking caused by anticholinergics in akinetic, 6-hydroxydopamine-treated rats. Science 199:1461–1463; 1978.
46. Schwaber, J. S.; Rogers, W. T.; Satoh, K.; Fibiger, H. C. Distribution and organization of cholinergic neurons in the rat forebrain demonstrated by computer-aided data acquisition and three-dimensional reconstruction. J. Comp. Neurol. 263:309–325; 1987.
47. Semba, K.; Fibiger, H. C. Organization of central cholinergic systems. Prog. Brain Res. 79:37–63; 1989.
48. Semba, K.; Fibiger, H. C.; Vincent, S. R. Neurotransmitters in the mammalian striatum: Neuronal circuits and heterogeneity. Can. J. Neurol. Sci. 14:386–394; 1987.
49. Sethy, V. H.; Hyslop, D. K. Effect of irreversible loss of muscarinic receptors on (^{3}H)-acetylcholine release from the hippocampus. Neuropharmacology 29:185–188; 1990.
50. Szerb, J. C. Cortical acetylcholine release and electroencephalographic arousal. J. Physiol. 192:329–343; 1967.
51. Szerb, J. C.; Hadhazy, P.; Dudar, J. D. Release of [^{3}H]acetylcholine from rat hippocampal slices: effect of septal lesion and of graded concentrations of muscarinic agonists and antagonists. Brain Res. 128:285–291; 1977.
52. Toide, K. Effects of scopolamine on extracellular acetylcholine and choline levels and on spontaneous motor activity in freely moving rats measured by brain dialysis. Pharmacol. Biochem. Behav. 33: 109–113; 1989.
53. Toide, K.; Arima, T. Effects of cholinergic drugs on extracellular levels of acetylcholine and choline in rat cortex, hippocampus and striatum studied by brain dialysis. Eur. J. Pharmacol. 173:133–141; 1989.
54. Watanabe, H.; Shimizu, H. Effect of anticholinergic drugs on striatal acetylcholine release and motor activity in freely moving rats studied by brain microdialysis. Jpn. J. Pharmacol. 51:75–82; 1989.
55. Wecker, L.; Dettbarn, W.-D. Relationship between choline availability and acetylcholine synthesis in discrete regions of rat brain. J. Neurochem. 32:961–967; 1979.
56. Wee, B. E. F.; Turek, F. W. Carbachol phase shifts the circadian rhythm of locomotor activity in the Djungarian hamster. Brain Res. 505:209–214; 1989.

57. Westerink, B. H. C.; Tuinte, M. H. J. Chronic use of intracerebral dialysis for the in vivo measurement of 3,4-dihydroxyphenylethylamine and its metabolite 3,4-dihydroxyphenylacetic acid. J. Neurochem. 46:181–185; 1986.
58. Wu, C. F.; Bertorelli, R.; Sacconi, M.; Pepeu, G.; Consolo, S. Decrease of brain acetylcholine release in aging freely-moving rats detected by microdialysis. Neurobiol. Aging 9:357–361; 1988.
59. Zatz, M.; Herkenham, M. A. Intraventricular carbachol mimics the phase-shifting effect of light on the circadian rhythm of wheel-running activity. Brain Res. 212:234–238; 1981.
60. Zis, A. P.; Nomikos, G. G.; Damsma, G.; Fibiger, H. C. In vivo neurochemical effects of electroconvulsive shock studied by microdialysis in the rat striatum. Psychopharmacology (Berlin); submitted.