The Rat Ventromedial Thalamic Nucleus and Motor Control: Role of N-Methyl-D-aspartate-Mediated Excitation, GABAergic Inhibition, and Muscarinic Transmission

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The rat ventromedial thalamic nucleus (VM) is a point of convergence of several pathways that are supposed to be involved in motor control. Cortical fibers terminating within this nucleus use an excitatory amino acid, possibly L-glutamate, as their transmitter. Excitatory amino acids are known to interact with N-methyl-D-aspartate (NMDA), kainate, and quisqualate receptors, the presence of which has been demonstrated within the thalamus, γ-Amino-butyrate (GABA) has been identified as the transmitter of the basal ganglia afferents to the VM, whereas cerebellar afferents to the VM are supposed to release ACh acting on muscarinic receptors. The present study investigates the behavioral and motor consequences of local injections of drugs into the VM, which specifically interact with NMDA, GABA, and muscarine receptors. Both the NMDA antagonist (−)2-amino-7-phosphonoheptanoate [(−)AP7], and the GABA agonist muscimol, but not the muscarinic antagonist scopolamine, induced catalepsy and limb rigidity. Both the (−)AP7- and muscimol-induced catalepsy were antagonized by coadministration of NMDA and the GABA antagonist bicuculline. The (−)AP7-induced catalepsy was characterized as an akinetic-rigid syndrome, in which the ability to induce a phasic activation of a set of muscles is lost and replaced by exaggerated tonic muscular responses. NMDA, bicuculline, and the muscarinic agonist bethanechol induced an increase in locomotor activity. The present study provides evidence that an imbalance between NMDA-mediated excitation and GABAergic inhibition within the rat VM leads to disturbances of motility, whereas muscarinic transmission within this nucleus appears to be of minor importance.

The rat ventromedial thalamic nucleus (VM) is characterized by its extensive neocortical projection and its complex afferentation, consisting of converging inputs from several brain regions that are supposed to be involved in motor control (Chevalier and Deniau, 1982; Herkenham, 1979; MacLeod and James, 1984).

The widespread neocortical projection arising from the VM is directed almost exclusively to the outer half of layer I of the neocortex (Herkenham, 1979). In turn, corticothalamic neurons located in layer VI of the cortex are the source of reciprocally organized projection to the VM. The major prethalamic inputs to the VM arise from the deep cerebellar nuclei, and the output

nuclei of the basal ganglia, in particular, the reticular part of the substantia nigra. In addition, the superior colliculus, the mesencephalic reticular formation, and the reticular thalamic nucleus contribute to the afferent input to the VM (Beckstead et al., 1979; Carter and Fibiger, 1978; Haroian et al., 1981; Herkenham, 1979; Jones, 1975). In contrast to the primate thalamus, where cerebellar and basal ganglia inputs are clearly segregated, the afferents to the rat VM are convergent (Asanuma et al., 1983; Herkenham, 1979; Schell and Strick, 1984).

In recent years, efforts have been made to identify the transmitters released by the afferent pathways terminating within the VM. There is evidence that GABA serves as the transmitter of the afferents arising from the basal ganglia, in particular, the nigrothalamic pathway (Di Chiara et al., 1979b; Kilpatrick et al., 1980; Penney and Young, 1981). In addition, the presence of GABA and glutamic acid decarboxylase, the synthesizing enzyme for GABA, has been demonstrated by immunocytochemical methods in a large number of neurons of the reticular thalamic nucleus and in local circuit neurons of the VM, suggesting that intrathalamic sources exist that contribute to the release of GABA within the VM (Houser et al., 1980; Ottersen and Storm-Mathisen, 1984). However, a recently published report on the anatomy of the cat motor thalamus indicates that most of the GABAergic inhibition within the VM is provided by basal ganglia afferents that synapse with thalamocortical projection neurons (Kultas-Ilinsky et al., 1985).

Acetylcholine has been tentatively suggested to act as the transmitter of the cerebellothalamic tract, since interruption of this tract by lesioning the brachium conjunctivum decreases the level of the ACh synthesizing enzyme choline acetyltransferase (CAT) within the VM (MacLeod et al., 1984; Nieoullon, 1984). In addition, there is electrophysiological evidence that thalamic neuronal activity evoked by stimulation of the cerebellothalamic tract can be attenuated by systemically or iontophoretically applied antimuscarinic drugs (Frigyesi and Purpura, 1966; MacLeod et al., 1984; Marshall and McLennan, 1972). Accordingly, binding studies have demonstrated the existence of muscarinic, but not of nicotinic cholinergic binding sites within the ventral thalamic nuclei (Hunt and Schmidt, 1978; Rotter et al., 1979).

An excitatory amino acid, possibly L-glutamate, is the most likely transmitter candidate of several corticofugal pathways, including the corticothalamic pathway (Fonnum et al., 1981; Young et al., 1981). Excitatory amino acids are supposed to interact with at least three different types of receptors, which have been classified as NMDA, kainate, and quisqualate receptors based on their preferential activation by one of these substances (McLennan, 1983; Watkins, 1984). Among these receptor subtypes, the NMDA receptor has been most clearly characterized, partly because it is only this type of receptor for which highly specific antagonists are available.

Following the discovery that the rat substantia nigra pars

reticulata serves as an output station of the basal ganglia (Di Chiara et al., 1977), the VM as the major thalamic target of the GABAergic nigrothalamic tract has gained increasing scientific interest. Several studies have been published in which the behavior of rats was studied following lesions of the VM or microinjections of GABA-specific compounds into the VM. The most consistent outcome of these studies has been that local injection of a GABAmimetic drug induces a state of catalepsy. On the other hand, it has been reported that blockade of GABAergic transmission within the VM antagonizes the catalepsy following systemically administered haloperidol (Di Chiara et al., 1979a, 1981; Garcia-Munoz et al., 1983; Kilpatrick et al., 1980; Klockgether et al., 1985; Reavill et al., 1981; Starr and Summerhayes, 1983a, b).

In these studies little attention was paid to the fact that the rat VM is a point of convergence of several pathways involved in motor function rather than a thalamic relay nucleus of the substantia nigra pars reticulata. Our present knowledge about the role of L-glutamate as the transmitter of the corticothalamic tract, GABA as the transmitter of the basal ganglia afferents to the VM, and ACh as the transmitter of the cerebellothalamic tract raises the question as to what extent glutamatergic, GABA-ergic, and cholinergic neurotransmissions within the VM are involved in the motor regulatory function of this nucleus. The present study therefore investigated the behavioral and motor consequences of manipulating NMDA-mediated excitation, GABAergic inhibition, and muscarinic transmission within the VM using the technique of intracerebral microinjection of transmitter-specific compounds.

Materials and Methods

Animals, surgery, and injection procedure

Male Wistar rats (F. Winkelmann, Borchen, FRG), weighing 200–240 gm at the time of surgery, were used throughout this study. Under pentobarbitone anesthesia (60 mg/kg, i.p.) two intracerebral stainless steel guide cannulae (23 gauge, 9.0 mm long) were stereotaxically implanted so that their tips were located 0.5 mm above the dura, which had been cautiously slit at this site before implantation. The present implantation procedure was chosen in order to avoid damage of brain tissue due to chronically implanted guide cannulae. After a recovery period of at least 5 d, bilateral intracerebral injections were performed in awake, unrestrained rats. To this end, a stainless steel injection cannula of appropriate length and diameter (29 gauge, 14.8 mm long), which was connected to a 2 μl Hamilton syringe via a polyethylene tubing, was carefully lowered through the guide cannula to the VM. The coordinates of the target site within the VM were AP 4.2–4.8, L 1.2, V −1.0, according to the atlas of König and Klippel (1963). All drugs were injected in a volume of 0.5 μl at a rate of 0.1 μl/min. After the end of the injection, the cannula was left in place for 1 min in order to allow adequate absorption by the surrounding tissue and thus lessen the likelihood of the injected drug being drawn back. Each rat was used only once. All behavioral tests took place between 0830 and 1400 hr and were performed in a well-lit quiet room, kept at a constant temperature of 22 ± 2°C.

Catalepsy

Catalepsy was assessed 15, 30, 60, and 90 min after beginning of the injection by means of the bar test, the bridge test, and the inversed bar test. For the bar test, animals were placed with both front paws on the edge of a wooden block of 9.0 cm height; for the bridge test, animals were suspended with their front and hindpaws between two wooden blocks; for the inversed bar test, the animals were placed with their hindpaws on a wooden block 9.0 cm high. For each test, an animal was considered to be cataleptic, if it maintained the respective position for at least 30 sec. In addition, in the bar test the time up to 180 sec was measured for which an animal maintained its position (descent latency).

Locomotor activity

Locomotor activity was recorded by means of an activity meter (Animex Activity Meter, Type O, Hägersten, Sweden) using six circular electro-

magnetic fields. Rats were individually placed into a Plexiglas cage (40 × 25 × 15 cm) on top of the meter. The activity meter was preadjusted to the same sensitivity before placing each animal on it. When the rat crossed an electromagnetic field a count was generated and recorded. Interruptions of the magnetic fields were cumulated over 5 min periods. Prior to intracerebral injection, each animal was habituated for 45 min and the spontaneous locomotor activity measured. After the habituation, animals were removed, injected intracerebrally, returned to the experimental cage, and drug-induced locomotor activity was recorded for 35 min.

Electromyographic recording

For registration of the tonic electromyogram (EMG) activity of the gastrocnemius muscle, rats were partly restrained in ventilated Plexiglas boxes with their hind limbs hanging free through slots in the bottom of the box. A pair of wire electrodes (Cooner Wire, Chatsworth, CA) was inserted percutaneously into the left gastrocnemius muscle. The signal was amplified, bandpass-filtered (5–10 kHz), rectified, and fed into an integrator. The EMG registration was started 15 min and continued up to 60 min after beginning the injection. The mean integrated activity of 5 min periods was calculated and expressed in arbitrary units. To ensure that only tonic EMG activity was measured, bursts of phasic activity lasting less than 1 min due to movements of the rat were discarded.

One group of animals was equipped with chronic EMG electrodes implanted into the gastrocnemius muscle and the tibialis cranialis muscle of the left hindleg. For this purpose, pairs of Teflon-insulated stainless steel wire electrodes (Cooner Wire) were passed subcutaneously from the head to a skin incision above the muscle. The wires were then passed through the muscle, leaving a 2–3 mm uninsulated portion within the muscle, and fixed by knotting their insulated ends together. A grounding electrode was subcutaneously implanted in the vicinity of the recording electrodes. The electrodes were connected to plugs fixed to the animal's skull. After a recovery period of at least 5 d, EMG recording was performed in awake, unrestrained animals. For this purpose, the EMG signals from chronically implanted electrodes were picked up with the help of flexible leads connected to the plugs on the rats' heads. The signals were amplified, bandpass-filtered (8–10 kHz), displayed on an oscilloscope, and stored on magnetic tape (CPR-4010, Bell & Howell, Pasadena, CA). The correct placement of the electrodes was verified by stimulating through the implanted electrodes and by visual inspection under pentobarbital anesthesia immediately after the recording session. Each animal was used for only one experiment, which consisted of a pre- and postdrug recording session.

Drugs

Muscimol (Sigma, St. Louis), bicuculline methiodide (bicuculline, Sigma), methyl scopolamine nitrate (scopolamine, Sigma), and carbamyl β-methylcholine chloride (bethanechol, Sigma) were dissolved in saline immediately before the injection. NMDA (Tocris Chemicals, Buckhurst Hill, Essex, UK), (−)2-amino-7-phosphonoheptanoate [(−)AP7] and (+)2-amino-7-phosphonoheptanoate [(+)AP7], obtained from Dr. R. Schwarcz, Baltimore, were brought into solution with a minimum quantity of 1 N NaOH, and the final volume was made up with saline. The pH was adjusted to 7.4.

Histology and statistics

After completion of the experiments, the precise localization of the injection sites was determined in serial 20 μm sections of the entire brain stained with cresyl violet. Statistical evaluation was carried out by means of the Mann-Whitney U test and Student's t test.

Results

Localization of thalamic microinjections

Figure 1*A* demonstrates typical localization of cannulae tips within the VM. Mild glial infiltration was observed in close proximity of the cannula tip (Fig. 1, *A* and *B*). Figure 1*C* indicates that the technique used for microinjections did not significantly alter the cytoarchitecture of the VM (Bold et al., 1984). The drawing in Figure 2*A* illustrates the topographical distribution of (−)AP7 (100 ng) injections into the VM at anteroposterior planes AP 4230–4890 μm (König and Klippel, 1963)
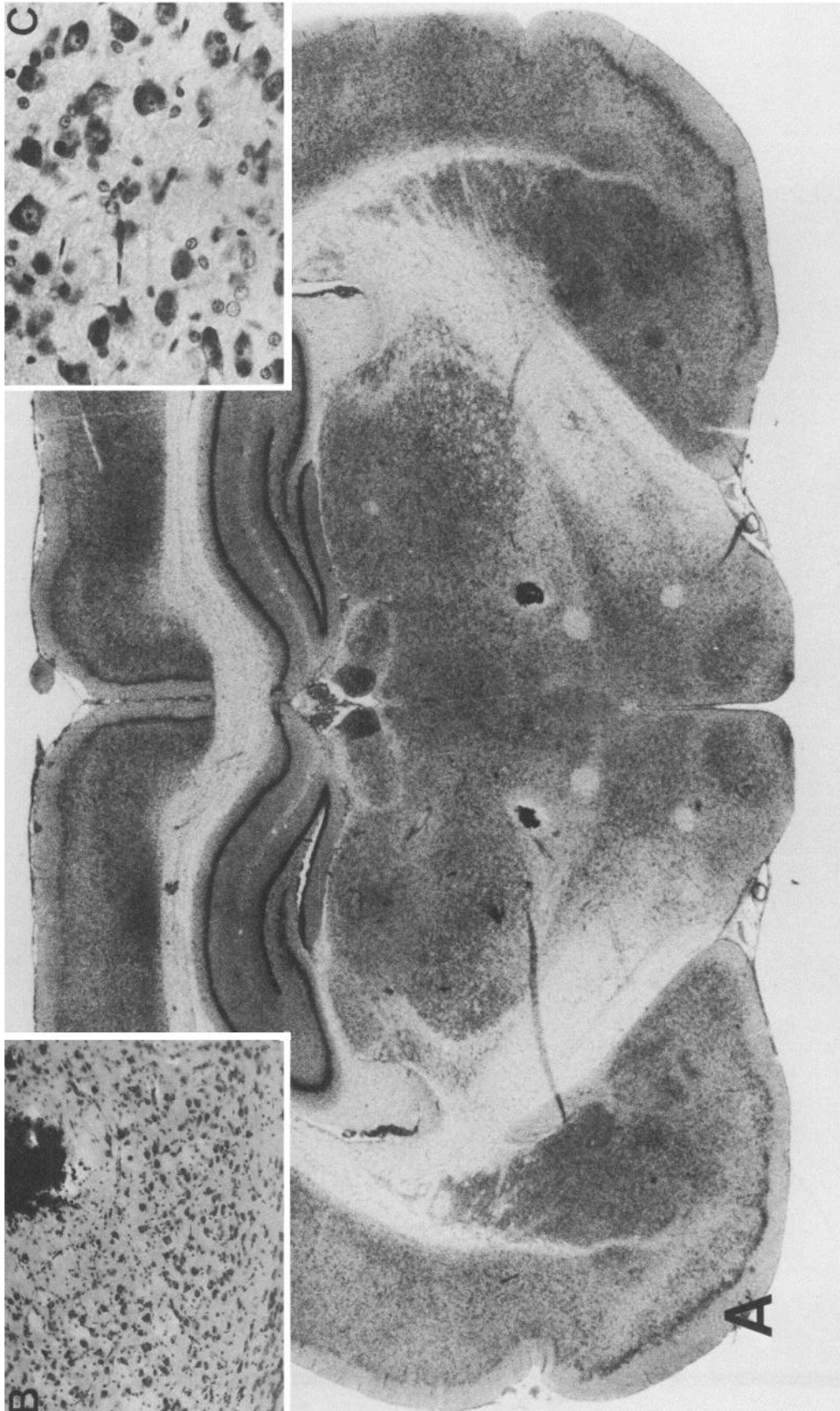
Figure 1. *A,* Low-power photomicrograph demonstrating the termination of cannulae tips within the rat VM at the frontal plane AP 4230 μm (König and Klippel, 1963). Cresyl violet stain. ×12. *B,* Photomicrograph of the VM demonstrating the type of tissue reaction in close proximity of the injection cannula tip. Note mild glial infiltration around the cannula tip. Cresyl violet stain. ×40. *C,* High-power photomicrograph demonstrating the cytoarchitecture of the VM in a rat which underwent bilateral microinjection of (−)AP7. Cresyl violet stain. ×186.

A

B
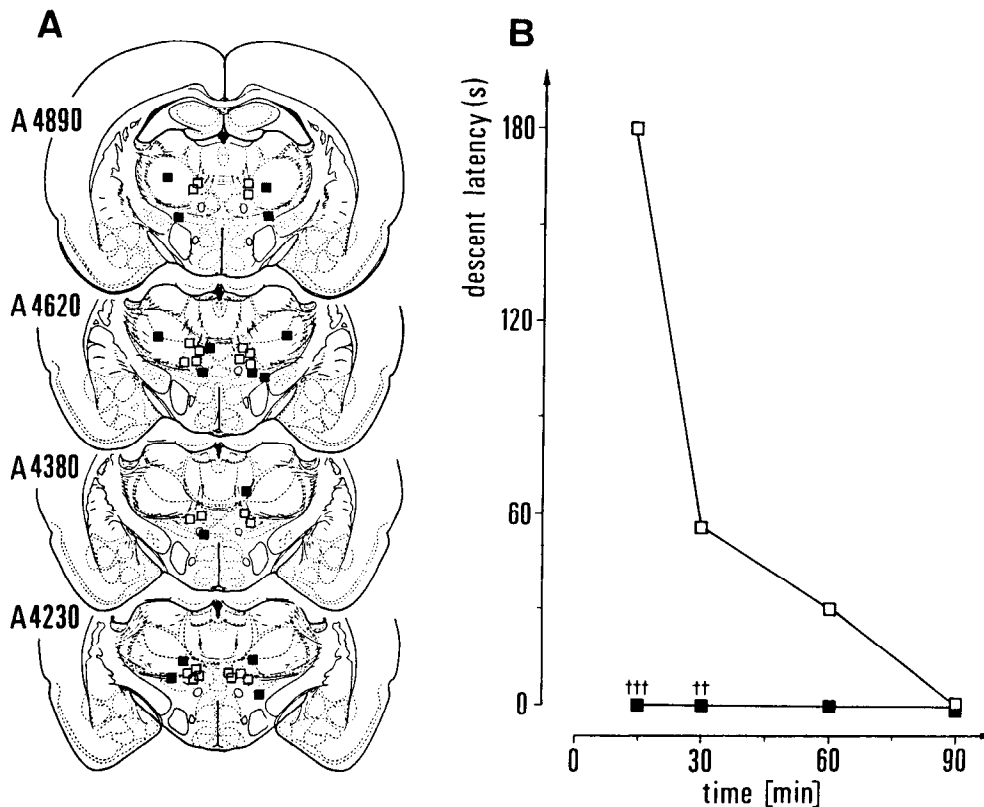
Figure 2. A, Topographical distribution of (−)AP7, 100 ng, injections into the VM (□) and areas outside the VM (■). *B,* Catalepsy after bilateral injections of (−)AP7, 100 ng, into the VM (□, *n* = 12) and areas outside the VM (■, *n* = 8). *Abscissa,* time (min) after beginning of the injection; *ordinate,* descent latency in the bar test (median values). ++, *p* < 0.02, +++, *p* < 0.002 vs injection of (−)AP7, 100 ng, into the VM (Mann-Whitney *U* test).

in comparison to control injections into the adjacent zona incerta and thalamic tissue outside the VM. The distribution of the (−)AP7 (100 ng) injection sites is representative for all experimental groups that received injections into the VM.

Catalepsy

Saline (0.5 μl) injected bilaterally into the VM was devoid of any obvious behavioral effect. In particular, it failed to elicit catalepsy, as measured in three behavioral tests (Table 1, Fig. 3).

The NMDA antagonist (−)AP7 injected bilaterally into the VM in doses of 25, 50, 100, and 250 ng induced catalepsy in a dose-dependent way. This behavioral effect had an immediate onset and lasted for about 30 to 60 min depending on the dose used (Table 1, Fig. 3*A*). The stereoisomer (+)AP7, 100 ng, which gave an almost maximal response when using (−)AP7, failed to induce catalepsy (Table 1, Fig. 3*B*). Injections of the active stereoisomer (−)AP7 (100 ng) into the zona incerta and into thalamic nuclei adjacent to the VM were found to be ineffective in inducing catalepsy (Fig. 2). Both NMDA (100 ng) and the GABA antagonist bicuculline (100 ng) prevented the development of catalepsy when injected in combination with (−)AP7 (100 ng) into the VM (Table 1, Fig. 3*B*).

The GABA agonist muscimol in the dose of 25 ng injected bilaterally into the VM induced catalepsy with an immediate onset and a duration of at least 90 min (Table 1, Fig. 3*C*). The GABA specificity, locus specificity, and dose dependency of this effect has been demonstrated in earlier publications (Di Chiara et al., 1979a; Klockgether et al., 1985). The development of the muscimol-induced catalepsy with 25 ng of the drug, was reversed by the coadministration of either the muscarinic agonist bethanechol (500 ng) or the excitatory amino acid NMDA (100 ng) for a period of about 30 and 15 min, respectively. After that time, a degree of catalepsy was assessed in both groups that was comparable to that after injection of muscimol (25 ng) alone (Table 1, Fig. 3*C*). The reoccurrence of catalepsy after coad-

ministration of muscimol with an excitatory drug, i.e., bethanechol or NMDA, into the VM is likely to be due to the prolonged action of muscimol in comparison to other drugs. The observation of this phenomenon excludes the possibility that the suppression of the muscimol-induced catalepsy by bethanechol or NMDA is caused by a chemical inactivation of muscimol.

The muscarinic antagonist scopolamine in doses of 500 ng and 2.0 and 5.0 μg did not induce catalepsy (Table 1, Fig. 3*C*).

Locomotor activity

Spontaneous locomotor activity did not differ in four experimental groups. Steady levels of activity were observed in all groups about 20 min after placing the animals into the experimental cage. The bilateral injection of NMDA (100 ng), bicuculline (100 ng), or bethanechol (500 ng) produced a significant increase of locomotor activity in comparison to animals treated with 0.5 μl saline (Fig. 4).

Tonic EMG activity

The tonic EMG activity of the rat gastrocnemius muscle is considered to be a measure of limb rigidity in this species (Ellenbroek et al., 1985). As shown in the original EMG recordings of Figure 5*A*, both (−)AP7, 250 ng, and muscimol, 50 ng, injected bilaterally into the VM induced tonic EMG activity, whereas saline, 0.5 μl, and scopolamine, 2 μg, failed to induce such an effect. The (−)AP7-induced tonic EMG activity returned to baseline levels 70 min after the injection, whereas the muscimol-induced activity did not show any apparent decline during the first 2 hr after the injection (Fig. 5*B*). Additional experiments revealed that the muscimol-induced tonic EMG activity lasted for about 4–6 hr (not shown).

EMG patterns in unrestrained animals

EMG recordings were made in awake, unrestrained rats from the gastrocnemius muscle, an extensor of the ankle joint, and
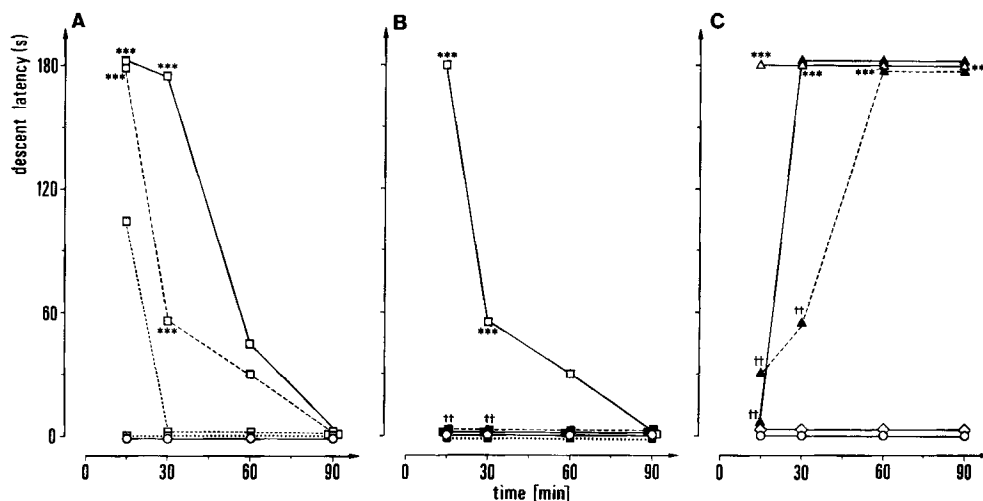
Figure 3. Catalepsy after bilateral injections into the VM. *Abscissa,* time (min) after beginning of the injection; *ordinate,* descent latency in the bar test (median values). *A,* —O—, saline (0.5 μl, $n = 12$); ··□··, (−)AP7 (25 ng, $n = 11$); --□--, (−)AP7 (50 ng, $n = 7$); --□--, (−)AP7 (100 ng, $n = 12$); —□—, (−)AP7 (250 ng, $n = 5$). ***, $p < 0.002$ vs. injection of saline (Mann-Whitney U test). *B,* —O—, saline (0.5 μl, $n = 12$); —□—, (−)AP7 (100 ng, $n = 12$); —■—, (+)AP7 (100 ng, $n = 6$); --■--, (−)AP7 (100 ng) + NMDA (100 ng, $n = 8$); ···■···, (−)AP7 (100 ng) + bicuculline (100 ng, $n = 9$). ***, $p < 0.002$ vs injection of saline; ++, $p < 0.02$ vs injection of (−)AP7 (100 ng) (Mann-Whitney U test). *C,* —O—, saline (0.5 μl, $n = 12$); —◇—, scopolamine (5 μg, $n = 5$); —△—muscimol (25 ng, $n = 10$); —▲—, muscimol (25 ng) + NMDA (100 ng, $n = 9$); --▲--, muscimol (25 ng) + bethanechol (500 ng, $n = 14$). ***, $p < 0.002$ vs injection of saline; ++, $p < 0.02$ vs injection of muscimol (25 ng) (Mann-Whitney U test).

the tibialis cranialis muscle, a flexor of the same joint, before and after bilateral injections of (−)AP7 (250 ng) into the VM.

Figure 6*A* shows the simultaneous EMG recordings of these muscles in an untreated rat during a period of steady forward locomotion when placed into an open field. In these recordings an alternating pattern of extensor and flexor activity typical for locomotion can be seen. After local injection of (−)AP7 into the VM, the rat maintained an akinetic position when placed into an open field, thereby exhibiting tonic EMG activity in both muscles. The degree of tonic EMG activity observed in unrestrained rats depended on the position into which the limb was passively brought.

When the hindleg of an untreated rat was gently placed on a 5 cm high wooden block, the rat quickly withdrew its leg. Figure 6*B* shows the underlying EMG activity in the tibialis muscle consisting of an initial phasic burst that resulted in the withdrawal of the limb followed by a series of EMG bursts reflecting subsequent stepping movements. In contrast, after injections of (−)AP7 into the VM, the hindleg remained in its imposed position on the block, thereby exhibiting tonic EMG activity. Pinching the hindpaw of such an animal resulted in a tonic increase of the EMG activity in the tibialis muscle, but the rat did not withdraw the limb (Fig. 6*B*). In general, phasic EMG bursts required to initiate limb movements or locomotion in

Table 1. Catalepsy after bilateral injections into the VM

Drug	Dose (ng)	n	15 min			30 min			60 min			90 min		
			a	b	c	a	b	c	a	b	c	a	b	c
Saline		12	0	0	0	0	0	0	0	0	0	0	0	0
(−)AP7	25	11	36	0	27	9	0	0	0	0	0	0	0	0
(−)AP7	50	7	71	0	29	14	0	0	43	0	0	14	0	0
(−)AP7	100	12	92	8	83	92	0	33	58	0	25	33	0	25
(−)AP7	250	5	100	20	100	100	20	80	60	20	20	20	20	20
(+)AP7	100	6	17	0	0	17	0	0	0	0	0	0	0	0
(−)AP7 + NMDA	100 + 100	8	25	0	12	25	0	12	12	0	0	0	0	0
(−)AP7 + BIC	100 + 100	9	11	0	0	11	0	0	11	0	0	11	0	0
MSC	25	10	100	50	80	100	60	90	90	60	70	80	40	70
MSC + NMDA	25 + 100	9	44	0	44	55	22	44	89	11	78	78	11	44
MSC + BCH	25 + 500	14	57	14	14	71	21	43	86	50	79	86	50	71
SCOP	500	5	0	0	0	0	0	0	0	0	0	0	0	0
SCOP	2.0 (μg)	6	17	0	0	0	0	0	0	0	0	0	0	0
SCOP	5.0 (μg)	5	20	0	0	20	0	0	0	0	0	0	0	0

Catalepsy was assessed 15, 30, 60, and 90 min after beginning of injections by means of the bar, bridge, and inversed bar tests. For each test, an animal was considered to be cataleptic if it maintained the respective position for at least 30 sec. BIC, bicuculline; MSC, muscimol; BCH, bethanechol; SCOP, scopolamine. *n,* number of animals. a, Percentage of cataleptic animals, as judged from the bar test; b, percentage of cataleptic animals, as judged from the bridge test; c, percentage of cataleptic animals, as judged from the inversed bar test.

(−)AP7-treated animals were not observed to occur either spontaneously or in response to external stimuli.

If an untreated animal was tilted 45° backward about a side-to-side axis, a synergistic tonic EMG response was recorded in the gastrocnemius and tibialis muscles. After (−)AP7 injections into the VM, a comparable tonic activation of both muscles occurred when the animal was tilted backward (Fig. 6C). All animals were subjected to a series of procedures that challenged their static equilibrium: 45° forward tilt about a side-to-side axis, roll tilt to both sides about a longitudinal axis, forward and backward pushing, and dropping. In these tests, both untreated and (−)AP7-treated animals showed principally the same reflexive adjustments of EMG activity. In detail, forward tilt and forward pushing led to a tonic EMG response in the tibialis muscle, whereas the muscular activity in the gastrocnemius muscle ceased. Backward pushing resulted in a synergistic activation of both the gastrocnemius and tibialis muscle, as has been documented for backward tilt. Roll tilt elicited an extensor response on the side tilted downward. During a drop, a prelanding EMG activity in the gastrocnemius muscle was recorded. Muscular responses of treated and untreated animals were nearly identical in these tests. Untreated animals, however, tended to initiate stepping movements, which were recorded as coordinated phasic EMG bursts subsequent to the initial reflexive muscular response.

These EMG patterns were observed consistently in all animals with successful injections into the VM and were stable in each animal for about 30 min after the injection of (−)AP7 into the VM.

Discussion

Role of NMDA-mediated excitation

The present study reports that the local injection of NMDA into the VM induces an increase of locomotor activity, whereas the local injection of the NMDA antagonist (−)AP7 results in the development of catalepsy. Evidence is presented that the latter effect is due to a specific blockade of NMDA receptors within the VM: (1) The development of the (−)AP7-induced catalepsy is dose-dependent. (2) The stereoisomer (+)AP7, which has been reported to be less effective, or ineffective, in blocking NMDA-mediated events (McLennan, 1982; Perkins et al., 1982), does not induce catalepsy. (3) (−)AP7-induced catalepsy is susceptible to an antagonism with NMDA. (4) Injections of (−)AP7 into thalamic tissue outside the VM fail to induce catalepsy. Since (−)AP7 is devoid of an intrinsic action on NMDA receptors (Watkins, 1984), the occurrence of a pharmacological effect after local injection of (−)AP7 into the VM points to the presence of an endogenous excitatory neurotransmission mediated by NMDA receptors within the VM.

The corticothalamic pathway is assumed to use an excitatory amino acid as its transmitter. The true identity of this substance remains uncertain although some evidence favors L-glutamate (Fonnum et al., 1981; Young et al., 1981). Both electrophysiological and receptor binding studies have revealed that L-glutamate is a mixed agonist interacting with NMDA, kainate, and quisqualate receptors (McLennan, 1983; Watkins, 1984). The existence of all three excitatory amino acid receptor subtypes within the thalamus has recently been demonstrated in autoradiographic studies (Monaghan and Cotman, 1982; Monaghan et al., 1984; Rainbow et al., 1984). Since apart from the corticothalamic tract no other afferent inputs to the VM have been convincingly demonstrated to use an excitatory amino acid as their transmitter (Ottersen and Storm-Mathisen, 1984), one may assume that the hypothesized excitatory transmitter compound acting on NMDA receptors within the VM is mainly derived from cortical afferents. The behavioral consequences of blocking kainate and quisqualate receptors remain to be investigated. At
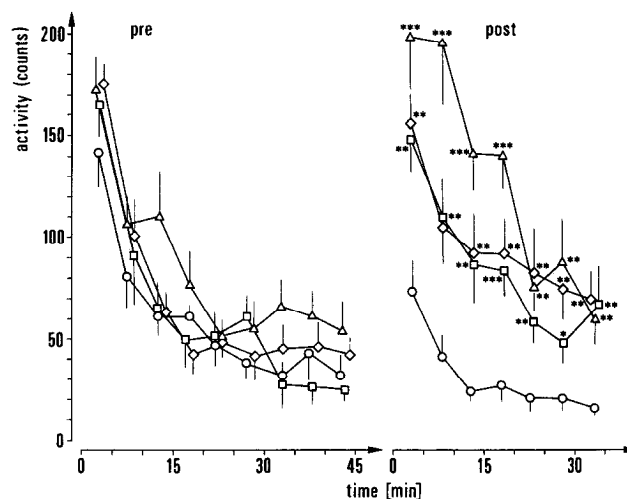

Figure 4. Locomotor activity before (pre) and after (post) bilateral injections into the VM. Abscissa, time (min) after placing the animal into the experimental cage; ordinate, activity counts per 5 min (mean ± SEM). Symbols: —O—, saline (0.5 μl, n = 12); —◇—, bethanechol (500 ng, n = 9); —□—, NMDA (100 ng, n = 8); —△—, bicuculline (100 ng, n = 10). *, p < 0.05, **, p < 0.01, ***, p < 0.001 vs injection of saline (Student's t test).

present, such experiments are hampered by the lack of specific antagonists acting at these receptor subtypes.

In order to achieve a better understanding of the role played by the VM in motor control, the mechanisms underlying the (−)AP7-induced catalepsy were analyzed using an EMG approach (De Ryck and Teitelbaum, 1983). It was found that the catalepsy in these animals is associated with the occurrence of tonic EMG activity of the gastrocnemius muscle, which is considered to be a measure of limb rigidity (Ellenbroek et al., 1985). Animals in the state of (−)AP7-induced catalepsy are unable to initiate limb movements either spontaneously or in response to external stimuli. It was found that the muscular mechanism underlying the animals' akinesia is an inability to perform a phasic activation of a set of muscles, whereas tonic EMG responses due to external stimuli do occur. The muscular responses that serve to maintain the animal's static equilibrium are left intact and can be observed in their purest form because they are not overlaid by limb movements and locomotor attempts. These responses are due to postural reflex mechanisms organized at the brain-stem and midbrain level. In conclusion, the (−)AP7-induced catalepsy may be characterized as an acute akinetic-rigid syndrome, in which the ability to induce a phasic activation of a set of muscles is lost and is replaced by exaggerated tonic muscular responses. Stabilization of an assumed posture instead of movement appears to be the primary goal of motor control in these animals.

Role of GABAergic transmission

The present study confirms that the local application of the GABA agonist muscimol to the VM produces catalepsy (Di Chiara et al., 1979a; Klockgether et al., 1985; Starr and Summerhayes, 1983a), whereas blockade of GABAergic transmission within the VM by local application of the GABA antagonist bicuculline results in an increase of locomotor activity. The catalepsy induced by injection of muscimol into the VM has been characterized in an earlier publication as an akinetic-rigid syndrome resembling that after injection of (−)AP7 into the VM (Klockgether et al., 1985). Since most of GABAergic inhibition within the VM is provided by the afferent pathways arising from the basal ganglia output nuclei (Kultas-Ilinsky et al., 1985), it seems justified to assume that facilitating or block-
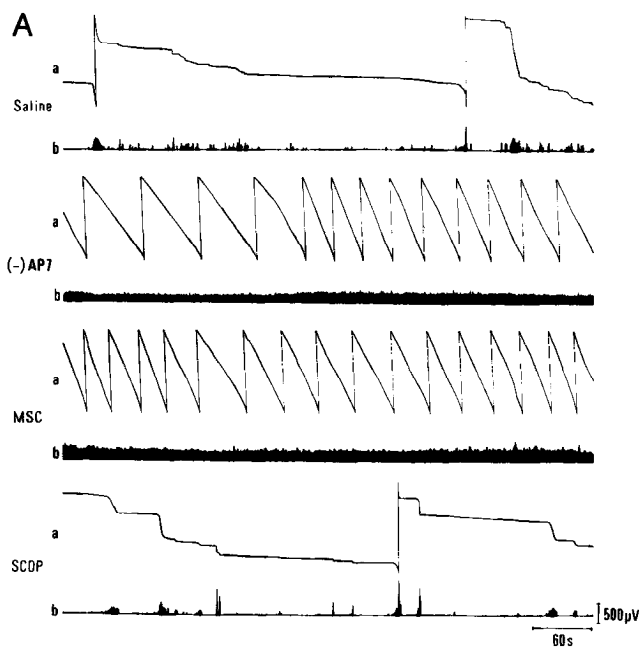
Figure 5. *A*, Original EMG recordings from the gastrocnemius muscle of partly restrained rats 30 min after bilateral injections of saline, 0.5 μl; (−)AP7, 250 ng; muscimol (*MSC*), 50 ng; and scopolamine (*SCOP*), 2 μg. The lower tracings (*b*) represent the rectified EMG activity, whereas the upper tracings (*a*) represent the integrated activity. *B*, Time course of tonic EMG activity after bilateral injections into the VM. *Abscissa*, time (min) after beginning of the injection; *ordinate*, integrated tonic EMG ativity of the gastrocnemius muscle (means ± SEM). Symbols: —O—, saline (0.5 μl, *n* = 12); —□—, (−)AP7 (250 ng, *n* = 9); —Δ—, muscimol (50 ng, *n* = 8); —◇—, scopolamine (2 μg, *n* = 7). *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ vs injection of saline (Student's *t* test).
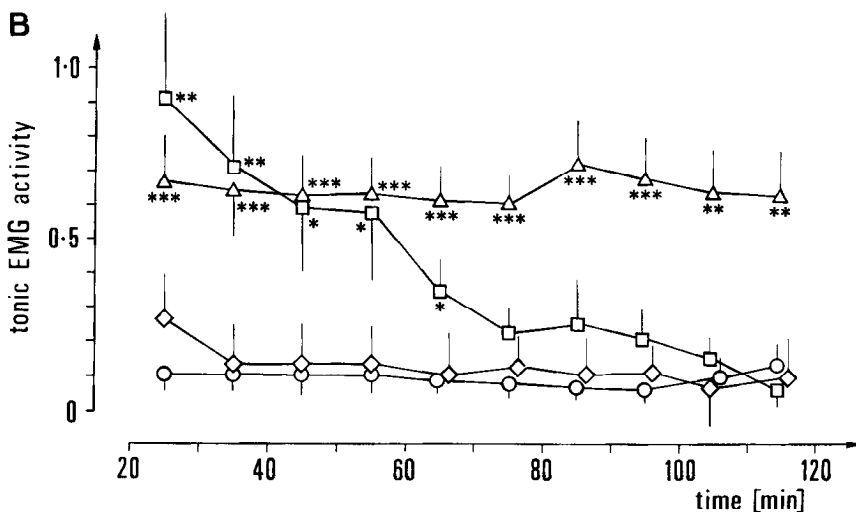
ing GABAergic transmission within the VM mimics the behavioral and motor effects of an increased or decreased nervous impulse flow in the GABAergic basal ganglia output pathways toward the VM. In fact, disinhibition of nigral and pallidal output neurons by local injection of a GABA antagonist into the reticular part of the substantia nigra or entopeduncular nucleus, the rodent and feline homologs of the primate internal segment of the globus pallidus, has been reported to result in catalepsy, whereas inhibition of these neurons by local application of a GABA agonist has been reported to result in locomotor activity (Di Chiara et al., 1981; Scheel-Krüger, 1983). These experimental data are consistent with the idea that the basal ganglia facilitate movements by releasing their target nuclei—i.e., the ventral thalamic nuclei, superior colliculus, and reticular formation—from a tonic GABA-mediated inhibition (Hikosaka and Wurtz, 1985). This principle seems to be valid in particular for the primate nigrotectal system, which is mainly engaged in oculomotor control, since nigrotectal cells have been found to decrease their discharge in relation to saccadic eye movements (Hikosaka and Wurtz, 1983).

There is a striking similarity between the cataleptic state occurring after blockade of NMDA-mediated events within the VM and that after facilitation of GABA-mediated events within the VM with respect to both the symptomatology and the underlying muscular mechanisms (Klockgether et al., 1985). The strict reciprocity of NMDA- and GABA-mediated behavioral responses within the VM is further underlined by the observations that the catalepsy induced by local injection of the NMDA antagonist (−)AP7 can be antagonized by coadministration of the GABA antagonist bicuculline, whereas the catalepsy induced by injection of the GABA-mimetic muscimol into the VM can be antagonized by coadministration of NMDA. Furthermore, both the local activation of NMDA receptors by NMDA and the local blockade of GABA receptors within the VM by bicuculline are capable of inducing increased locomotor activity.

Role of muscarinic transmission

The present study reports that the local application of the specific muscarinic agonist bethanechol into the VM has a motor-activating effect in two behavioral paradigms. Bethanechol leads
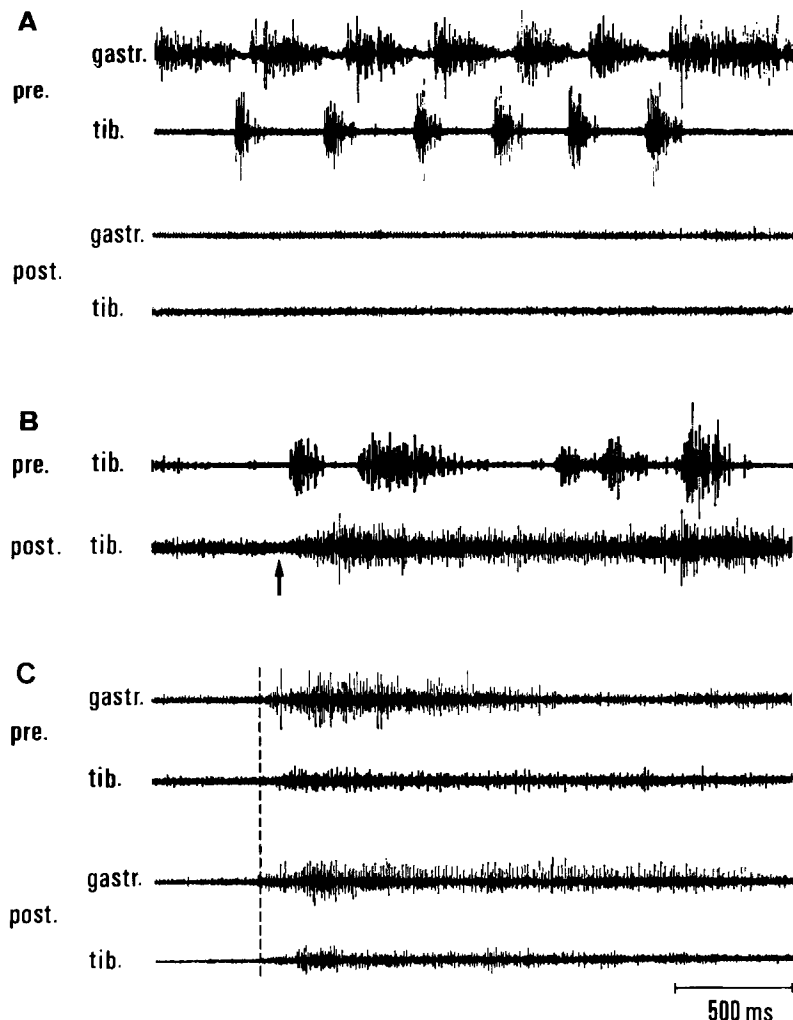
Figure 6. A, Original EMG recordings from the gastrocnemius (*gastr*) and tibialis cranialis (*tib*) muscle in an unrestrained rat placed into an open field before (*pre*) and after (*post*) bilateral injection of 250 ng (−)AP7 into the VM. *B,* Original EMG recording from the tibialis cranialis muscle (*tib*) in an unrestrained rat, the hindleg of which was placed on a 5 cm high block before (*pre*) and after (*post*) bilateral injection of 250 ng (−)AP7 into the VM. During the postinjection trial, the hindpaw was pinched by the experimenter. The onset of the stimulus is marked by an *arrow. C,* Original EMG recordings from the gastrocnemius (*gastr*) and tibialis cranialis (*tib*) muscle in an unrestrained rat during 45° backward tilt about a side-to-side axis before (*pre*) and after (*post*) bilateral injection of 250 ng (−)AP7 into the VM. The onset of the tilt is marked by the *dotted line.*

to an increased locomotor activity and counteracts the development of the catalepsy induced by local application of muscimol to the VM. Since iontophoretically applied cholinomimetics excite neurons of the ventral thalamic nuclei in a muscarine specific way (MacLeod et al., 1984; McLennan et al., 1968), the above-mentioned behavioral effects may be ascribed to a pharmacological excitation of VM neurons due to an interaction of bethanechol with muscarinic receptors within the VM.

On the other hand, the muscarinic antagonist scopolamine, when locally injected into the VM, is devoid of an obvious behavioral effect even at high doses. This negative finding suggests that the degree of muscarinic transmission within the VM is low or even absent. This suggestion seems surprising in the light of the cholinergic nature of cerebellothalamic transmission. However, at present it is impossible to be too definitive about the role of ACh as the cerebellothalamic transmitter. As outlined by MacLeod et al. (1984), the biochemical and electrophysiological data presented as evidence for such a role have to be interpreted with great reservation. The decrease of CAT levels following brachium conjunctivum lesions might be due to the concomitant damage of the cholinergic Ch5 cell group located within the brachium conjunctivum (Wainer et al., 1984). Furthermore, the sensitivity of cerebellothalamic transmission to antimuscarinic drugs might be related to an overall reduction of neuronal excitability induced by atropine-like drugs. The fact that all studies in which the central cholinergic pathways of the

rat brain were mapped using specific antibodies to CAT failed to demonstrate the existence of cholinergic neurons within the deep cerebellar nuclei (Satoh et al., 1983; Wainer et al., 1984) casts further doubt on the cholinergic nature of the cerebellothalamic tract. The matter has grown still more complicated, since Nieoullon (1984) has shown that hemicerebellectomy in cats does not only result in a decrease of CAT within the ventrolateral thalamus but also decreases the high-affinity glutamate uptake, at least in the caudal part of the ventrolateral nucleus, suggesting that an excitatory amino acid might be involved in cerebellothalamic synaptic transmission. One should also take into account that ACh, although involved in cerebellothalamic transmission, might be released only in small quantities within the VM due to a low tonic discharge rate of cerebellothalamic neurons. This latter proposal, however, seems less probable, since recordings of neurons within the deep cerebellar nuclei in awake rats and monkeys show that these neurons discharge tonically at a relatively high firing rate (Hernandez-Mesa and Bures, 1978; Thach, 1968).

Functional considerations

The rat VM appears to represent a brain site from which the animal's motility can be influenced in both directions: Decreasing neuronal activity within the VM by blocking excitatory or enhancing inhibitory neurotransmission within the VM induces a state of rigid catalepsy in which the animals are unable to initiate limb movements, whereas facilitation of neuronal ac-

tivity induces an increase in locomotor activity.

The present study provides evidence that an imbalance between NMDA-mediated excitation and GABA-mediated inhibition within the VM leads to disturbances of motility, whereas muscarinic transmission appears to be of minor importance in this respect. In view of the role of an excitatory amino acid as the transmitter of the corticothalamic tract (Fonnum et al., 1981; Young et al., 1981) and GABA as the transmitter of the thalamic afferents arising from the basal ganglia (Di Chiara et al., 1979b; Kilpatrick et al., 1980; Penney and Young, 1981), it is conceivable that an altered integration of excitatory nervous impulses arising from the cortex and inhibitory impulses arising from the basal ganglia at the thalamic level might represent a pathophysiological factor in the development of movement disorders accompanied by disturbances of motility. Of particular interest in this context, a recent investigation reported a close overlap of high levels of glutamate and high levels of GABA within the human medial thalamus, suggesting that this overlap might form the neurochemical basis for a functional interaction between cortical and basal ganglia afferents to the thalamus (Muramoto et al., 1984).

The major output pathway arising from the VM is directed diffusely toward layer I of the neocortex (Herkenham, 1979). The observation that electrical stimulation of the VM depolarizes neuronal elements in the superficial layers of wide neocortical areas (Glenn et al., 1982), indicates that the VM plays a crucial role for the regulation of cortical excitability. Such a role of the rat VM is corroborated by the recent finding that a chronic lesion of the VM results in a severe and long-lasting depression of metabolic activity in wide cortical areas, as measured with the deoxyglucose technique (Girault et al., 1985). It is tempting to speculate that the profound changes in the animal's motility observed after chemically manipulating the neuronal activity within the VM are due to acute changes in cortical excitability.

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