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Anatomical Analysis of Frontal Cortex Sites at Which Carbachol Induces Motor Seizures in the Rat

J. A. STIVERS, L. R. SKIRBOLL, R. LONG AND J. N. CRAWLEY[1]

Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892

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STIVERS, J. A., L. R. SKIRBOLL, R. LONG AND J. N. CRAWLEY. *Anatomical analysis of frontal cortex sites at which carbachol induces motor seizures in the rat.* PHARMACOL BIOCHEM BEHAV **30**(1) 129–136, 1988.—High amplitude spiking representative of seizures, accompanied by an unusual motor behavior pattern of rearing and forelimbic clonus resembling "boxing," was elicited by microinjection of the cholinergic agonist, carbachol, 4 μg, into the medial prefrontal cortex of the rat. A rating scale devised to score the behavior revealed a motor pattern elicited by carbachol from the medial anterior cortex which was similar to that described by Racine [24] for electrical stimulation of the amygdala. Topographical analysis of the areas surrounding the medial anterior cortex region revealed that the motor manifestations of seizures were elicited over a wide region of the anterior cortex, with scores significantly lower at carbachol microinjection sites greater than 1 mm rostral, 2 and 3 mm caudal, and 2 mm lateral to the standard medial prefrontal cortex site. Unilateral microinjection of carbachol yielded motor seizures primarily from the contralateral forepaw, suggesting involvement of a crossed pathway. Retrograde tracing with fast blue dye, combined with immunostaining for choline acetyltransferase and NADPH-diaphorase, found that the cholinergic neurons innervating the standard microinjection site were the dorsolateral tegmental cells, as previously reported, which have been shown to also contain substance P and corticortropin releasing factor. In addition, cholinergic neurons of the nucleus basalis of Meynert region were found to innervate the standard microinjection site. These findings implicate cholinergic innervation of the rostral cortex in classical limbic seizures.

Seizures Carbachol Acetylcholine Dorsolateral tegmentum Prefrontal cortex Nucleus basalis of Meynert

PREVIOUS studies of the coexistence of acetycholinesterase, substance P and corticotropin releasing factor in the dorsolateral tegmental nucleus (NTDL), projecting to the medial anterior cortex, revealed that the cholinergic agonist carbachol [7,8], microinjected into the medial anterior cortex, elicited motor seizures. These carbachol-induced seizures were potentiated by substance P, and inhibited by corticotropin releasing factor, suggesting a modulatory role for the two peptides in this triple coexistence [7,32].

Since these previous studies represented the first report of motor seizures induced by a cholinergic agonist in the rostral cortex, and since most seizure paradigms suggest a limbic focus, further studies were initiated to topographically analyze brain regions from rostral cortex caudally to the caudate and hippocampus. These studies were designed to determine whether carbachol-induced motor seizures were elicited from the medial anterior cortex simply due to spreading of the injectate to limbic structures classically associated with seizures.

Electroencephalographic analysis of epileptic seizures has localized the focal site primarily in limbic structures [22]. A small fraction of patients showing partial seizures have been designated as suffering from frontal lobe epilepsy. While seizures initiated in the frontal lobes often spread to limbic structures, seizure onset in the frontal lobes can be distinguished from seizure onset in the temporal lobes both by EEG pattern and by behavioral manifestations [22]. We have previously reported that microinjection of carbachol into the medial anterior cortex of rats induces high amplitude spiking in the frontal cortex, and a pattern of motor behaviors consisting of an upright posture and repetitive forepaw treading [7,8]. The "boxing"-like motor behavior is always accompanied by the EEG manifestations [8], suggesting that the rostral regions of the cortex may contain sites sensitive to cholinergic agonists for the induction of seizures. The carbachol-induced motor seizures were blocked by the muscarinic antagonist, atropine, and by anticonvulsant doses of benzodiazepines [8]. The first aim of the present study was to design a simple, reliable, and descriptive scoring system to characterize the behavioral manifestations elicited by carbachol from the frontal cortex, and to compare these motor seizures to the clonus described for amygdala kindled seizures by Racine [24].

The only animal studies of seizures elicited from the rostral cortex used electrical rather than chemical stimulation [23,24]. Racine [23,24] described epileptiform after-

[1]Requests for reprints should be addressed to Dr. Jacqueline N. Crawley, Clinical Neuroscience Branch, National Institute of Mental Health, Building 10, Room 4N214, Bethesda, MD 20892.

discharges and a corresponding motor behavior syndrome consisting of (1) mouth and facial movements, (2) head nodding, (3) forelimb clonus, (4) rearing, and (5) rearing and falling. This syndrome was elicited after repeated electrical stimulation of the anterior neocortex, pyriform cortex, entorhinal cortex, and cingulate cortex. Excitatory agents have been shown to induce seizures at several limbic sites. Kainic acid has been extensively used as a tool for studying temporal lobe epilepsy [17]. Intraperitoneal, intravenous, intraventricular, and intracerebral injections of kainic acid have been shown to induce local seizures in the hippocampus, amygdala and striatum, and to produce neuronal loss and gliosis particularly in the CA3 hippocampal subfield, as well as increasing regional brain glucose metabolism in the hippocampus and amygdala [1–3, 12, 16, 26]. Kainic acid, bicuculline, and carbachol elicited generalized seizures when microinjected at very low doses into the deep prepiriform cortex, lateral to the nucleus accumbens [21]. Repeated microinjections of the cholinergic agonist, carbachol, into the amygdala, caudate and hippocampus, at doses of 0.5–5 μg, elicited kindled seizures [35, 36]. The second aim of the present study was to map the anatomical sites adjacent to the medial prefrontal cortex site to delineate the anatomical specificity of carbachol-induced motor seizures. Since most seizures are of limbic origin, it was likely that microinjection of carbachol produced a considerable amount of spread in the caudal direction, to areas of the cingulate cortex, caudate nucleus amygdala and hippocampus, previously implicated in limbic seizures.

Cholinergic projections to the medial anterior cortex of the rat arise from cell bodies in the dorsolateral tegmentum and nucleus basalis of Meynert [11,31]. Studies of neurons in the dorsolateral tegmentum have revealed that substance P [18,34] and corticotropin releasing factor [7,19] coexist with acetylcholine, as determined by immunocytochemical staining for catechol acetyltransferase or acetylcholinesterase. We have found that microinjection of substance P into the medial anterior cortex potentiates carbachol-induced motor seizures, while microinjection of corticotropin releasing factor into the medial anterior cortex inhibits carbachol-induced motor seizures [7]. Neither peptide produced motor seizures when microinjected without carbachol, suggesting that the two peptides act as modulators of the actions of acetylcholine. In this triple coexistence, it appears that one peptide increased and one peptide decreased the response to carbachol. In addition, two substance P antagonists inhibited carbachol-induced motor seizures, supporting the possibility of a modulatory function for endogenous substance P [32]. The third aim of the present study was to determine whether the precise anatomical coordinates for microinjection of carbachol which induce motor seizures in the present study would correspond only to the terminal field of the dorsolateral tegmentum, source of the triple coexistence, or whether other sources of cholinergic projections might be involved in motor seizures.

METHOD

Behavior

Male Sprague-Dawley rats, 200 grams starting weight, were housed in a temperature and humidity controlled vivarium on a 7 a.m.–7 p.m. lighting schedule, with food and water constantly available. After cannulae implantation, rats were individually housed. Each animal was used only once, except for the unilateral injection study, where each rat was injected first on the left side and then on the right side.

Indwelling 24 gauge guide cannulae were stereotaxically implanted under sodium pentobarbital anesthesia as previously described [7]. Coordinates for the medial anterior cortex region containing terminals from the dorsolateral tegmentum were AP +3.6 mm, LAT ±0.8 mm, and VERT −3.5 mm from bregma, incisor bar at −3.5 [6,19]. Six sites were chosen for analysis of anatomical specificity of carbachol-induced "boxing": (1) Lateral 1 mm (AP +3.6 mm, LAT ±1.8 mm, VERT −3.5 mm); (2) Lateral 2 mm (AP +3.6 mm, LAT +2.8 mm, VERT −3.5 mm); (3) Rostral 1 mm (AP +4.6 mm, LAT ±0.8 mm, VERT −3.5 mm); (4) Caudal 1 mm (AP +2.6 mm, LAT ±0.8 mm, VERT −3.5 mm); (5) Caudal 2 mm (AP +1.6 mm, LAT ±0.8 mm, VERT −3.5 mm); and (6) Caudal 3 mm (AP +0.6 mm, LAT ±0.8 mm, VERT −3.5 mm). These sites were selected to provide a gradient away from the standard medial anterior cortex site, and to include limbic structures adjacent in the caudal direction, such as the cingulate cortex and caudate nucleus, which have been found to elicit seizures. A site medial to the standard medial anterior cortex site could not be tested, since the cortical hemispheres are slightly separated in this very anterior region, and cannulae implanted medial to 0.8 mm would have yielded injections into the cerebrospinal fluid space. At the end of each experiment, each rat was injected with fast green dye as previously described [7] for histological analysis of the guide cannulae and microinjection site. Data were grouped according to histological findings of the exact injection site for each rat. Six animals for each of the seven anatomical groups described above were obtained. In addition, post facto histological analysis found 3 rats with microinjection sites which were almost 1 mm dorsal to the standard medial anterior cortex site, and 7 rats with microinjection sites which were approximately 1 mm ventral to the standard medial anterior cortex site.

One week after surgical implantation of the cannulae, rats were microinjected bilaterally with 4 μg carbachol (carbamylcholine, Sigma Chemical Co., St. Louis, MO) in a saline vehicle. Since saline-treated animals never showed any incidence of "boxing" at any injection site [7,8], saline control treatments were not routinely performed in this series of studies. Injections were performed in awake, gently restrained rats, using a 31 gauge stainless steel injection tube (Small Parts, Inc., Miami, FL) inserted through each cannula into the medial prefrontal cortex, 1 mm ventral to the ventral tip of the cannula. The microinjection tube was attached by PE-20 tubing to a 10 μl Hamilton syringe, advanced by a Sage microinfusion pump (Orion Research, Inc., Cambridge, MA), to deliver 0.2 μl per injection site over a one minute period, with 5 additional seconds after cessation of drug delivery to allow complete diffusion. The microinjection parameters were chosen to minimize tissue damage and to prevent spread away from the injection site. The parameters of 0.2 μl per one minute produced spread of fast green dye over a radius of 0.5 mm. These parameters were previously found to be effective in distinguishing caudal from rostral regions of the nucleus accumbens, a space of less than 1 mm radius [6].

In previous reports of carbachol-induced "boxing" elicited from the medial anterior cortex, these motor seizures were scored by determining the number of seconds of a fifteen minute period in which the animal was observed in an upright vertical stance accompanied by repetitive forepaw treading [7, 8, 32]. The criteria for scoring a "boxing" bout

TABLE 1
RATING SCALE FOR "BOXING" BEHAVIORS

Score	Description
0	Normal exploratory activity (exploring cage, grooming, resting)
1	Hyperactivity, "wet dog" shakes, stereotyped sniffing and licking, with or without forepaw tremors
2	"Boxing" with one forepaw; rhythmic contractions of the body; rhythmic opening and closing of the mouth with no "boxing"
3	Rearing and "boxing" bouts of less than 5 seconds; total cumulative "boxing" less than 15 seconds/30 second interval
4	"Boxing" bouts of longer than 5 seconds; total cumulative "boxing" greater than 15 seconds, but with some pauses between bouts
5	Thirty seconds of continuous "boxing" with no pauses

Rating scale for "boxing" behaviors. Carbachol (1–6 μg) was microinjected bilaterally into the medial anterior cortex of rats. All behaviors were observed and analyzed for frequency and duration. A rating scale of 0–5 was then designed to score "boxing" behaviors using an ethologically descriptive, quantitative, and replicable system. See text for further description of methodology, and differentiation of the "boxing" behavior pattern to other motor behavior patterns elicited in other types of seizure paradigms.

was simply a minimum duration of five seconds. In the course of testing various anticonvulsants and peptides for their potential antagonist activity on the motor seizures it became evident that this method of scoring was inadequate. While some of the compounds injected together with carbachol effectively limited the number of seconds spent in the standard "boxing" posture accompanied by forepaw treading, some treatments affected only part of the behavioral manifestation of seizures. Behaviors were observed which could not be accounted for by the scoring system, such as partial upright posture and forepaw treading with only one forepaw. To address these problems, and to fully characterize the qualitative components of the "boxing" syndrome, an ethogram of "boxing" behaviors was developed. A rating scale was then designed to carefully quantitate a variety of motor behaviors and grade both the severity and duration of each of these motor patterns. As described below in the Results section, every thirty seconds the rat received a rating from 0 to 5, where 0 indicated normal exploratory behavior and 5 indicated that the rat spent the full 30 seconds in a continuous "boxing" posture with forepaw treading. Scoring began 15 minutes after microinjection of carbachol and session length was 15 minutes [7, 8, 32]. Thus, the "boxing" score was the sum of each thirty-second rating score over the fifteen minute period. Rats were injected bilaterally with 1–6 μg carbachol and scored simultaneously by two independent observers, uninformed of the treatment condition, using both the old and new scoring systems.

To determine whether carbachol-induced motor seizures were restricted to the injected hemisphere, six rats were microinjected with carbachol (6 μg) into the left medial anterior cortex site and scored for "boxing." Right and left forepaw treading were recorded separately. Four days later, the same rats were microinjected with carbachol (6 μg) into the right medial anterior cortex site and similarly scored.

Anatomy

Male albino rats 250–300 g were used. Fast blue dye (0.2 μl) was injected bilaterally or unilaterally into the medial anterior cortex (coordinates 3.6 mm anterior to bregma; 0.8 mm lateral to the midline; and 3.5 mm ventral from the dura) according to the atlas of Paxinos and Watson [20]. The dye was allowed to transport 4 days and the animals were prepared for either immunohistochemisty or NADPH-diaphorase staining. In immunohistochemical studies, colchicine was injected (60 μg in 10 μl 0.9% saline) into the lateral ventricle 24 hours before perfusion. Colchicine, a mitotic inhibitor, is known to arrest axoplasmic transport and to cause accumulation of transmitters and enzymes in cell bodies [9]. For both diaphorase and immunohistochemical procedures, animals were perfused through the ascending aorta with 10% ice-cold formalin-picric acid. The brains were dissected out, immersed in the same fixative for 90 minutes and rinsed in 0.1 M phosphate buffered saline (PBS) with 10% sucrose added. After at least 24 hours rinsing, the brains were cut on a cryostat (section thickness 10–15 μm) and the sections processed for either indirect immunohistochemistry or diaphorase.

Immunohistochemical procedures have been described in detail elsewhere [14,29]. Briefly, the sections were incubated with monoclonal antibodies specific for choline acetyltransferase (Boehringer Mannheim 770981) at +4 degrees C for 24–48 hours, rinsed in PBS, incubated with goat anti-rat IgG, rhodamine conjugated (Accurate Chemical ΔJGR2603), for 1 hour at 25 degrees C, rinsed in PBS mounted in a mixture of glycerol and PBS (3:1) and examined under epi-fluorescence in a Zeiss microscope under appropriate filters for rhodamine and fast blue [29]. Photographs were taken using Ilford HP5, 400 ASA black and white film.

NADPH-diaphorase staining has been described in detail by Sherer-Singler and co-workers [25]. Sections were first cut as described above and visualized for fast blue. Photographs were taken of labelled cells and the sections were subsequently processed for diaphorase. Briefly, sections were incubated in a 0.1 M Tris-Cl (pH 8.0) containing 1.0 mM NADP, 0.2 mM nitroblue tetrazolium and 15 mM sodium malate at 37 degrees C for 15–30 min, then rinsed in PBS and mounted. Vincent and co-workers [34] report that this procedure selectively stains the cholinergic neurons of the brainstem reticular formation including the dorsolateral tegmental nucleus and pedunculopontine nucleus. Sections were visualized and photographed under bright field conditions using a Zeiss microscope and HP5 ASA 40 black and white film.

RESULTS

Behavior

A rating scale of 0–5 was developed to yield both qualitative and quantitative scoring of the "boxing"-like motor seizures. Continuous scores of 3 and 4 were observed primarily in rats microinjected bilaterally with doses of 4 μg carbachol, while scores of 1 and 2 were observed primarily in rats microinjected with doses of 3 μg carbachol. Figure 1 provides a

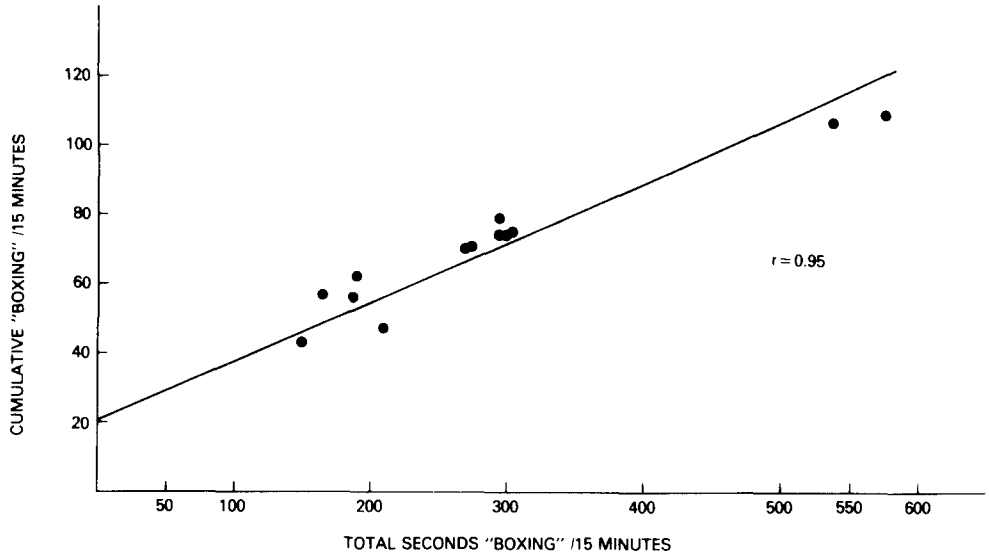

FIG. 1. Plot of "boxing" scores obtained by two scoring methods, by two independent observers, following microinjection of carbachol, 1–6 μg, into the medial anterior cortex. The scores along the abscissa represent cumulative total number of seconds spent in the "boxing" mode over a fifteen minute session [7, 8, 32]. The scores along the ordinate represent the rating scale for "boxing," which takes into account the serverity and duration of a variety of behavior patterns described in Table 1, over a fifteen minute period.

comparison of scores for a group of animals which were simultaneously rated by both scoring systems, and by two independent observers. The correlation coefficient obtained was 0.95, $t=7.16$, $p<0.001$.

Figure 2 illustrates the anatomical sites of microinjection of carbachol, 4 μg bilaterally, and the resulting scores of "boxing"-like motor seizures. The top panel shows the results of microinjection sites aimed at sites lateral to the medial anterior cortex. Each point represents 1–4 rats with identical injection sites. Motor seizures were significantly reduced or absent in rats microinjected at sites 3 mm lateral to the midline, i.e., 2 mm lateral to the medial anterior cortex site (see Table 2). The bottom panel shows the results of microinjection sites rostral and caudal to the medial anterior cortex. Each point represents 1–4 rats with identical injection sites. Motor seizures were significantly reduced or absent in rats microinjected at sites more than 4.6 mm rostral, and 1.5 mm rostral to bregma, i.e., greater than 1.0 mm rostral or 2 mm caudal to the medial anterior cortex site (see Table 2).

Table 2 provides statistical analysis of motor seizures from sites lateral, rostral, and caudal to the medial anterior cortex. Each group contains data from six rats. One way analysis of variance yielded a significant value, $F\ 6,36=5.11$, $p<0.01$. Newman-Keuls a posteriori test for significance of individual means yielded significant differences for sites 2 mm lateral ($p<0.01$), 1 mm rostral ($p<0.01$), 2 mm caudal ($p<0.05$), and 3 mm caudal ($p<0.01$) as compared to the standard medial anterior cortex site. Caudal 3 mm represented sites in the cingulate cortex, corpus callosum, lateral septum, and medial dorsal caudate nucleus.

Post facto analysis of microinjection sites revealed 3 rats with sites 1 mm dorsal and 7 rats with sites 1 mm ventral to the standard site of 3.5 mm ventral to the surface of the skull. The motor seizure "boxing" scores for rats microinjected with carbachol at these three sites were not significantly different. These three sites all fall within the terminal regions of the dorsolateral tegmental neurons [7].

Unilateral microinjections of carbachol were found to induce repetitive forepaw treading primarily contralateral to the injection site. Six rats injected unilaterally with carbachol showed "boxing" scores of 39.3 ± 3.8 by the contralateral forepaw as compared to "boxing" scores of 5.3 ± 1.5 by the ipsilateral forepaw, $t(22)=8.30$, $p<0.001$.

Anatomy

Four days after bilateral injections of fast blue into the prefrontal cortex, numerous cells with strong blue fluorescence could be seen bilaterally in many areas of the brain including thalamus, locus coeruleus, ventral tegmental area, hippocampus, dorsolateral tegmental area (NDTL), and the nucleus basalis of Meynert (nbM). Subsequent staining of sections for either ChAT immunohistochemistry or NADPH-diaphorase revealed that only in the NTDL and nbM were the fast blue-labelled cells found to be cholinergic. A detailed topographic analysis of multiple dye injection sites within the prefrontal cortex and subsequently labelled cells was not performed. However, not all immuno- or diaphorase-stained cells contained retrograde dye (Fig. 3).

Unilateral injections of fast blue dye resulted in primarily ipsilateral but some contralateral staining of both NTDL and nbM.

DISCUSSION

Careful mapping studies have identified the sites within the motor cortex from which electrical stimulation elicits movements of the forelimbs and of the hindlimbs [4, 5, 10, 13, 27, 28]. In the rat, these sites are in the lateral and ventral aspects of the motor cortex [5, 10, 13]. Higher current levels [5] or application of penicillin [4] or tungstic acid gel [33] elicit motor manifestations of seizures similar to the motor pattern reported in the present study. It is likely, therefore, that the cholinergic induction of seizures in the present study represents one of many excitatory inputs to motor cortex and limbic structures which can elicit forelimb movements

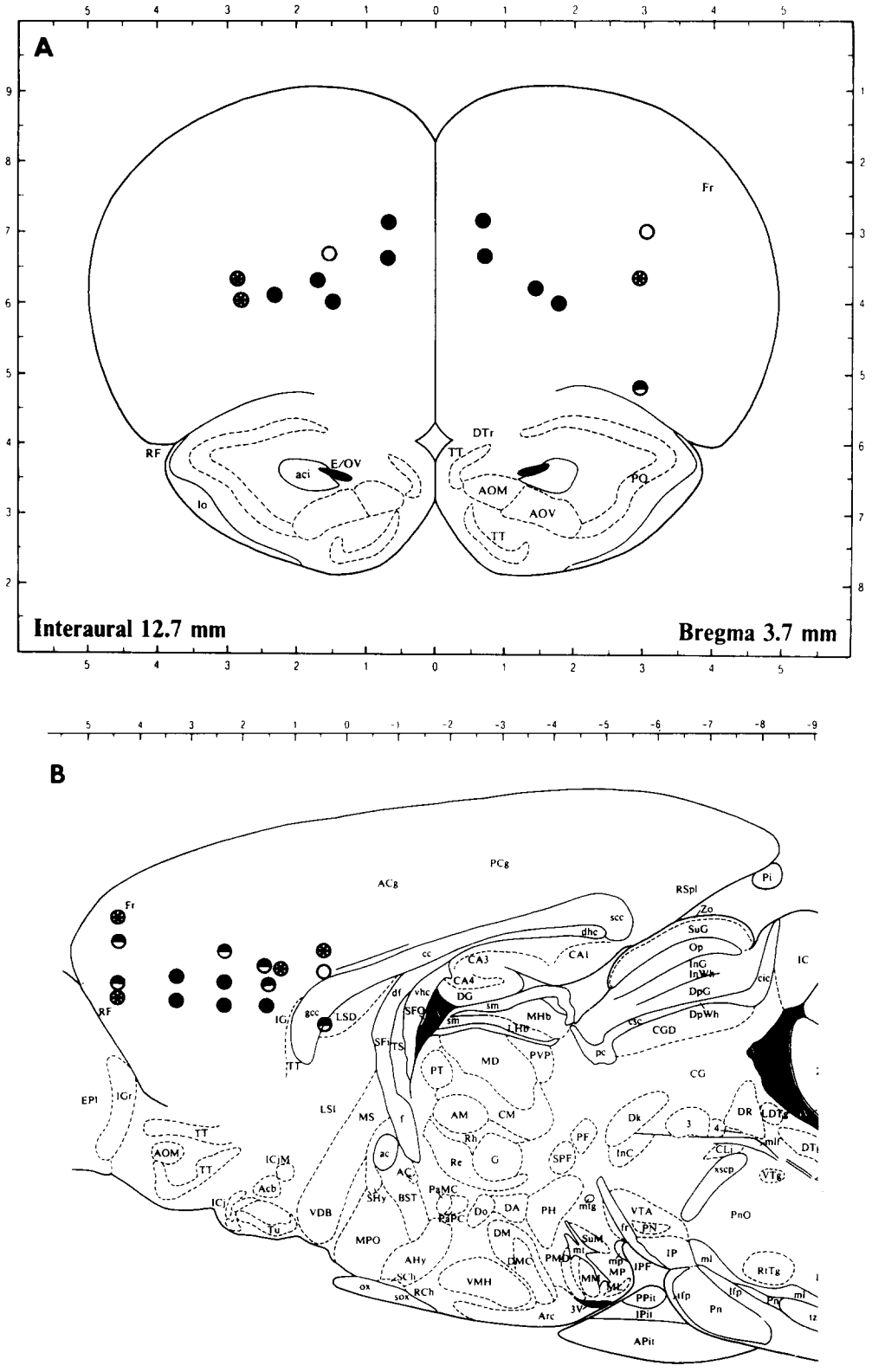

FIG. 2. Anatomical sites of microinjection of carbachol, 4 μg bilaterally, and the resulting scores of "boxing" behavior. (A) Frontal section showing sites lateral to the standard medial anterior cortex site. (B) Sagittal section showing sites rostral and caudal to the standard medial anterior cortex site. Diagrams modified from Paxinos and Watson [15]. Each point represents 1–4 animals with identical injection sites. Legend: ●≥56; ⊖=37–55; ⊗ 19–36; ○≤18 "boxing" score, using the rating scale of Table 1. Statistical analysis is presented in Table 2.

TABLE 2

Cannula Site	"Boxing" Score
Medial Anterior Cortex (AP +3.6 mm, LAT ± 0.8 mm, VERT 3.5 mm)	74.2 ± 4.1
Lateral 1 mm (AP +3.6, LAT ± 1.8, VERT 3.5)	65.0 ± 12.7
Lateral 2 mm (AP +3.6, LAT ± 2.8, VERT 3.5)	32.6 ± 9.2†
Rostral 1 mm (AP +4.6, LAT ± 0.8, VERT 3.5)	37.6 ± 3.9†
Caudal 1 mm (AP +2.6, LAT ± 0.8, VERT 3.5)	58.7 ± 4.2
Caudal 2 mm (AP +1.6, LAT ± 0.8, VERT 3.5)	46.6 ± 3.8*
Caudal 3 mm (AP +0.6, LAT ± 0.8, VERT 3.5)	33.6 ± 10.0†

Topographical analysis of "boxing" scores following microinjection of carbachol, 4 μg, at anatomical sites lateral, rostral, and caudal to the standard microinjection site. Sites were categorized post facto from histological analysis. Data are expressed as mean ± standard error of the mean. N=6 for each group. ANOVA: $F(6,36)=5.11$, $p<0.01$; Newman-Keuls: $^*p<0.05$, $^\dagger p<0.01$.

and motor seizures of varying intensity, duration, and behavioral parameters.

The behavioral description of the motor patterns exhibited by rats microinjected with carbachol into the medial prefrontal cortex are qualitatively somewhat different from other reported types of seizures. Kindled seizures of the amygdala or hippocampus are manifested by sniffing, chewing movements, head nodding, rearing and forepaw tremor, exophthalamos and ptosis, and loss of postural control [1, 23, 24, 35]. Electrical stimulation of the anterior neocortex of rats produced behaviors including myoclonic contraction of the forelimbs, a loss of postural control, and a mild extension [23]. The myoclonus described by Racine [23,24] appears to correspond to the forepaw treading described in Table 1. Lateral movements of the head have been described in conjunction with forelimb movements after the electrical stimulation of the anteromedial cortex [27]. However, the oral movements and whisker twitching described by Racine [24] for amygdala and hippocampal electrical stimulation were not uniformly present in carbachol-induced motor seizures elicited from the medial anterior cortex. The manifestation of seizures most similar to the present report has been described by Takahashi and co-workers [33] and Ito and coworkers [15] as "kangaroo posturing" following application of tungstic acid gel subdurally or to the motor cortex of rats. The medial anterior cortex appears to initiate a combination of motor behaviors similar to, but not identical to, kindled limbic seizures. This motor pattern is significantly different from the tonic-clonic convulsion induced by drugs which inhibit GABA receptors, e.g., pentylenetetrazol and bicuculline, or activate glutamate receptor, e.g., strychnine, in which the motor manifestations are tremor, rigidity, and falling to a posture of exposed ventral surface [30]. However, receptors for excitatory amino acids such as glutamate have not been tested for seizure activity in the rostral cortex. Further studies involving stimulation of cholinergic neurons innervating the medial anterior cortex are necessary to determine the function of endogenous acetylcholine in the rostral cortex in seizure activity.

Carbachol-induced "boxing" behavior appears to be elicited from a broad frontal cortical region circumscribed within a 2 mm diamter in the anterior cortex of the rat. High "boxing" scores were obtained at microinjection sites from 4.0 to 2.0 mm rostral to bregma, and 0.5 to 2.0 mm lateral to the midline. In addition, high motor seizure scores were obtained at sites at least 3.5 to 5.5 mm ventral to the surface of the skull, suggesting that all cortical regions in the dorsoventral axis are active. The medial anterior region of the cortex corresponds closely with the terminal region of the dorsolateral tegmental neurons which have been shown to contain acetylcholinesterase or choline acetyltransferase, substance P and corticotropin releasing factor [7, 20, 34]. "Boxing" scores from microinjection sites in the cingulate cortex, corpus callosum, lateral septum, and medial anterior caudate nucleus were between 0 and 50% of scores for microinjection sites in the prefrontal cortex. "Boxing" scores in the posterior regions were only occasionally zero, indicating either (1) spread of the microinjected carbachol to distant structures, (2) recruitment of neuronal activation away from the initiation point of the seizures, or (3) a role for other nuclei in components of the "boxing" behavior syndrome. However, since the highest "boxing" scores were obtained from microinjections in the medial anterior cortex, with gradual diminution of effect in three directions away from this region, it seems likely that the primary site of action of carbachol in eliciting motor seizures is in the general area of the medial anterior cortex, rather than in the limbic system. The present data argue against a primary site of action in the hippocampus, amygdala, prepiriform cortex, caudate nucleus, and other limbic structures which are known to directly initiate and sustain seizure activity [1, 2, 35].

The findings from the retrograde tracing study support and extend our previous results showing that the NTDL projects to the medial anterior cortex. The present study verifies that the exact microinjection site chosen for further studies does, in fact, receive innervation from the NTDL. In addition, the anatomical finding of a cholinergic projection from the nucleus basalis of Meynert to the medial prefrontal cortex raises the possibility that carbachol-induced motor seizures are elicited from cholinergic receptors innervated by the nbM, instead of or as well as, by the NTDL. No thalamic, hippocampal, or tegmental sites of retrograde dye localization were found to be cholinergic. Specific lesion studies of the NTDL and the nbM are necessary to determine the relative contributions of each of these nuclei to carbachol-induced motor seizures. However, it is relevant to note that cholinergic neurons of the nbM do not contain substance P or CRF, as do the NTDL neurons. We have previously found that substance P or CRF modulate carbachol-induced seizures when microinjected into the medial anterior cortex [7,32]. This indirect information lends support to the notion that the NTDL is the primary source of the cholinergic projections mediating seizures elicited from the medial anterior cortex.

The contralateral forepaw treading seen after unilateral carbachol injections into the medial anterior cortex suggests that interhemispherical connections are activated by the carbachol microinjected into the medial anterior cortex. The retrograde tracing study showed primarily ipsilateral projections from the NTDL to the medial anterior cortex. It seems likely, therefore, that the contralateral motor behavior rep-

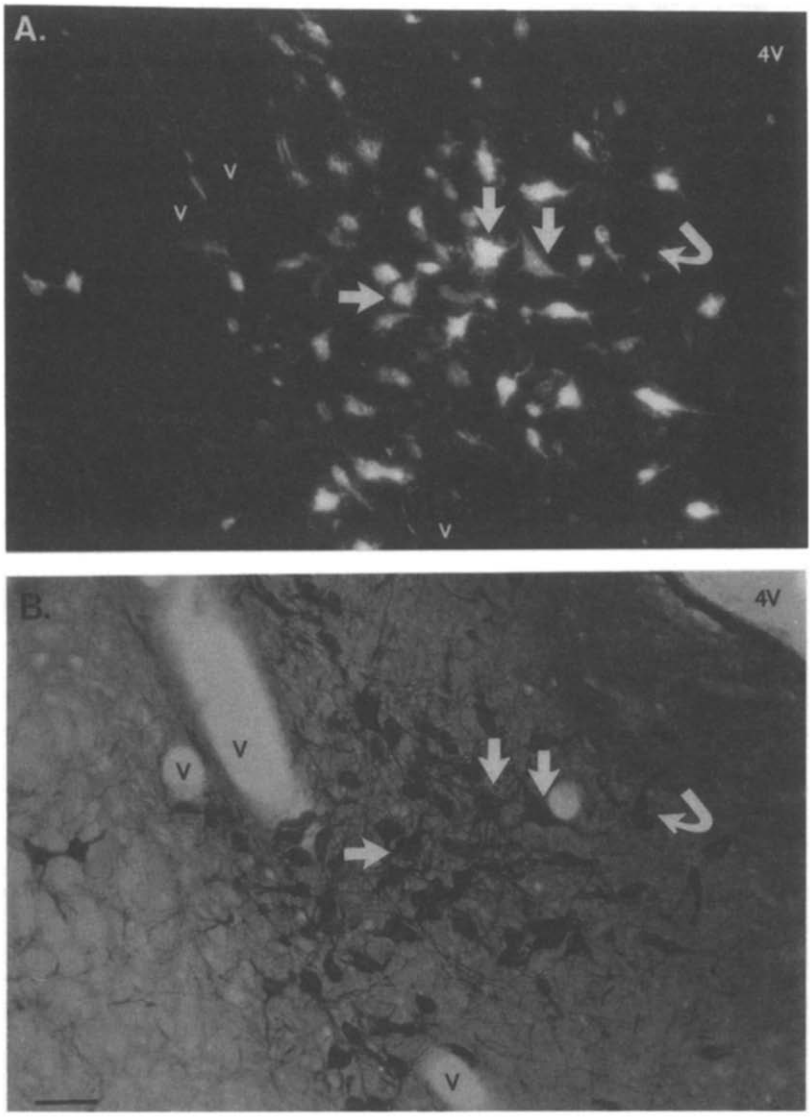

FIG. 3. Fluorescent (A) and bright field (B) micrographs of frontal sections through the pontine tegmentum of the rat. (A) Shows fast blue filled cells in the lateral dorsal tegmental nucleus in the floor of the fourth ventricle after dye injection into the medial anterior cortex. (B) Shows the same section histochemically stained for NADPH-diaphorase (a marker for cholinergic neurons). Short arrows designate some of the many cells which are positive for both fast blue and NADPH-diaphorase. Curved arrow points out cell which is diaphorase positive but does not contain retrograde dye. Bar=30 μm; v=blood vessel; 4v=fourth ventricle.

resents a crossed projection somewhere between the medial anterior cortex and the motor nuclei regulating forelimb movements.

In conclusion, the data reported herein represent the first demonstration of cholinergic induction of motor seizures from the rostral cortex. The anatomical substrate appears to involve the medial anterior cortex, rather than a uniform distribution of active sites into limbic regions classically associated with kindled seizures. Cholinergic innervation of the medial anterior cortex was found to include the NTDL and the nbM. These findings raise the possibility that one or more cholinergic pathways have the potential for initiating cortical seizures. Since the motor manifestations observed are similar to amygdala-kindled seizures, it appears likely that the medial anterior cortical region can either activate, or be activated by, the well-known limbic seizure circuitry.

REFERENCES

1. Ben-Ari, Y., E. Tremblay and O. P. Ottersen. Injections of kainic acid into the amygdaloid complex of the rat: an electrographic, clinical and histological study in relation to the pathology of epilepsy. *Neuroscience* **5:** 515–528, 1980.
2. Ben-Ari, Y., E. Tremblay, O. P. Ottersen and B. S. Meldrum. The role of epileptic activity in hippocampal and 'remote' cerebral lesions induced by kainic acid. *Brain Res* **191:** 79–97, 1980.
3. Ben-Ari, Y., E. Tremblay, D. Riche, G. Ghilini and R. Naquet. Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline or pentetrazole: metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. *Neuroscience* **6:** 1361–1391, 1981.
4. Collins, R. C. Kindling of neuroanatomic pathways during recurrent focal penicillin seizures. *Brain Res* **150:** 503–517, 1978.
5. Collins, R. C., E. M. Santori, T. Der, A. W. Toga and E. W. Lothman. Functional metabolic mapping during forelimb movement in rat. 1. Stimulation of motor cortex. *J Neurosci* **6:** 448–462, 1986.
6. Crawley, J. N., D. W. Hommer and L. R. Skirboll. Topographical analysis of nucleus accumbens sites at which cholecystokinin potentiates dopamine-induced hyperlocomotion in the rat. *Brain Res* **335:** 337–341, 1985.
7. Crawley, J. N., J. A. Olschowka, D. I. Diz and D. M. Jacobowitz. Behavioral investigation of the coexistence of substance P, corticotropin releasing factor, and acetylcholinesterase in lateral dorsal tegmental neurons projecting to the medial frontal cortex of the rat. *Peptides* **6:** 891–901, 1985.
8. Crawley, J. N., J. A. Stivers, J. V. Martin and W. B. Mendelson. Cholinergic induction of seizures in the rat prefrontal cortex. *Life Sci* **38:** 2347–2354, 1986.
9. Dahlstrom, A. Effect of colchicine on transport of amine storage granules in sympathetic nerves of the rat. *Eur J Pharmacol* **5:** 111–112, 1964.
10. Donoghue, J. P. and S. P. Wise. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. *J Comp Neurol* **212:** 76–88, 1982.
11. Edstrom, J. P. and J. W. Phillis. A cholinergic projection from the globus pallidus to cerebral cortex. *Brain Res* **189:** 524–529, 1980.
12. Evans, M. C. and B. S. Meldrum. Regional brain glucose metabolism in chemically-induced seizures in the rat. *Brain Res* **297:** 235–245, 1984.
13. Hall, R. D. and E. P. Lindholm. Organization of motor and somatosensory neocortex in the albino rat. *Brain Res* **66:** 23–38, 1974.
14. Hökfelt, T., L. Skirboll, J. Rehfeld, M. Goldstein, K. Markey and O. Dann. A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing. *Neuroscience* **5:** 2093–2124, 1980.
15. Ito, T., M. Hori, K. Yoshida and M. Shimizu. Effect of acid gel in rats. *Arch Int Pharmacodyn* **241:** 287–299, 1979.
16. Lothman, E. W., R. C. Collins and J. A. Ferrendelli. Kainic acid-induced limbic seizures: electrophysiologic studies. *Neurology* **31:** 806–812, 1981.
17. Nadler, J. V. Kainic acid as a tool for the study of temporal lobe epilepsy. *Life Sci* **19:** 2031–2042, 1981.
18. Olschowka, J. A., D. I. Diz and D. M. Jacobowitz. Coexistence of substance P, CRF and ACHE in neurons of the nucleus tegmenti dorsalis lateralis. *Soc Neurosci Abstr* **9:** 167.5, 1983.
19. Olschowka, J. A., T. L. O'Donohue, G. P. Mueller and D. M. Jacobowitz. The distribution of corticotropin releasing factor-like immunoreactive neurons in rat brain. *Peptides* **3:** 995–1015, 1982.
20. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. Sydney: Academic Press, 1982.
21. Piredda, S. and K. Gale. A crucial epileptogenic site in the deep prepiriform cortex. *Nature* **317:** 623–625, 1985.
22. Quesney, L. F. and P. Gloor. Localization of epileptic foci. In: *Long-Term Monitoring in Epilepsy*, edited by J. Gotman, J. R. Ives and P. Gloor. North Holland: Elsevier Science Publishers, 1985.
23. Racine, R. J. Modification of seizure activity by electrical stimulation: cortical areas. *Electroencephalogr Clin Neurophysiol* **38:** 1–12, 1975.
24. Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr Clin Neurophysiol* **32:** 281–294, 1972.
25. Scherer-Singler, U., S. R. Vincent, H. Kimura and E. G. McGeer. Demonstration of a unique population of neurons with NADPH-diaphorase histochemistry. *J Neurosci Methods* **9:** 229–234, 1983.
26. Schwartz, R., R. Zaczek and J. T. Coyle. Microinjection of kainic acid into the rat hippocampus. *Eur J Pharmacol* **50:** 209–220, 1978.
27. Sharp, F. R. Regional (^{14}C)2-deoxyglucose uptake during forelimb movements evoked by rat motor cortex stimulation: cortex, diencephalon, midbrain. *J Comp Neurol* **224:** 259–285, 1984.
28. Sinnamon, H. M. and B. S. Galer. Head movements elicited by electrical stimulation of the anteromedial cortex of the rat. *Physiol Behav* **33:** 185–190, 1984.
29. Skirboll, L., T. Hökfelt, G. Norell, O. Phillipson, H. Kuypers, M. Betivoglio, C. Catsman-Berrovoets, T. Visser, H. Steinbusch, A. Verhofstad, A. Cuello, A. Godstein and M. Brownstein. A method for specific transmitter identification of retrogradely labeled neurons: immunofluorescence combined with fluorescence tracing. *Brain Res Rev* **8:** 99–127, 1984.
30. Skolnick, P., P. Syapin, B. Paugh, V. Moncada, P. Marangos and S. Paul. Inosine, an endogenous ligand of the brain benzodiazepine receptor, antagonizes pentylenetetrazole-induced seizures. *Proc Natl Acad Sci USA* **76:** 1515–1518, 1979.
31. Stewart, D. J., D. F. MacFabe and L. W. S. Leung. Topographical projection of cholinergic neurons in the basal forebrain to the cingulate cortex in the rat. *Brain Res* **358:** 404–407, 1985.
32. Stivers, J. A. and J. N. Crawley. Substance P antagonists block carbachol-induced "boxing" behavior at a site of coexistence in the rat prefrontal cortex. *Peptides* **8:** Suppl 1, 117–121, 1988.
33. Takahashi, S., T. Yamauchi and Y. Hirabayashi. Experimental seizures produced by tungstic acid gel. *Life Sci* **11:** 1011–1020, 1972.
34. Vincent, S. R., K. Satoh, D. M. Armstrong and H. C. Fibiger. NADPH-diaphorase: a selective histochemical marker for the cholinergic neurons in the pontine reticular formation. *Neurosci Lett* **43:** 31–36, 1983.
35. Vosu, H. and R. A. Wise. Cholinergic seizure kindling in the rat: comparison of caudate, amygdala and hippocampus. *Behav Biol* **13:** 491–495, 1975.

34. Vincent, S. R., K. Satoh, D. M. Armstrong and H. C. Fibiger. NADPH-diaphorase: a selective histochemical marker for the cholinergic neurons in the pontine reticular formation. *Neurosci Lett* **43:** 31–36, 1983.
35. Vosu, H. and R. A. Wise. Cholinergic seizure kindling in the rat: comparison of caudate, amygdala and hippocampus. *Behav Biol* **13:** 491–495, 1975.

36. Wasterlain, C. G. and V. Jonec. Chemical kindling by muscarinic amygdaloid stimulation in the rat. *Brain Res* **271:** 311–323, 1983.