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The Role of Ventrolateral Striatal Acetylcholine in the Production of Tacrine-Induced Jaw Movements

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COUSINS, M. S, M. FINN, J. TREVITT, D. L. CARRIERO, A. CONLAN, AND J. D. SALAMONE. *The role of ventrolateral striatal acetylcholine in the production of tacrine-induced jaw movements.* PHARMACOL BIOCHEM BEHAV **62**(3) 439–447, 1999.—The anticholinesterase tacrine induces tremulous jaw movements in rats, and considerable evidence indicates that this response is dependent upon ventrolateral striatal mechanisms. Three experiments were conducted to study the relation between ventrolateral striatal acetylcholine and the production of tremulous jaw movements. In Experiment 1, intracranial microinjection of the acetylcholine synthesis inhibitor hemicholinium-3 into the ventrolateral neostriatum reduced tremulous jaw movements induced by 5.0 mg/kg tacrine. Microinjection of hemicholinium into a cortical site dorsal to striatum (Experiment 2) was without significant effect upon tacrine-induced tremulous jaw movements. In Experiment 3, rats were implanted with dialysis probes in the ventrolateral striatum to measure extracellular levels of acetylcholine during tacrine-induced jaw movements. Tacrine (2.5–5.0 mg/kg) increased both extracellular acetylcholine and tremulous jaw movements. The 5.0 mg/kg dose of tacrine produced a substantial increase in ventrolateral striatal acetylcholine levels (324% of baseline within 30 min). Across all tacrine-treated rats there was a significant linear correlation between tremulous jaw movements and acetylcholine levels ($r = +0.56$) during the first 30-min postinjection period. This correlation was largely due to the group that received 5.0 mg/kg tacrine; within this group, there was a very high correlation ($r = +0.87$) between tremulous jaw movements and acetylcholine levels in the first sample after injection. These data are consistent with the notion that tremulous jaw movements induced by tacrine are mediated by ventrolateral striatal acetylcholine. Moreover, these results suggest that dialysis methods could be used to monitor the relation between striatal acetylcholine and tremulous movements induced by a variety of different conditions. © 1999 Elsevier Science Inc.

THA Cognex Dialysis Tremor Vacuous Chewing Orofacial Parkinson Alzheimer Caudate putamen

SEVERAL lines of evidence indicate that brain acetylcholine (ACh) is involved in the production of parkinsonian symptoms. Idiopathic and neuroleptic-induced parkinsonism are often treated with muscarinic antagonists (2,15,32,34,53). The muscarinic agonists bethanecol and RS86 have been shown to produce or worsen parkinsonian symptoms in humans (20,36). Physostigmine has been shown to exacerbate parkinsonian symptoms (15); this anticholinesterase also enhances the neuroleptic-induced oral tremor known as "rabbit syndrome" (57). Tacrine (Cognex), an acetylcholinesterase inhibitor that is used for the treatment of Alzheimer's disease (52), can produce parkinsonian side effects such as bradykinesia, rigidity, and tremor (26,38).

Research with animals has demonstrated that cholinomimetic drugs produce motor effects that are similar to parkinsonian symptoms. Cholinomimetic drugs impair operant lever pressing (7,11), produce catalepsy (18,29) and suppress locomotor activity (7,48). Cholinomimetic drugs such as tremorine, oxotremorine, physostigmine, and tacrine are known to be tremorogenic agents (22,30,37). One of the motor effects produced by cholinomimetics is tremulous jaw movements (also known as vacuous jaw movements or purposeless chewing). This response is characterized by rapid, vertical deflections of the lower jaw that resemble chewing but are not directed at any stimulus. Although there is some disagreement

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about the clinical relevance of drug-induced oral behaviors (43,55), it has been suggested that tremulous jaw movements in rats share some characteristics with human parkinsonian symptoms (23,46). Tremulous jaw movements can be induced by dopamine (DA) depletion and cholinergic stimulation (5,6,8, 16,22,23,31,40,44,46,47,50). The jaw movements induced by acute interference with DA systems can be alleviated by antiparkinsonian muscarinic antagonists (41,42,45,49). Cholinomimetic-induced jaw movements can be reduced by antiparkinsonian drugs such as apomorphine, bromocriptine, L-DOPA, and amantadine (9,51). The full D_1 agonist SKF 82958 also suppresses cholinomimetic-induced jaw movements, while the partial agonist, SKF 38393, does not (9). Recent evidence indicates that tacrine induces tremulous jaw movements in the dose range of 1.25 -10.0 mg/kg (3,7,33). Slow motion videotape analyses demonstrated that the local frequency of tacrine-induced jaw movements was in the 3–7 Hz frequency range (10,33), and electromyographic methods demonstrated that the jaw movements induced by 2.5 mg/kg tacrine were accompanied by rhythmic 4-Hz oscillations of activity in the temporalis muscle, which is involved in jaw closing (10). These findings are consistent with the tremor frequency of 3–7 Hz that is observed in parkinsonian patients (1). Tacrine-induced jaw movements are not blocked by methylscopolamine, but are blocked by systemic injections of scopolamine, and by microinjection of scopolamine directly into the ventrolateral neostriatum [VLS; (33)]. This is consistent with reports demonstrating the involvement of the VLS, but not the anterior, medial, or dorsal neostriatum, in the production of tremulous jaw movements by physostigmine (25), pilocarpine (28,46), and DA depletion (23).

In the present study, three experiments were conducted to investigate the role of VLS ACh in the production of tacrine-induced tremulous jaw movements in rats. In the first experiment, the high affinity choline uptake inhibitor hemicholinium-3 (HC-3) was microinjected into the VLS to determine its effect on tacrine-induced tremulous jaw movements. The blockade of choline uptake produced by HC-3 results in a decrease in ACh synthesis (17,21), and this experiment was conducted to assess the role of endogenous ACh synthesis in the VLS on tacrine-induced jaw movements. To control for the spread of HC-3 to overlying cortex, the effects of HC-3 injected into a cortical site dorsal to the VLS were assessed in the second experiment. In Experiment 3, the effect of systemic tacrine on VLS ACh levels was determined with in vivo microdialysis in awake, freely moving rats. An observer simultaneously recorded tremulous jaw movements. The VLS was selected as the site for probe implantation based upon the results of Experiment 1, and also because of the previous work showing that the VLS, but not the anterior, medial, or dorsal neostriatum, was important for the production of tremulous jaw movements (16,23,25,28,33,46). Previous work has employed microdialysis methods to study the effects of tacrine on extracellular ACh or DA in neocortex (35), hippocampus (24), and neostriatum (3,56,59). However, extracellular ACh levels in VLS have not previously been assessed following systemic tacrine administration in awake and freely moving rats. The present methods allowed for assessment of the correlation between the neurochemical and behavioral effects of tacrine.

METHOD

Animals

A total of 89 male Sprague–Dawley rats (Harlan–Sprague–Dawley, Indianapolis, IN) were used. Rats were maintained on a 12 L:12 D cycle (lights on 0700 h), and all drug treatments were given 3–7 h after lights on. Food and water were available ad lib. The animals were housed and cared for in accordance with established University and NIH guidelines.

Drugs

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine) and physostigmine were obtained from Sigma (St. Louis, MO) and dissolved in a 0.1% ascorbic acid solution. HC-3 (bromide salt; Sigma) was dissolved in 0.9% saline.

Cannulations for Hemicholinium Experiments

Rats were anesthetized with 50.0 mg/kg sodium pentobarbital and treated with 1.0 mg/kg methylscopolamine before being placed in the stereotax. Animals were implanted with bilateral stainless steel 23-ga guide cannulae aimed either 2.0 mm (VLS site) or 5.0 mm (dorsal cortical control site) above the VLS target site (target site was AP +1.4 mm, ML +4.0 mm, DV −7.2 mm; nose bar raised 5.0 mm above the interaural line). The guide cannulae were secured to the skull by stainless steel screws and cranioplastic cement. The patency of the 23-ga guide cannulae was maintained with 30 ga stylets.

Experimental Procedure for Intracranial Drug Injections

Seven to 10 days following surgery, animals received bilateral microinjections of HC-3 or saline vehicle (0.5 μl total volume; pH of the high-dose solution of HC-3 = 6.7). The injections were made with 30-ga injectors that were set to extend 2.0 mm beyond the tip of the guide cannulae. Injectors were placed into either the VLS (saline vehicle, $n = 12$; 5.0 μg/side HC-3, $n = 7$; 10.0 μg/side HC-3, $n = 8$) or the dorsal control site (saline vehicle, $n = 14$; 10.0 μg/side HC-3, $n = 12$) through the guide cannulae. The drug was pushed at 1.0 μl/min by a Harvard Apparatus syringe pump for 30 s. Following microinjection, the injectors were left in place for 2 min to allow for diffusion of drug into the tissue. Eight minutes following the end of the diffusion period, all rats received 5.0 mg/kg tacrine IP. Immediately after the tacrine injection, the rats were placed in an elevated observation chamber (28 cm^3) for a 10-min habituation period.

At the end of the habituation period an observer blind to the treatment condition counted tremulous jaw movements with a mechanical counter for 5 min (i.e., 10–15 min after tacrine injection). Tremulous jaw movements were defined as rapid deflections of the lower jaw that resembled chewing but were not directed at any apparent physical stimulus (33). Each individual vertical deflection of the jaw was counted as a jaw movement. If an animal groomed or exhibited directed chewing, there was a 5-s time-out period during which tremulous jaw movements were not counted. As defined above, tremulous jaw movements can be distinguished from gaping, which is a large, slow opening of the jaw (46,47), and masseter tremor, which is a very rapid shaking of the cheek muscles that does not necessarily involve jaw openings. Using these behavioral methods, the observer used in these experiments was shown to have a high degree of interrater reliability with a second observer in scoring tremulous jaw movements ($r = 0.92$).

Surgery for Microdialysis

Rats were anesthetized with sodium pentobarbital (50.0 mg/kg), and also received methylscopolamine. Animals received unilateral implantation of a 16-ga stainless steel hollow tube 4.2 mm above the left or right VLS target site (see

above), and each tube was secured to the skull by stainless steel screws and cranioplastic cement. A 19-ga stainless steel tube was used to maintain the patency of the 16-ga guide cannula. Approximately 10–20 days after implantation of the guide cannula the rats were again anesthetized for implantation of the dialysis probe. The dialysis probe was inserted into, and was set to extend 4.0 mm beyond, the 16-ga guide cannula. Loop style dialysis probes were constructed in the laboratory from a dialysis fiber (200 μM, 15,000 mol wt cutoff) with an active probe surface of 6.0 mm (3.0 mm on either side of the probe tip, with the loop set very tightly). The dialysis probe was set in place with cranioplastic cement. The polyethylene tubing attached to the dialysis probe was fed through a metal tether and attached to a fluid swivel. Artificial cerebrospinal fluid (ACSF; 147.2 mM NaCl, 1.4 mM $CaCl_2$, and 4.0 mM KCl, 50 nM neostigmine) was continuously perfused through the tubing and probe by a syringe pump at 2.0 μl/min during testing. At the end of the experiment, histological analyses were performed to confirm placement of the dialysis probes.

Neurochemical Analysis of ACh

Microdialysis samples were analyzed for ACh using a high-performance liquid chromatography (HPLC) with electrochemical detection (ESA, New Bedford, MA). Approximately 10 μl of dialysate was injected into the ACh HPLC system, and the output measured on a chart recorder according to previously described methods (19). The applied potential was +0.3 V (working vs. reference) using a solid-state analytical cell containing a PEEK/platinum working electrode. The mobile phase [100 mM sodium phosphate, 0.5 mM tetramethylammonium chloride, 0.005% microbicide (Reagent MB), and 2.0 mM octanesulfonic acid] was pumped at a flow rate of 0.35 ml/min. The polymeric column was maintained at 35°C. Standards and spiked dialysis samples with choline and ACh (Sigma) were assayed each test day.

Experimental Procedure for Microdialysis Studies

Rats were surgically implanted with the dialysis guide cannulae, and 10–20 days later were implanted with dialysis probes. The flow rate of the ACSF was adjusted to 1.0 μl/min overnight before being returned to 2.0 μl/min the morning of the test day, 30 min before beginning to collect the first dialysis sample. Dialysate samples were collected every 30 min. Immediately after collection, 10 μl was injected into the ACh HPLC. Observations of tremulous jaw movements (see above) were conducted for 5 min at the beginning of every 30-min neurochemical period. After at least two 30-min behavioral and neurochemical baseline periods, rats received an IP injection of saline vehicle ($n = 9$), 2.5 ($n = 8$), or 5.0 mg/kg ($n = 7$) tacrine. Behavioral observations and neurochemical data were gathered for 3.0 h following IP injections. Immediately after testing, rats were sacrificed for histological verification of placements. A correlational study also was performed to study the relation between physostigmine-induced changes in extracellular ACh and the induction of jaw movements. A group of 12 rats received injections of either saline vehicle, 0.0625, 0.125, or 0.25 mg/kg physostigmine ($n = 2$–4 per dose). The same behavioral and dialysis methods described above were used to perform this experiment.

Statistics

For the hemicholinium experiments, a one-way analysis of variance (ANOVA; Systat version 5.03, Evanston, IL) was conducted for each anatomical group (VLS and dorsal cortical control site). In the tacrine microdialysis experiment, behavioral (number of tremulous jaw movements) and neurochemical (picograms expressed as percentage of baseline) data were separately analyzed by a 3 × 6 (treatment group × time period) repeated-measures ANOVA. Planned comparisons that used the error term from the overall analysis were used to test for differences between vehicle and tacrine treatments. The number of comparisons at each time period was limited to two [see (27), pp. 109–118, 209–212]. The Wilcoxon rank-sign test was used to test for differences between the predrug baseline and the first postinjection period for each neurochemical measure. The Pearson product-moment correlation was used to identify significant correlations between neurochemical and behavioral variables. Two types of correlational analyses were performed. Jaw movements and VLS ACh levels were correlated for the first sample after injection, and additional correlations also were performed that included data points from all eight samples. Significance level was set at $p < 0.05$.

RESULTS

Effects of Striatal and Cortical Injections of Hemicholinium

As shown in Table 1, direct injection of HC-3 into the VLS produced a dose-related decrease in tacrine-induced tremulous jaw movements. ANOVA indicated a significant effect of dose, $F(2, 24) = 6.50$, $p < 0.05$]. Planned comparisons determined that 10.0 μg/side of HC-3 suppressed the tremulous jaw movements induced by tacrine. In Experiment 2, there was no significant effect of HC-3 injected into the dorsal cortical control site on tacrine-induced tremulous jaw movements, $F(1, 24) = 3.86$, NS. Figure 1 shows injector sites from Experiments 1 and 2.

Microdialysis Studies

Figure 2 shows the location of probe placements in the VLS. Tremulous jaw movements did not differ between the treatment groups during the two 30-min baseline periods. Figure 3 shows that during the six postinjection time periods there was a significant effect of tacrine treatment on tremulous jaw movements, $F(2, 21) = 20.23$, $p < 0.0001$. There was also a significant effect of time periods, $F(5, 105) = 17.81$, $p < 0.0001$, and a significant tacrine treatment by time period interaction, $F(10, 105) = 4.74$, $p < 0.0001$. Planned comparisons

TABLE 1

EFFECTS OF INTRASTRIATAL AND CORTICAL INJECTIONS OF HC-3 ON THE MEAN (±SEM) NUMBER OF TREMULOUS JAW MOVEMENTS INDUCED BY TACRINE (5.0 mg/kg)

	Mean	SEM
1. VLS injections		
Vehicle	157.6	27.9
5.0 μg/side HC-3	110.6	11.3
10.0 μg/side HC-3	*64.0	9.7
2. Cortical injections		
Vehicle	141.6	16.5
10.0 μg HC-3	103.2	8.7

*Significantly different from vehicle control.

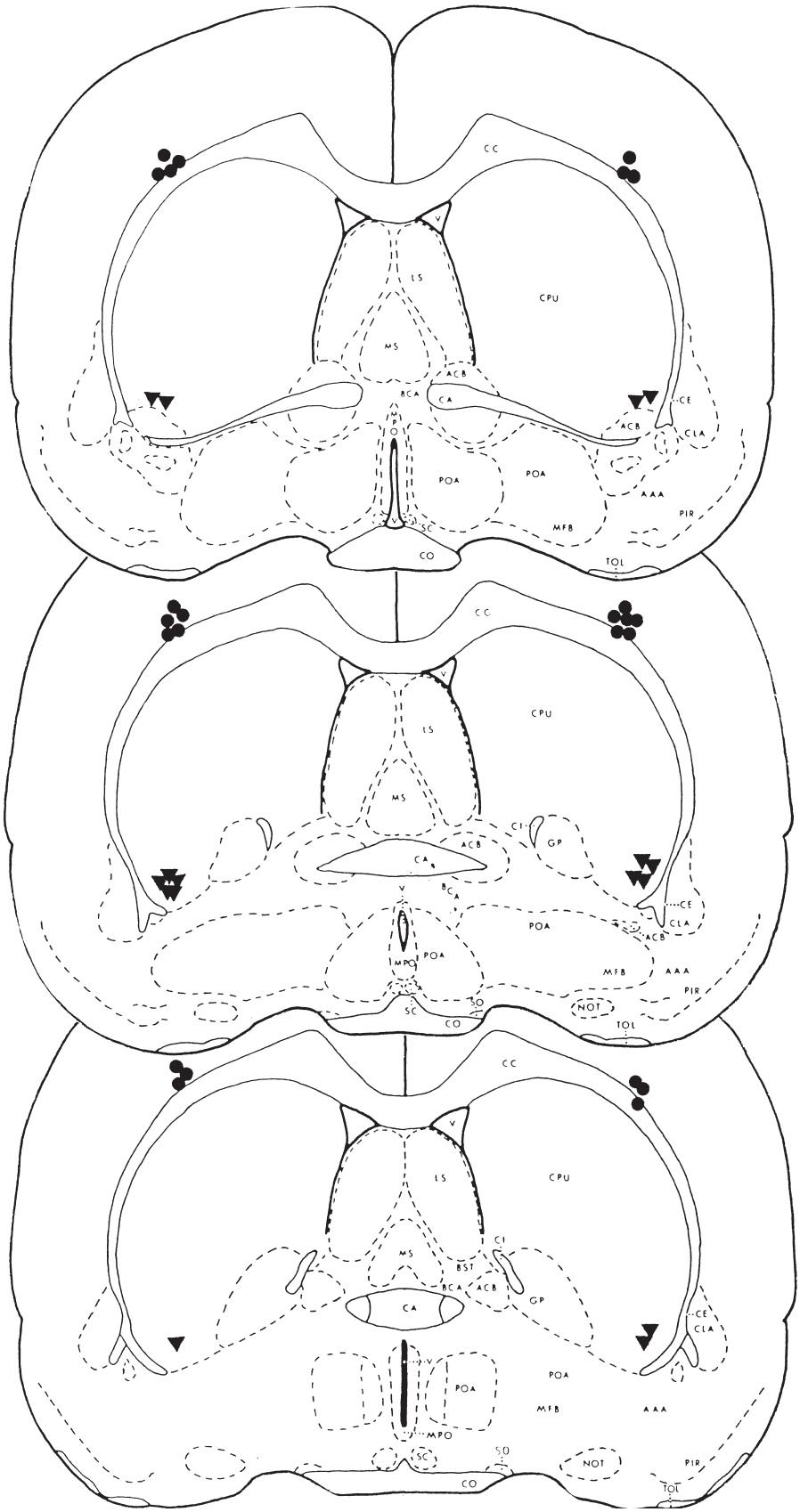

FIG. 1. Injector placements in ventrolateral striatum (triangles) and overlying neocortex (circles), from Experiments 1 and 2; placements are shown for rats that received 10 μg HC-3. cp—caudate putamen.

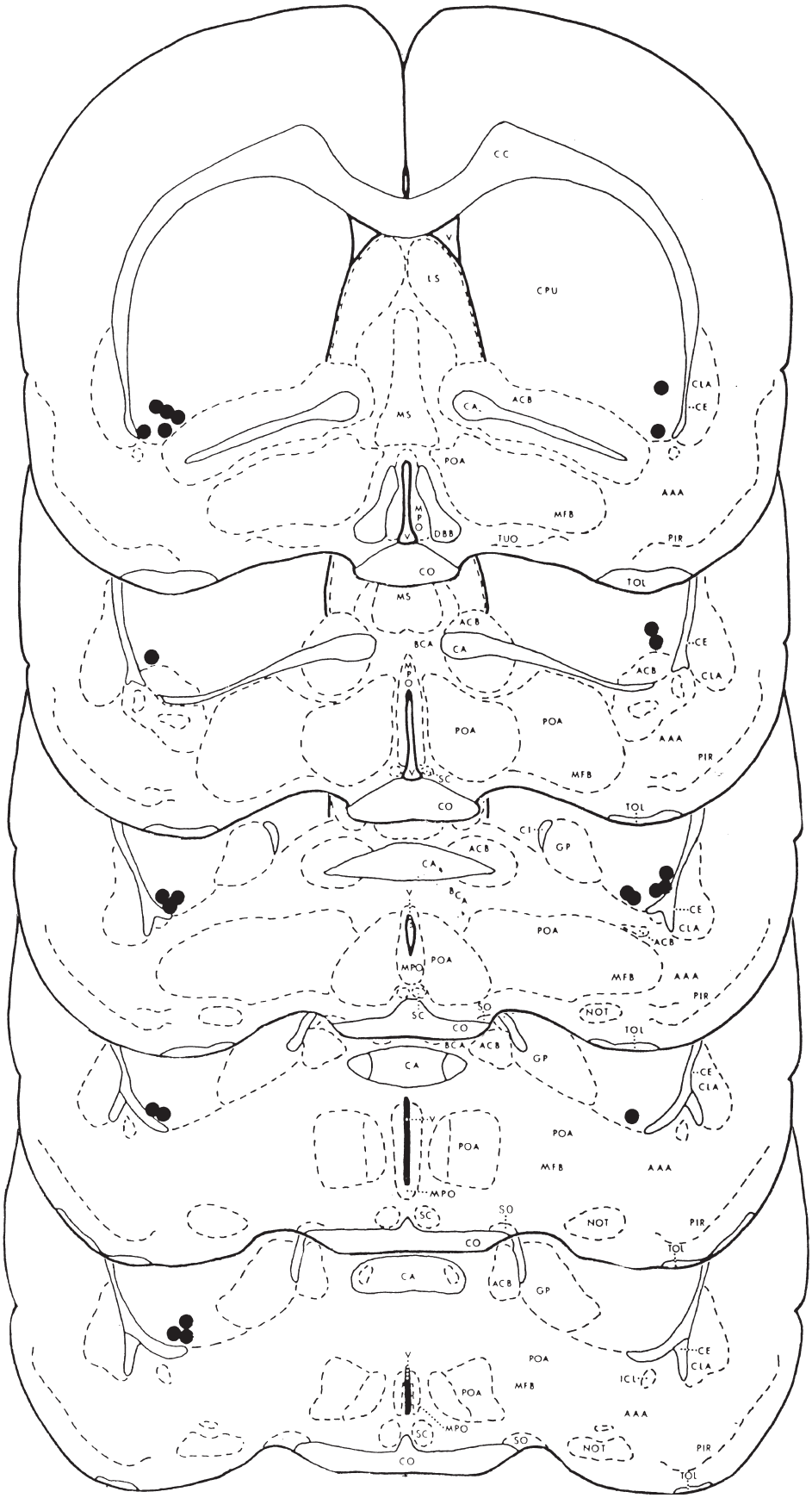

FIG. 2. Probe placements in ventrolateral striatum from Experiment 3; placements (circles) are shown for rats that received vehicle, 2.5 and 5 mg/kg tacrine. cp—caudate putamen.

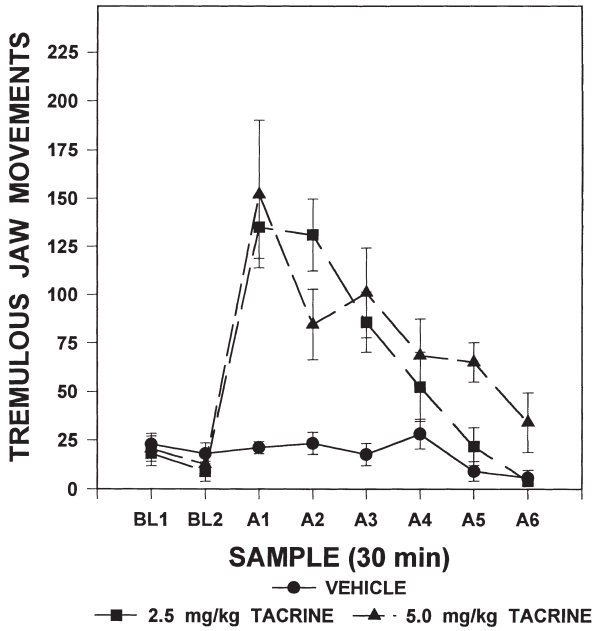

FIG. 3. Mean (±SEM) number of tremulous jaw movements observed during the first 5 min of each dialysis sample (BL, baseline; A1–A6, samples 1–6 after injection). The group that received 2.5 mg/kg tacrine showed significant increases in jaw movements relative to the control group during the first three samples after injection. The group that received 5.0 mg/kg tacrine showed significant increases in jaw movements relative to the control group during the first five samples after injection.

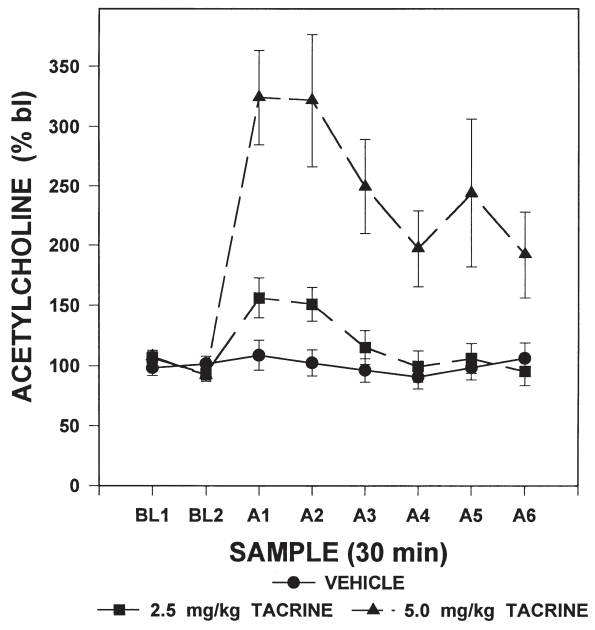

FIG. 4. Mean (±SEM) amount of ACh (as % of baseline) in each dialysis sample (BL, baseline; A1–A6, samples 1–6 after injection). The group that received 5.0 mg/kg tacrine showed significant increases in jaw movements relative to the control group during the first three samples after injection. Both groups that received tacrine showed significant differences between the predrug baseline and the first sample after injection.

determined that 2.5 mg/kg tacrine and 5.0 mg/kg differed from vehicle during the first three and first five 30-min postinjection periods, respectively.

Baseline extracellular ACh levels between the three treatment groups did not differ, $F(2, 21) = 0.96$, NS; mean pg ACh/10 μl (±SEM): vehicle = 2.89 (±0.73), tacrine 2.5 mg/kg = 4.17 (±1.44), tacrine 5.0 mg/kg = 2.06 (±0.72). As shown in Fig. 4, there was a significant effect of tacrine on ACh levels during the six postinjection samples, $F(2, 21) = 15.86$, $p < 0.001$. There was a significant time period effect, $F(5, 105) = 9.75$, $p < 0.0001$, and a significant time period by tacrine treatment interaction, $F(10, 105) = 2.92$, $p < 0.01$. Planned comparisons showed that tacrine 5.0 mg/kg differed from vehicle during the first three postinjection periods. With the vehicle control group, there was no significant difference between the predrug baseline and the first sample after injection. There were significant differences between the predrug baseline and the first sample after injection for the groups that received 2.5 mg/kg tacrine ($T = 0$, $p < 0.05$) and 5.0 mg/kg tacrine ($T = 0$, $p < 0.05$).

Two types of correlations between jaw movements and extracellular ACh were performed; data for the first 30-min sample after injection were analyzed, as well as data from all eight samples (two baseline samples, six after injection). Some of the correlational analyses combined data across treatment groups, whereas additional correlations also were calculated separately for each of the groups that received tacrine (i.e., both 2.5 and 5.0 mg/kg). Figure 5 is a scatterplot showing the relation between jaw movement activity and increases in extracellular ACh during the first 30-min sample after injection. Correlational analyses during the first 30-min period after tacrine injection showed that there was a linear relation between ACh levels and tremulous jaw movements across animals from all three injection groups [$r(22) = +0.70$, $p < 0.0001$]. For the analysis that included only animals treated with vehicle and 2.5 mg/kg tacrine, the correlation was not significant [$r(15) = +0.45$]. However, for the analysis that included only animals treated with vehicle and 5.0 mg/kg tacrine, there was a high, statistically significant correlation between jaw movements and VLS ACh [$r(14) = +0.90$, $p < 0.001$]. There was a significant correlation between jaw movements and ACh levels if the two groups that received 2.5 and 5.0 mg/kg tacrine were combined [$r(13) = +0.56$, $p < 0.05$]. Within the group that received 2.5 mg/kg tacrine, there was not a significant correlation between tremulous jaw movements and increases in VLS ACh [$r(6) = +0.07$] but there was a high, significant correlation between tremulous jaw movements and VLS ACh in the group that received 5.0 mg/kg tacrine [$r(5) = +0.87$, $p < 0.05$]. Additional correlations were performed that included data from all eight dialysis samples. There was a significant correlation between ACh levels and tremulous jaw movements during all eight sample periods if both groups of tacrine-treated animals were combined [$r(190) = +0.56$, $p < 0.0001$]. There also were significant correlations between ACh levels and tremulous jaw movements within the tacrine 2.5 mg/kg [$r(62) = +0.57$, $p < 0.01$] and 5.0 mg/kg treatment groups [$r(54) = +0.63$, $p < 0.01$]. As described above, a correlational study also was performed in a separate group of rats, in which various doses of physostigmine were injected (0.0625–0.25 mg/kg), and neurochemical and behavioral data were gathered. During the first 30-min period after injection of physostigmine or vehicle, there was a significant correlation between increases in extracellular ACh and the

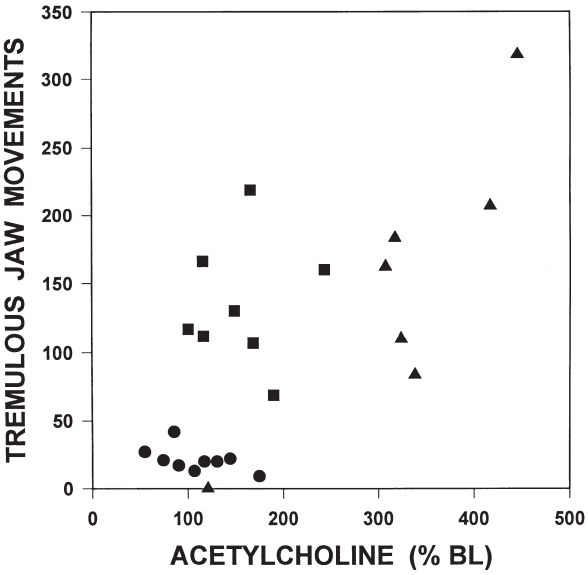

FIG. 5. This figure is a scatterplot showing the relation between increases in acetylcholine (as % of baseline) and the number of tremulous jaw movements in the first sample after injections. Data are shown for each individual animal that received vehicle (circles), 2.5 mg/kg tacrine (squares), and 5.0 mg/kg tacrine (triangles).

number of tremulous jaw movements [$r = 0.79$, $n = 12$, $p < 0.05$; data not shown).

DISCUSSION

The purpose of the present study was to characterize the relation between VLS ACh and tacrine-induced tremulous jaw movements. There is substantial evidence that the VLS subregion of the neostriatum is involved in tremulous jaw movements. Kelley et al. (25) demonstrated that the VLS was the most effective site for the induction of tremulous jaw movements by ACh and physostigmine. Microinjection of ACh and physostigmine into the dorsolateral or ventromedial striatum had no effect upon oral behavior (25). The VLS, but not the ventromedial striatum, was the most effective site for pilocarpine-induced jaw movements (46). DA depletions in the VLS, but not the dorsolateral or anteroventromedial striatum, led to the induction of tremulous jaw movements (23). Consistent with these findings, tacrine-induced jaw movements in the present study were significantly reduced by microinjection of HC-3 into the VLS, but not by injections into overlying cortex. These results demonstrate that inhibition of ACh synthesis in the VLS substantially reduced tacrine-induced jaw movements. The lack of effect of cortical injections of HC-3 indicates that VLS injections are not having their effects due to spread to more dorsal regions. These data strongly support the hypothesis that the VLS is critically involved in tremulous jaw movements. Thus, the results of Experiments 1 and 2 indicate that it is warranted to study the relation between jaw movement activity and extracellular ACh using microdialysis probes directly implanted into the VLS.

Although previous work has focussed separately on the behavioral and neurochemical effects of tacrine, the present work directly studied the relation between cholinergic neurochemistry in the VLS and tacrine-induced tremulous jaw movements. In the microdialysis experiment, tacrine increased both tremulous jaw movements and extracellular ACh in the VLS. Systemic administration of 5.0 mg/kg tacrine produced a substantial increase (324% of baseline within 30 min) in extracellular ACh levels. Although injections of 2.5 mg/kg tacrine did not significantly increase ACh levels relative to control injections, this group did show a moderate increase that was significantly higher than its predrug baseline. It is likely that tacrine affected extracellular ACh because of its actions as an anticholinesterase, although other effects (e.g., enhancement of release) cannot be ruled out by the present methods (4,39, 58). The group averages seem to suggest that the behavioral response at the low dose of tacrine was generally more sensitive than the neurochemical response to tacrine. For example, the group that received 2.5 mg/kg tacrine showed a large increase in jaw movements despite showing only a moderate increase in ACh levels. In addition, there was not a significant correlation between jaw movements and VLS ACh within the group that received 2.5 mg/kg tacrine. It is possible that this lack of sensitivity to the neurochemical effects of the low dose of tacrine is occurring because the dialysis method is measuring overflow from a large number of synapses around the probe, rather than directly measuring synaptic levels. Thus, it is possible that 2.5 mg/kg tacrine is increasing cholinergic stimulation at the synapse, but there is not enough overflow to measure substantial changes in dialysate levels. Also, it is possible that the presence of neostigmine in the perfusate reduced the apparent increase in extracellular ACh at the low dose of tacrine (12), or altered the extraction of acetylcholine (54). Future research should be conducted to determine if the neurochemical sensitivity at low doses of tacrine could be enhanced.

Correlational analyses demonstrated that there was strong relation between tremulous jaw movements and increases in ACh among those animals that received the higher dose of tacrine. Several lines of evidence indicate that VLS ACh levels were positively correlated with the production of tremulous jaw movements at the 5.0-mg/kg dose of tacrine. There was a significant correlation between tremulous jaw movements and increases in VLS ACh during the first sample after injection if all animals were included in the analysis ($r = +0.70$), if rats treated with vehicle and 5.0 mg/kg tacrine were included ($r = +0.90$), and even if the 5.0-mg/kg group alone was analyzed ($r = +0.87$). In the analysis that included data from all eight samples, there was a significant correlation between VLS ACh levels and tremulous jaw movements in the group that received 5.0 mg/kg tacrine ($r = +0.63$). Thus, although there was variability in both the neurochemical and behavioral responses to tacrine, correlational analyses demonstrated substantial covariability between these measures; animals that showed more jaw movement activity also tended to show greater increases in ACh after injection of 5.0 mg/kg. Evidence also indicated that there was a correlation between extracellular ACh and the number of vacuous jaw movements in physostigmine-treated rats, indicating that this may be a general characteristic of the jaw movements induced by anticholinesterases. Together with the pharmacological data reviewed above, and the results of the hemicholinium experiments, these neurochemical findings are consistent with the notion that VLS ACh is important for the production of tremulous jaw movements in rats. In addition, the overall pattern of correlational results indicate that dialysis methods can be used to investigate the relations between the neurochemical and behavioral effects of drugs acting on ACh.

Together with previous results, the present experiments indicate that interference with VLS ACh reduces tacrine-induced jaw movements, and also demonstrate that there is a significant correlation between VLS ACh levels and jaw

movement activity. These data indicate that VLS ACh is very important for cholinomimetic-induced tremulous jaw movements. It also is possible that ACh in the VLS is important for the jaw movement activity induced by interference with DA. Tremulous jaw movements are induced as a result of acute treatment with haloperidol (49), and also after pharmacological or neurotoxic depletion of DA (16,23,45). The jaw movements induced by acute haloperidol or reserpine are reduced by the muscarinic antagonist scopolamine (41,45,49). Thus, it has been suggested that interference with DA may produce tremulous jaw movements indirectly via enhancement of VLS ACh release (45). Cholinergic neurons in neostriatum have been characterized as large choline acetyltransferase-positive aspiny interneurons, which receive synaptic inputs from several different cells, including nigrostriatal DA terminals (14). Evidence indicates that DA antagonism or depletion can increase striatal ACh release (13). Future research should examine the neurochemical correlates of the jaw movements induced by neuroleptic drugs or DA depletion, to determine if VLS ACh is related to the production of jaw movements resulting from compromised function of striatal DA.

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