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AMINERGIC CONTROL OF NEURONAL FIRING RATE IN THALAMIC MOTOR NUCLEI OF THE RAT

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INTRODUCTION

Clinical observations and physiological studies suggest that ventral-anterior (VA) and ventrolateral (VL) thalamic nuclei play a role in initiating and controlling muscle activity and postural changes associated with movement (8, 25, 65, 69) and participate in spatial learning and recall of motor sequences (20, 53, 66). In fact, the VA-VL complex forms part of two motor networks, subject to cortical control (49) and delivering weighted contributions from cerebellum and basal ganglia to the sensorimotor cortex (62).

The most extensive projection from the cerebellum originates in the lateral and interposed nuclei, and is conveyed to distinct but partially overlapping thalamic areas coinciding with contralateral VL and, to a lesser extent, with a dorsolateral area of VA (4, 50, 59, 68, 70). The termination zones for the more restricted fastigial input lie in bilateral VL (4, 6) so that information from two or more cerebellar nuclei may converge on the same thalamic neurons (64).

In contrast, the main pallidal projection reaches the medio-ventral VA and the pars oralis and caudalis of the VL, with the density of the projections decreasing along an anterior to posterior gradient in the thalamus (63). Therefore, massive cerebellar and pallidal projections directed to the VA-VL complex overlap only in a lateral area of the VL (50), even if single thalamic nuclei receive differentially weighted inputs from both sources (63).

Furthermore, sensory information from the pretectal area of the rostral midbrain (37) and, to a minor extent, from the spinothalamic tract (74) is also delivered to the same VA-VL zones that receive cerebellar output.

Like many other sensory and motor structures thalamic motor nuclei receive a relevant aminergic projection. In fact they are innervated by noradrenergic terminals originating in the *locus coeruleus* (10) and by serotonergic fibers coming from dorsal, medial and pontine raphe nuclei (14). A moderate concentration of serotonin (5-HT) was detected in both VA and VL, at least in man, whereas the NA content appears more relevant in magnocellular VA than in lateral structures like VL or lateral geniculate nucleus (54). A network of noradrenergic and serotoninergic fibers was described near soma and dendrites of thalamic neurons. Noradrenergic innervation appeared denser than serotoninergic innervation in the ventrobasal complex (52), but was less dense

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than serotoninergic innervation in the geniculate (47). A synaptic organization was not observed (52) and in fact only a minority of NA labeled varicosities made synaptic contacts (27). Noradrenaline (NA) and 5-HT can modulate neuronal firing in some thalamic specific and unspecific nuclei including lateral and medial geniculate nuclei, ventrobasal complex and reticular nucleus (29, 44, 45).

It has been suggested that one function of neuroamines in the thalamus is to inhibit the rhythmic bursting neuronal firing characteristic of slow wave sleep, and to evoke a distinct firing mode, typical of arousal (55). Both NA and 5-HT could evoke these effects by suppression of a potassium leak current and enhancement of the hyperpolarization-activated cation current (43).

However, some data suggest that the effects of aminergic pathways on thalamocortical processing could have more specific functions. In fact, dopamine exerts a mostly suppressive action on ventrolateral thalamic input to motor cortex (24) and NA has complex interactions with some neurotransmitters in thalamic specific nuclei, e.g. inducing phenomena of occlusion with acetylcholine in the lateral geniculate nucleus (40).

The aim of this work was to study and compare the effects induced by NA and 5-HT on the firing of single neurons in thalamic motor nuclei to ascertain whether both amines exert the same type of modulation on VA and VL.

METHODS

Animal preparation

Experiments were performed on Wistar rats deeply anesthetized with urethane (1.5 g/kg). Acquisition and care of laboratory animals conformed to the guidelines published in the NIH *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1985), to the European Community Council Directive (86/609/EEC) and to Italian law. Experimental protocol was approved by the IACUC of the University of Catania.

Loss of toe-pinch reflex was used to indicate surgical anesthesia. Heart rate was monitored continuously during the experiments and supplementary doses of anesthetic were administered whenever the heart rate exceeded 370-380 beats min^{-1}. Body temperature was maintained with a heating pad, and a gel of agar-agar (2%) was used to cover the exposed tissue and to prevent desiccation.

The head was held in a stereotaxic frame, small holes were drilled in the skull and a multi-barrel glass microelectrode was positioned by a micromanipulator at coordinates corresponding to VA or VL thalamic nuclei (56).

The final point of each electrode penetration in thalamic motor nuclei was marked by iontophoretic application of Pontamine sky blue (Sigma) ejected for 5-10 min by the recording electrode at a cathodal current of 20 μA.

At the end of the experiment the brain was removed and fixed in 10% formalin. The electrode tracks and recording sites were identified in serial coronal sections of the diencephalon (60 μm thick) stained with Neutral Red.

Recording and drug microiontophoresis

Three- or five-barrel glass microelectrodes were used to record extracellular single unitary neuronal activity and to apply pharmacological agents by microiontophoresis.

The recording barrel was filled with a 4% solution of Pontamine sky blue (Sigma) in 3M NaCl (resistance: 7-12 MΩ). Action potentials, recorded extracellularly from single VL and VA neurons, were rated as unitary and used for data only if they had a signal-to-noise ratio of at least 3:1 and

remained unmodified in amplitude during the tests. Recorded potentials were amplified and filtered, then fed to a PC for discrimination, storage and quantitative analyses (interface: Cambridge Electronic Design 1401; software: SPIKE2).

One barrel of the micropipette (three in five-barrel electrodes) was used for microiontophoresis and contained one of the followings drugs: noradrenaline hydrogen tartrate (NA, Sigma, 200 mM, pH 4.0), clonidine hydrochloride (CLO, Tocris, 50 mM, pH 5.0), L-isoproterenol hydrochloride (ISO, Sigma, 50 mM, pH 5.0), 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma, 30 mM, pH 4.5), 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT, Sigma, 20 mM, pH 4.5-5.0), alpha-methyl-5-hydroxytryptamine (ALPHA-MET-5-HT, Tocris, 20 mM, pH4.5-5.0). In experiments performed using five-barrel micropipettes, one barrel was filled with a noradrenergic or serotoninergic receptor antagonist, yohimbine hydrochloride (YO, Sigma, 20 mM, pH 4.5-5.0), timolol maleate (TIM,Tocris, 20 mM, pH 4.5-5.0), ketanserin tartrate (KET, Janssen, 10 mM, pH 4.5), 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine (nan-190, Tocris Cookson, 10 mM, pH 4.5-5.0). All drugs were dissolved in water except 5-HT (in 165 mM NaCl). Preceding each penetration, pH values of the solutions were routinely controlled before barrel filling and adjusted if necessary.

The microiontophoretic system (Neurophore BH-2) balanced currents automatically through a barrel filled with 3 M NaCl to neutralize any voltage shift due to the applied currents. As a control, we routinely verified that pure positive or negative currents up to 120 nA, applied through the barrel filled with 3 M NaCl (balance), had no effect on the background firing rate. Whenever such a current ejection induced variations of more than the standard deviation of the mean firing rate, the unit was excluded from further analysis.

Drugs were retained by negative currents (2-10 nA), delivered to the barrels, and ejected by brief (30 s) positive pulses at various current steps (up to 120 nA). Antagonists were applied with longer-lasting currents (up to 20 min, 2-20 nA).

Whenever a unit was isolated, applications (30 s pulses) of a drug (either NA or 5-HT or an agonist) were performed at various current intensities (5-100 nA) to identify the maximal response and more applications were repeated at the corresponding maximal intensity. The interval between applications was not standardized; instead we waited for the firing rate to return to the initial value and then ejected the drug after a delay of 1-2 min.

Data analysis

The sequence of inter-spike intervals of each recorded unit was used to calculate the firing rate over 1 s bins for analyses and 5 s for display. It was also used to calculate the frequency distribution of intervals (interval histogram). The mean value of the firing rate recorded over a sequence of 180 values (3 min) in the absence of any drug application was defined as the mean background activity (MBA). If the standard deviation (SD) exceeded 50% of the MBA the unit was excluded from analysis. In contrast, if the unit gradually increased or decreased its firing rate in the course of the recording session a new MBA was calculated.

A modification of the firing rate by at least 2 SD from the MBA for at least 20 s was defined as a response to a drug application. The parameters used to define a response were the magnitude M, the contrast C and the duration D. The magnitude M expressed the intensity of the effect in number of spikes and was defined as the difference between the number of spikes recorded during the response and during an equal period of time preceding drug ejection. The contrast C (expressed in %) was the ratio between these values and indicated the signal-to-noise value i.e. the incidence of the response with respect to the background activity. The parameter D estimated the duration of the effect in seconds (s).

Statistical analyses of firing rate and discharge pattern modifications were performed with a statistical software package (GRAPH PAD Prism, version 4.00).

Whenever a single unit was isolated, three applications of an amine (NA or 5-HT) or an agonist at different intensities were routinely followed by three applications of the same drug performed at the maximal intensity able to evoke reversible responses. The mean values of M, C, and D parameters referred to these three trials and were used to describe the response of the neuron.

Different sets of responses (e.g. to an amine and to a respective agonist or to the same amine in different nuclei) were compared with a two-tailed Student *t* test (Mann-Whitney *U* test for *C* val-

ues). An antagonist was considered effective if it reduced at least two of the three parameters of a response by 50-70% (partial antagonism) or more (blockade).

In two groups of units responsive to NA or 5-HT and in all those showing no significant modification of the firing rate following NA or 5-HT application, normalized frequency distributions (bin: 10 ms, max: 1 s) of inter-spike intervals were calculated and the relative histograms (ISIH) plotted, for periods of 120-240 s, before and following drug application and for periods of at least 30 s during drug application. Then, to test whether in a unit the discharge pattern changed significantly, the ISIH calculated before drug application was used as a control and compared to each of the ISIHs referred to successive periods of time. A procedure of non-linear regression was applied to fit each pair of ISIHs independently, and then a global fit was tested with the aim of finding shared best fit values between data sets. In other words, data were fitted in two ways and results compared (F test) to test whether the individual ISIHs were statistically distinguishable or not, i.e. whether one curve sufficed for both data sets.

RESULTS

The unitary activities of 218 thalamic neurons, 95 localized in VA and 123 in VL, were identified and recorded as long as their spike characteristics satisfied the criteria described in Methods. In a majority of neurons (about 55%) the firing pattern was characterized by bursting (6-8 spikes) but a tonic single spike discharge was observed in a conspicuous group of units (45%). The mean firing rate was 4 spikes/s (SD: 2 spikes/s, modal value: 1-2 spikes/s) in VA and 6 spikes/s (SD: 6 spikes/s, modal value: 1-2 spikes/s) in VL.

Responses to NA application

Unitary activities of 33 thalamic units, 22 located in VL and 11 in VA, were studied during application of NA. The firing rate of 82% of VL units (18 out of 22) and of all the 11 VA units tested was modified by NA application, the effect being a depression of the firing rate in 26 out of 29 responsive neurons (90%). Modifications of the firing rate in response to NA application were found in similar percentages both in units bursting discharge and in those with tonic single spike discharge.

Two types of inhibitory responses were however detected in both nuclei. In fact, the normalized distribution of durations D of inhibitory responses to NA recorded in the VA-VL complex (Fig. 1, a1) shows that the duration of a majority of inhibitory effects induced by NA clustered around values of less than two minutes, whereas a minority of reversible firing depressions ranged from 2 min to more than 20 min. On the basis of their duration (less or more than 2 min) the responses of thalamic units inhibited by NA were classified into two subsets, short-lasting inhibitions (SLI) and long-lasting inhibitions (LLI). The percentages of the types of effects detected in VA and in VL neurons in response to NA application indicate that in VL more than two thirds of inhibitions (11 out of 16) were SLI (Fig. 1, b1) whereas in VA (Fig. 1, b2) SLI and LLI responses were recorded in equal number. In 3 neurons (2 in VL and 1 in VA) NA ejection induced enhancements of the firing rate lasting 128, 130 and 245 s respectively.

The mean values of M, C and D parameters, calculated for each subset of responses in VA as well as in VL are reported in Table 1. The magnitudes M of NA-induced SLI were significantly larger in VL than in VA ($P = 0.040$) and the same trend could

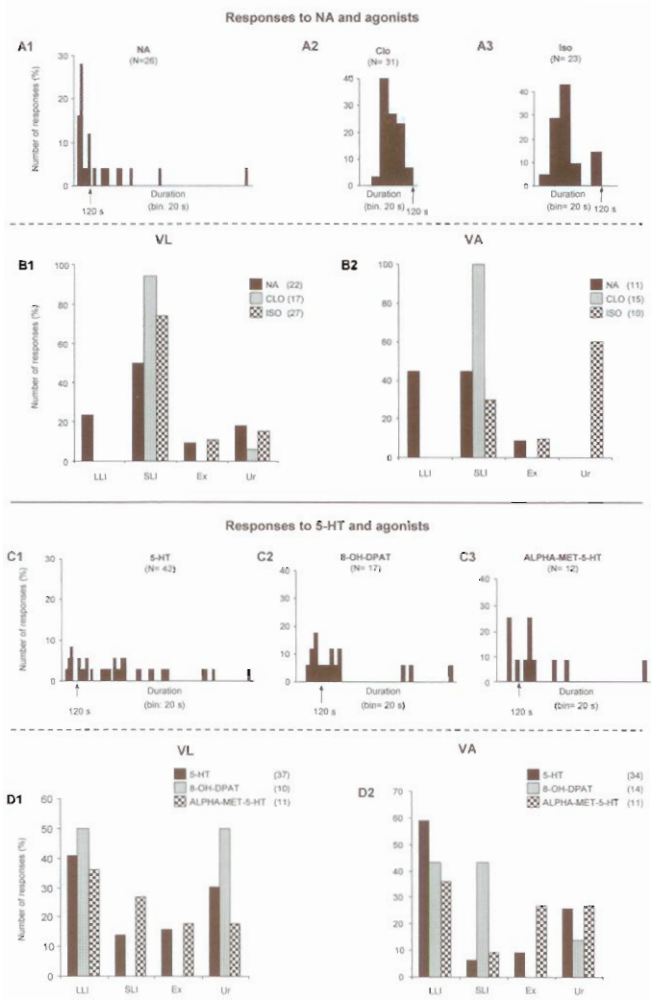


Fig. 1. - Duration and types of neuronal responses in ventrolateral (VL) and ventral anterior (VA) thalamic nuclei to microiontophoretic application (30 s) of noradrenaline (NA), 5-hydroxytryptamine (5-HT) and their agonists.

A1-A3. Normalized distributions of duration of inhibitory responses evoked in motor thalamic neurons by NA (A1), clonidine (CLO, A2) and isoproterenol (ISO, A3). Whereas NA application evoked either short-lasting inhibitions (SLI, less than 120 s) or long-lasting inhibitions (LLI, more than 120 s), both NA agonists induced SLI only.

B1-B2. Percentages of LLI, SLI, excitations (Ex) and unresponsive units (Ur) detected in VL (B1) and in VA (B2) following application of NA and its agonists. For each type of response, black, stippled and checkered columns indicate respectively NA-evoked, CLO-evoked and ISO-evoked effects. The total number of units tested for each drug is indicated in parentheses in each drawing.

C1-C3. Normalized distributions of duration of inhibitory responses evoked in motor thalamic neurons by 5-HT (C1), 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT, C2) and alpha-methyl-5-hydroxytryptamine (ALPHA-MET-5-HT, C3). All the drugs evoked both SLI and LLI.

D1-D2. Percentages of LLI, SLI, excitations (Ex) and unresponsive units (Ur) detected in VL (D1) and in VA (D2) following application of 5-HT and its agonists. For each type of response, black, stippled and checkered columns indicate respectively 5-HT-evoked, 8-OH-DPAT-evoked and ALPHA-MET-5-HT-evoked effects. The total number of units tested for each drug is indicated in parentheses in each drawing.

be observed with regard to the LLI. Examples of responses to NA application in VL and VA are shown in Figure 2a. In both nuclei depressive responses to NA were dose-dependent (Fig. 2b).

The alpha$_2$ adrenergic receptor agonist clonidine (CLO), tested in 32 thalamic neurons was effective in inducing depression of the firing rate lasting less than 120 s (Fig. 1, a2) and comparable to the NA-evoked SLI. These effects, recorded in 16 of the 17 VL units tested and in all 15 VA units (Fig. 1, b1-b2), were totally or partially blocked by simultaneous application of yohimbine, an alpha$_2$ noradrenergic receptor antagonist, in 12 out of the 14 neurons tested. In VA the SLI induced by NA application and those evoked by CLO were comparable in intensity (Table 1) and the latter appeared significantly longer lasting ($P = 0.044$). In contrast, in VL, CLO application induced SLI having magnitudes M smaller than those evoked by NA ($P = 0.0003$) even if the C values of responses to CLO were higher than in VA ($P = 0.011$).

The beta adrenergic receptor agonist isoproterenol (ISO) was tested in 37 thalamic neurons, 27 in VL and 10 in VA. In the majority of cases (23 out of 37 units, 62%) the response to ISO application was a depression of the firing rate, and all the inhibitory responses lasted less than 120 s (Fig. 1, a3). Differences were observed between VL and VA. In VL the effect of ISO application was a SLI in 20 out of 27 neurons (74%), an enhancement of the firing rate in 3 units and no apparent response in the remaining 4 units (Fig. 1, b1). In contrast, the majority of VA units (6 neurons) were unresponsive to ISO application (Fig. 1, b2), a SLI being recorded in 3 units and an increase in firing rate in the remaining unit. ISO-evoked SLI were partially antagonized by application of timolol, a beta noradrenergic receptor antagonist, in 2 of 4 cases tested.

The mean values of the parameters of responses to ISO ejection are shown in Table 1. In VA the few inhibitory responses to ISO were not significantly different from the SLI evoked by NA application, whereas in VL the magnitudes M of the ISO-evoked SLI were smaller ($P = 0.002$) than those of the NA-evoked SLI.

On the whole the parameters of the responses to application of NA agonists were comparable, but CLO application was able to evoke effects in a higher percentage of neurons and these effects appeared longer lasting in VA.

Examples of responses to NA receptor agonists and antagonists are shown in Figure 3.

Responses to 5-HT application

The number of thalamic neurons tested for 5-HT application was 71, of which 34 were in VA and 37 in VL. Thalamic neurons that modified their firing rate following 5-HT application were 25 out of 34 (74%) in VA and 26 out of 37 (70%) in VL. Modifications of the firing rate in response to 5-HT was observed in units discharging in bursts as well as in those characterized by tonic single spike discharge.

The most frequent response was a depression of the firing rate, seen in 22 out of 25 VA units and in 20 out of 26 VL units. The normalized distribution of duration of inhibitory responses to 5-HT recorded in the VA-VL complex appears almost flat

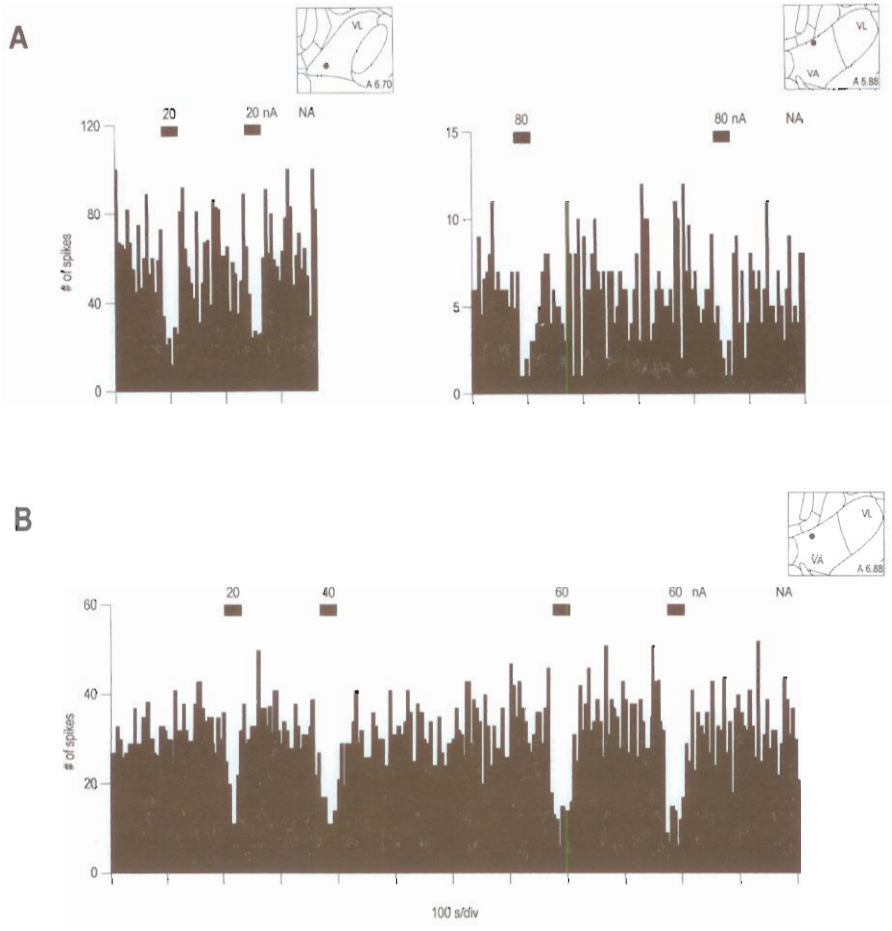


Fig. 2. - Modulation of background firing rate of thalamic neurons by application of noradrenaline (NA). Histograms (5-s bins) illustrate the firing rate of three units whose position is shown on schematic coronal thalamic sections drawn from stereotaxic atlas (56) at the coordinates (A: anterior) indicated in the respective insets. The horizontal bars above the histograms indicate the duration of the ejection periods of NA at the currents given.

A) Inhibitory effects induced by NA in two thalamic units, respectively located in the ventrolateral (VL) nucleus (left) and in ventral-anterior (VA) nucleus (right), were indicated by a depression of the mean firing rate whose value was described by the three parameters M, C and D defined in text. In both neurons the NA evoked effects were fully reversible.

B) Dose-dependency of the response of a VA neuron to NA applications at various current intensities (20, 40 and 60 nA).

(Fig. 1, c1) indicating that 5-HT-induced depressions of the firing rate could last from 30 sec to 10-15 min and no clustering of responses was apparent. Maintaining the criterion adopted with regard to the responses to NA application, thalamic units inhibited by 5-HT application were also classified into two subsets, SLI and LLI on the basis of their duration (less or more than 2 minutes respectively). In both nuclei LLI was the most frequent response whereas SLI were observed in a minority of VL

Table 1 - *Responsiveness of VA-VL neurons to NA, 5-HT and their agonists.*

	NA (33)				Clonidine (32)				Isoproterenol (37)			
	M	C	D	n	M	C	D	n	M	C	D	n
Short lasting inhibitions												
VA	-71 ± 30	-43 ± 8	49 ± 7	5	-83 ± 11	-35 ± 3	70 ± 6 *	15	-81 ± 28	-53 ± 6	71 ± 25	3
VL	-160 ± 20 °	-45 ± 5	65 ± 10	11	-56 ± 8 ***	-47 ± 3	66 ± 5 °	16	-77 ± 13 **	-42 ± 4	50 ± 6	20
Long lasting inhibitions												
VA	-362 ± 49	-38 ± 7	361 ± 89	5								
VL	-1008 ± 40	-37 ± 8	530 ± 264	5								
Enhancements												
VA	651	41	245	1					VA	405	74	188	1
VL	170/150	43/36	138/146	2					VL	507 ± 87	61 ± 18	322 ± 148	3

	5-HT (71)				8-OH-DPAT (24)				alpha-methyl-5-HT (22)			
Long lasting inhibitions												
VA	-743 ± 20	-43 ± 4	576 ± 90	20	-793 + 44	-54 ± 7	260 ± 105 *	6	-183 ± 12 *	-44 ± 12	153 ± 9 ***	4
VL	-1002 ± 19	-42 ± 5	516 ± 91	15	-272 ± 93**	-28 ± 5	330 ± 93 °	5	-1393 ± 48	-53 ± 7	362 ± 12	4
Short lasting inhibitions												
VA	-99/69	-54; -32	39/64	2	-171 ± 93	-50 ± 4	77 ± 8	6	-71	-46	89	1
VL	-254 ± 78	-39 ± 9	75 ± 12	5					-286 ± 83	-45 ± 4	51 ± 3	3
Enhancements												
VA	345 ± 18	151 ± 77	243 ± 68	3					270 ± 56	79 ± 27	93 ± 17	3
VL	626 ± 11	214 ± 14	220 ± 53	6					4380/47	139/59	388/31	2

Values are means ± SE. VA: ventral-anterior thalamic nucleus; VL: ventrolateral thalamic nucleus; M: Magnitude (number of spikes); C: Contrast (%); D: Duration (s); n: number of neurons. ° Significant differences between responses in VA and VL; * Significant difference between responses to an amine and its agonist; $p < 0.05$ = °/*; $p < 0.01$ = °°/**; $p < 0.001$ = °°°/***.

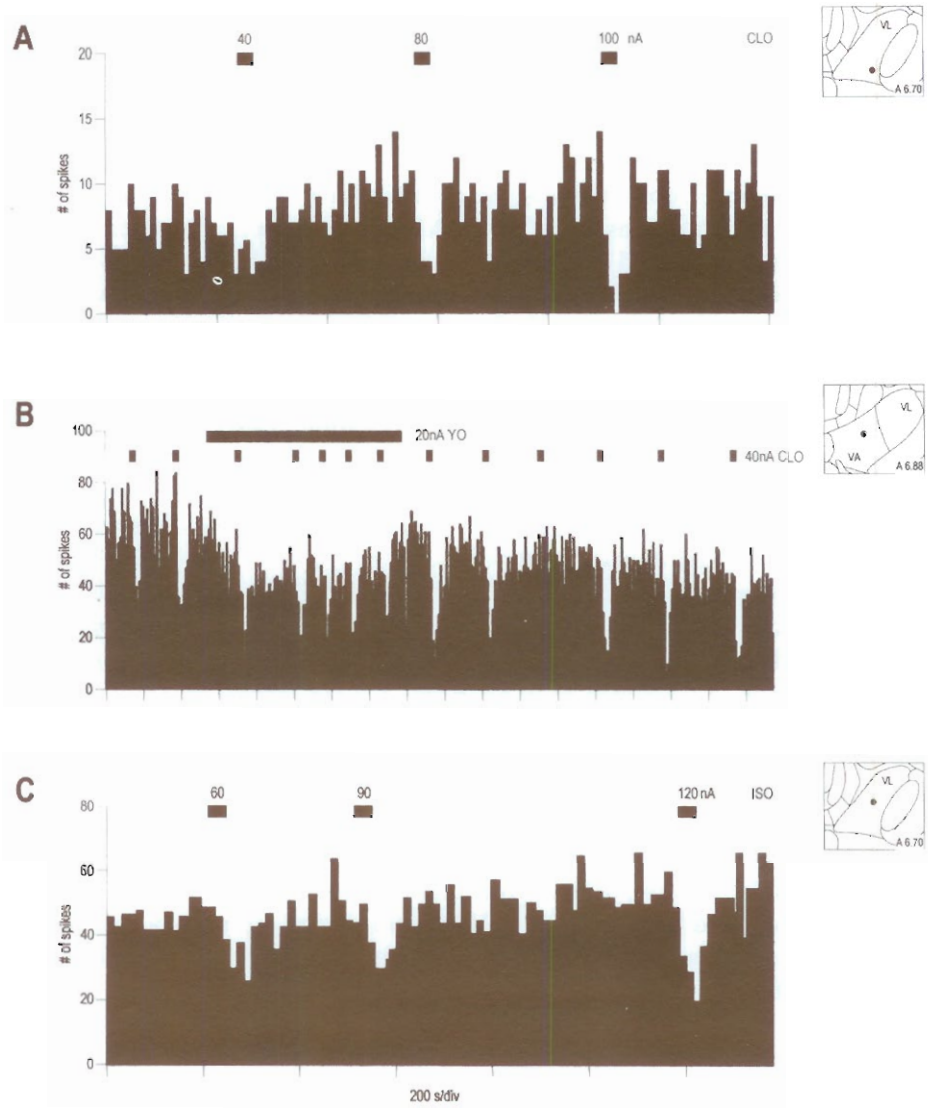


Fig. 3. - Ratemeter records (10-s bin) illustrating the responses of three thalamic neurons in ventrolateral (VL) and ventral-anterior (VA) nuclei to application of noradrenergic receptor agonists. A schematic location of recording sites is indicated in the respective insets.

A) The histogram shows the mean firing rate of a VL neuron in the course of three successive applications of alpha$_2$ noradrenergic receptor agonist clonidine (CLO) ejected at increasing current intensities, indicated above the horizontal bars of the ejection periods. The drug induced short lasting depressions of the firing rate that appeared dose-dependent.

B) CLO-evoked depression of the firing rate, recorded in a VA thalamic neuron, and partially antagonized by application of alpha$_2$ noradrenergic receptor antagonist yohimbine hydrochloride (YO).

C) Dose-dependent depression of the firing rate induced in a VL neuron by application of beta receptor agonist isoproterenol (ISO) ejected at increasing current intensities.

neurons and appeared sporadic in VA (Fig. 1, d1-d2). After recovery following a LLI, a response could not be evoked again before 20 minutes or more. An opposite type of response to 5-HT application, an increase of the firing rate, was recorded in 9 thalamic units, 3 located in VA and 6 in VL.

Parameters of the three types of responses (LLI, SLI, excitations) in the two nuclei are reported in Table 1, examples of responses are shown in Figure 4.

Application of the 5-HT_{1A} receptor agonist 8-OH-DPAT, tested in 24 thalamic neurons, induced only depressions of the firing rate in 12 out 14 VA units and 5 out of 10 VL units. The durations of 8-OH-DPAT-evoked effects, clustered around 120 s (Fig. 1, c2), were classified as above as LLI or SLI. In VL, half of the tested units were unresponsive to 8-OH-DPAT application whereas the responses evoked in the remaining neurons were LLI (Fig. 1, d1). In contrast, LLI and SLI appeared in the same percentage in VA (Fig. 1, d2) and the C value of 8-OH-DPAT-evoked LLI was smaller ($P = 0.019$) in VL than in VA (Table 1). However, responses to 8-OH-DPAT had a smaller magnitude M in VL and smaller duration D in VA ($P = 0.004$ and $P = 0.040$ respectively) than those evoked by 5-HT application. In two experiments the 5-HT_{1A} receptor antagonist nan-190 was tested in 3 neurons, 2 in VA and 1 in VL. These were inhibited by application of 8-OH-DPAT. A partial antagonism (60%, 65%, 69%) was verified in each case.

Application of the 5-HT_2 receptor agonist ALPHA-MET-5-HT, tested in 22 thalamic neurons, induced depression of the firing rate in 12 units and enhancement in 5 units. The frequency distribution of duration (Fig. 1, c3) shows no clustering of responses. Similar percentages of responses to ALPHA-MET-5-HT were observed in VA and VL with the exception of SLI that was sporadic in VA (Fig. 1, d1-d2). However, in VA the responses to ALPHA-MET-5-HT appeared less intense than to 5-HT. In fact, LLI evoked by ALPHA-MET-5-HT in VA had M and D values smaller ($P = 0.030$ and $P = 0.0003$ respectively) than those induced by 5-HT application. Examples of neuronal responses to application of 5-HT agonists are shown in Figure 5.

Application of ketanserin, a 5-HT_2 receptor antagonist, was tested in 4 neurons (3 inhibited and 1 excited by alpha-met-5-HT) and induced a total block of the excitatory response and of an inhibitory response, and partially antagonized (65% and 68% respectively) the remaining two inhibitions.

Modes of firing

The neuronal discharge pattern before and after application of either amine was studied in units whose firing rate was modified by NA or 5-HT application as well as in units whose firing rate was not modified. The analysis of frequency distributions of ISIH over consecutive periods of activity (see *Data Analysis*), showed that if the background firing was characterized by bursting, both NA and 5-HT ejection desynchronized neuronal activity independently of their effect on the firing rate. However, this modification of discharge pattern usually only lasted for less than 1 min (Fig. 6a).

In thalamic units characterized by tonic single spike discharge, no significant pattern modification appeared if NA application was ineffective on the firing rate or

induced LLI. In contrast, in 6 of the units exhibiting a SLI, the discharge pattern was also modified in response to NA ejection as the slope of the ISIH appeared significantly different from the control (Fig. 6b). Unfortunately, this type of analysis could not be extended to all units because the firing rate of some neurons, mostly during the response, was too low to provide a valid ISIH.

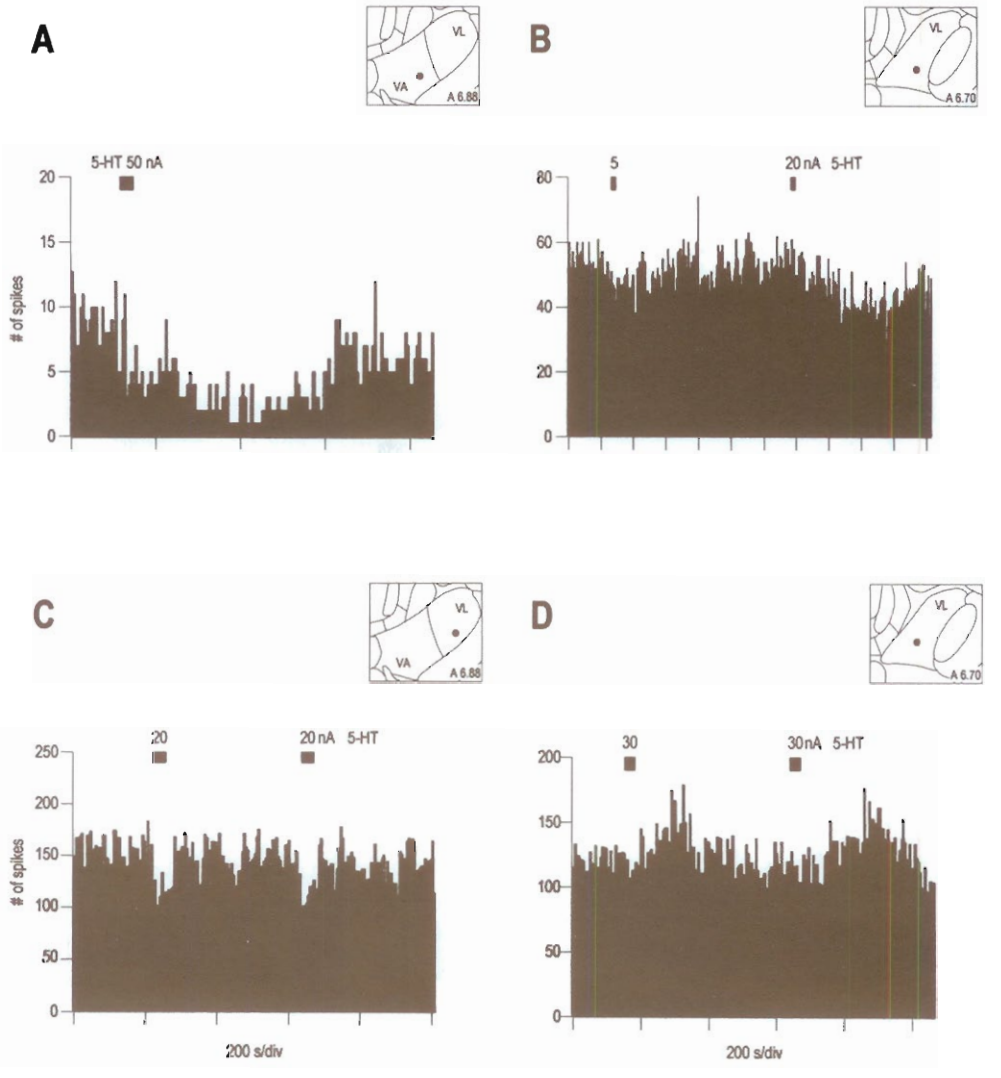


Fig. 4. - Examples of various types of responses to application of 5-hydroxytryptamine (5-HT), recorded in four thalamic neurons, one belonging to ventral-anterior (VA) nucleus and three to ventrolateral (VL) nucleus, whose location is schematically indicated in the respective insets.
Ratemeter records (5-s bins) referred to these neuronal units show the most frequent effect, a long-lasting inhibition recorded in a VA (A) and in a VL unit (B) respectively, and examples of less frequent responses such as short-lasting inhibitions (C) and excitatory effects (D), both mostly observed in VL.

The discharge pattern of thalamic units that showed short- or long-lasting inhibitions following 5-HT application appeared unmodified during and after the drug application. In contrast 4 of the units excited by 5-HT also modified their discharge pattern (Fig. 6c). Finally, significant and reversible modifications of the firing pattern were observed in 14 out of 20 neurons whose firing rate was not altered by 5-HT application. The latency of these effects ranged from a few seconds to 8 min after 5-HT application, and lasted from 30-60 s to more than 30 minutes. The most

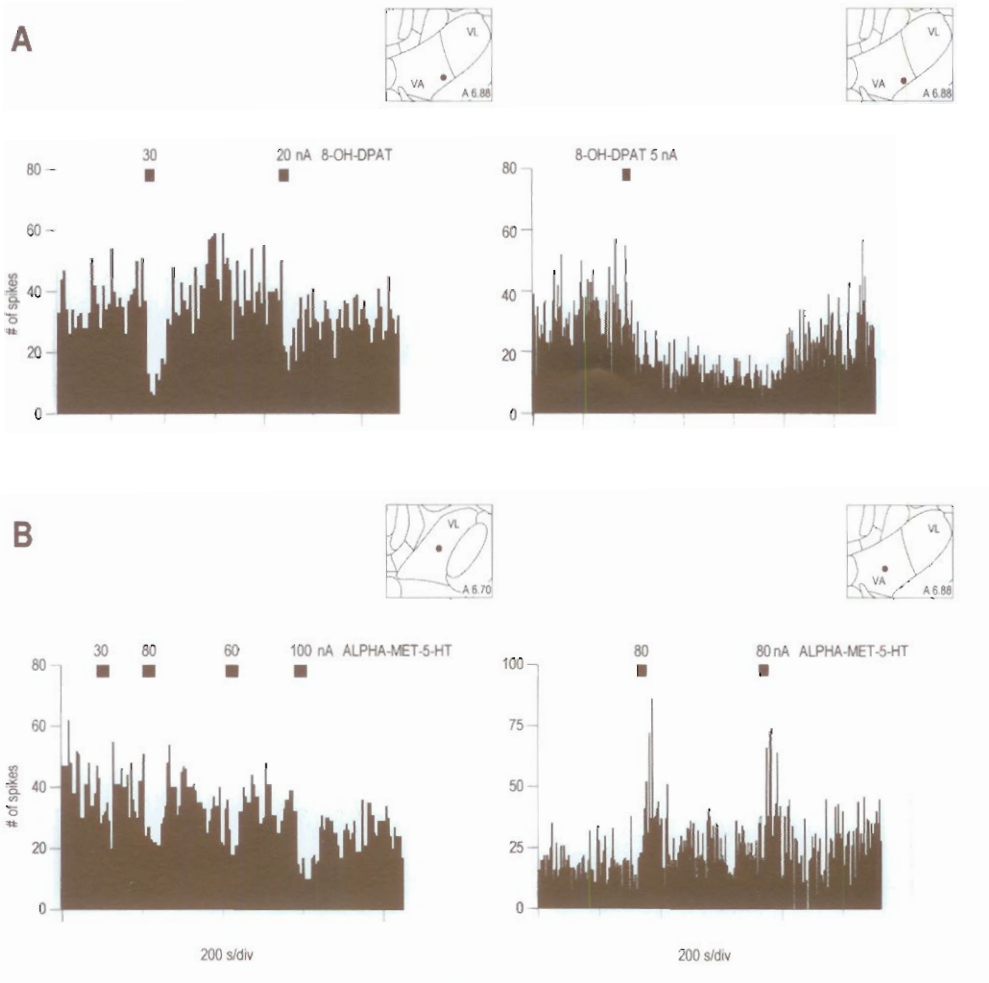


Fig. 5. - Responses of thalamic ventrolateral (VL) and ventral-anterior (VA) neurons to application of the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) and 5-HT$_2$ receptor agonist alpha-methyl-5-hydroxytryptamine (ALPHA-MET-5-HT).
A. Ratemeter records (5-s bins) showing examples of the two types of inhibitory responses (short-lasting on the left and long-lasting on the right) induced by application of 8-OH-DPAT in two VA neurons. B. Ratemeter records (5-s bins) showing short-lasting inhibitory responses of a VL unit (left) and excitatory responses of a VA unit (right) to ALPHA-MET-5-HT application.

frequent modification in the pattern was a rearrangement in the percentage of intervals included between 10-200 ms with respect to longer and shorter intervals. In some cases a significant change in the discharge pattern appeared after the second but not the first 5-HT application (Fig. 6d). No significant difference was detected between VA and VL.

DISCUSSION

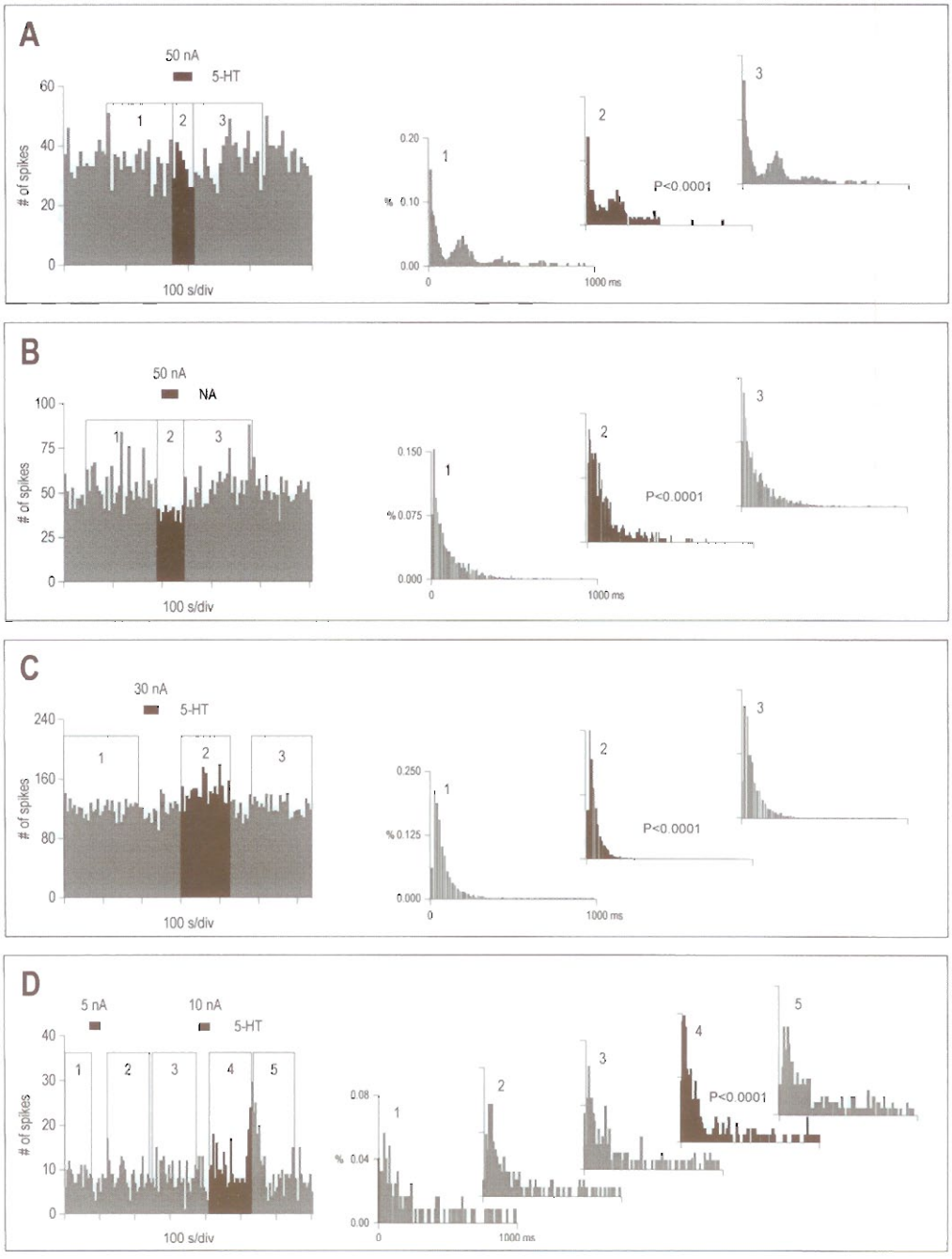
These results demonstrate that NA and 5-HT can modify the firing of a high percentage of neurons in VA and VL thalamic nuclei. The action of neuroamines is mostly depressive in both structures, but some differences exist between the responses evoked by NA and those induced by 5-HT application.

No previous systematic study has been carried out on the effects of amines on neurons of the VA-VL complex. Several studies on the effects of NA (44) and 5-HT (42) have been carried on other thalamic nuclei, mostly on the dorsal lateral geniculate nucleus in guinea pig and cat, and extended to monkey and ferret with regard to 5-HT-evoked responses (45, 46). Results presented in these papers indicated a depolarising action of both amines due to a decrease in potassium conductance (44) and an enhancement of a mixed Na+/K+ current, activated by hyperpolarization (55). The authors hypothesized that these effects induced the suppression of rhythmic burst firing and a switch to the single spike firing mode. However, later work (45, 46) gave evidence of strong hyperpolarizing effects of 5-HT.

Our results indicate that in the VA-VL complex both amines induce a depression of the firing rate in a majority of cases in addition to suppression of burst firing. With regard to 5-HT-evoked responses, these data are in line with those detected in other thalamic nuclei in vitro (45) where the usual response to an increase of 5-HT levels was an inhibition, with the exception of lateral geniculate neurons that were mostly depolarised by 5-HT. Furthermore, in vivo stimulation of the dorsal raphe (26, 75) also evoked long-latency and long-lasting inhibitory responses in this nucleus, in many ways similar to the long-lasting inhibitions we describe in both VA and VL.

In contrast, the NA-induced inhibitions we recorded in thalamic motor nuclei are different from NA responses described in other thalamic nuclei, including the lateral geniculate nucleus, in which neurons were depolarised by direct application of NA in vivo (60) and in vitro (44) and upon stimulation of locus coeruleus (23, 60, 61).

In fact, at least one interspecies difference was observed in the pigeon, where NA application induced inhibition in the homolog of the geniculate nucleus (19). Significant species-dependent differences, and even opposite neuronal behaviours, were also recorded in the pulvinar nucleus of the thalamus in response to 5-HT application (46). However, no previous data are available on the effects of NA on the neuronal firing rate in the VA-VL complex of any species, so we cannot comment on whether the hyperpolarizing action of NA recorded in the VA-VL complex of the rat *is peculiar to this species, or a common characteristic of thalamic motor nuclei*.


A
50 nA
5-HT
# of spikes
100 s/div
P<0.0001
1000 ms
B
50 nA
NA
# of spikes
100 s/div
P<0.0001
1000 ms
C
30 nA
5-HT
# of spikes
100 s/div
P<0.0001
1000 ms
D
5 nA
10 nA
5-HT
# of spikes
100 s/div
P<0.0001
1000 ms

There is no reason to suppose that all thalamic neurons, or even all thalamocortical neurons, are influenced by the amines in the same way. How representative the action of an amine in a thalamic nucleus is with respect to other thalamic nuclei is a question that was carefully considered by Monckton and McCormick (45). They studied the action of 5-HT and recorded a depolarizing effect of the amine in some lateral geniculate neurons and a diffuse hyperpolarizing response in ten different thalamic nuclei, with a response magnitude varying greatly across and within nuclei (45). However, the amounts of NA and 5-HT vary between thalamic nuclei and even within the same nucleus (54), suggesting that the function of these amines may not be the same in all thalamic nuclei.

Depressions of the firing rate evoked by amines in thalamic motor nuclei in vivo do not give unequivocal information on membrane mechanisms activated by NA or 5-HT in the VA-VL complex. In fact our experimental data suggest a combination of various direct and indirect effects induced by local modification of the 5-HT level (including a variety of pre- and post-synaptic mechanisms) but do not allow identification of single contributions. Indirect inhibition mediated by the activation of GABAergic interneurons was proposed by McCormick (41) to explain the discrepancy between the decrease in spontaneous firing rate detected in the thalamic geniculate nucleus in vivo in the presence of 5-HT (38), and depolarization recorded in vitro (42).

On the other hand, functional characteristics of the VA-VL complex suggest that these nuclei can be regarded as motor nuclei rather than relay or unspecific thalamic nuclei. The background firing rate of almost all the motor structures depends on the levels of NA and 5-HT, with the exception of *globus pallidus*, whose sponta-

Fig. 6. - Examples of firing pattern modifications induced in thalamic neurons belonging to ventral-anterior (VA) and ventrolateral (VL) nuclei by application of noradrenaline (NA) and 5-hydroxytryptamine (5-HT).

Each ratemeter record shows the firing rate of a unit before, during and after the ejection periods indicated by the horizontal bars above the histograms. Numbered histograms on the right of each ratemeter record are normalized frequency distributions of interspike intervals, each referred to the periods indicated in the corresponding numbered and shaded sections of the ratemeter record. The black histogram in each group is significantly ($p < 0.0001$) different in shape from the number 1 of the same group that is referred to the firing during a period preceding the amine ejection (control). Therefore during the period of time indicated in black on the ratemeter record the firing pattern of the studied unit was significantly different from control.

A. In a VL unit characterized by bursting (histogram 1) a 5-HT ejection did not modify firing rate, but partially desynchronized its firing (histogram 2). The effect lasted as long as the application time of the amine (30 s).

B. In a VA unit, NA application induced a depression of the mean firing rate and a significant change in the shape of the interval distribution (histogram 2). Both effects were reversible and had the same duration.

C. Following 5-HT application this VL unit enhanced its firing rate and modified its discharge pattern. Both effects had long latency and similar duration.

D. In this VL unit, 5-HT application had no effect on the firing rate at any current intensity. Although ejection at a low current intensity (5 nA) was followed by no significant pattern modification (histograms 2-3), application at a higher intensity (10 nA) did modify the pattern of discharge for about 2 min (histogram 4).

neous firing appears scarcely influenced by the amines (57). However, the effects induced by the amines on neuronal firing are similar in some structures and opposite or different in others. For example, both amines evoke the same effect, an increase of the firing rate, in subthalamic neurons (3, 21) and in spinal (1, 72) as well as hypoglossal (7) motoneurons. However, different responses are evoked by the two amines in a variety of functionally disparate motor structures where NA induces quite stereotyped responses (inhibitory with one exception (9)), while 5-HT evokes effects which vary in sign and intensity. Such different behaviour is observed in motor cortex (22, 39), *substantia nigra* (9, 67), red nucleus (12, 33), vestibular complex (30, 31, 32, 34), cerebellar cortex (51, 73) and cerebellar nuclei (17).

In the VA-VL complex, NA modified the firing rate of a higher percentage of neurons than 5-HT. However, the latter could modify the discharge pattern even when it did not affect the firing rate. Furthermore, NA evoked more short- than long-lasting depressions of the firing rate in VL and the same number of short- and long-lasting responses in VA. In contrast, 90% and 75% of inhibitions evoked by 5-HT in VA and VL nuclei respectively were long-lasting effects.

NA-evoked responses

Responsiveness and sign of responses to NA were similar in VA and in VL neurons. Short-lasting inhibitions (SLI), together with modifications of the firing pattern, were more numerous in VL and were also characterized by higher magnitudes than in VA, in spite of the low concentration of the amine detected in VL and other important thalamic nuclei, at least in man (54). In contrast, the content of NA was higher in VA, where both SLI and LLI appeared in equal number.

The differences observed between VA and VL with regard to percentages and magnitudes of the two types of inhibitory responses (short and long lasting) to NA could be due to an uneven distribution of noradrenergic receptors in thalamic nuclei. Our results demonstrate that alpha$_2$ and to a lesser extent, beta receptor agonists mimicked SLI. Both types of receptors are present in the thalamus (2, 76) and thought to be involved in responses to NA in some thalamic nuclei (11, 43). Thus it is plausible that alpha$_2$ and beta receptors also make a significant contribution to the NA-evoked SLI in thalamic motor nuclei. Furthermore, alpha$_2$ and beta receptors were implicated in the NA-evoked effects observed in cerebellar nuclei (17) and in neural structures such as the red nucleus and the vestibular complex that, like VL, are targets of cerebellar output (12, 13, 30).

In thalamic nuclei where NA application induced depolarization, these effects were mediated by alpha$_1$ and, to a lesser extent, beta adrenergic receptors (42). In VL, application of alpha$_2$ as well as beta receptor agonists induced smaller effects than those induced by NA. It is likely that two or more mechanisms mediated by different receptors contribute to the SLI evoked by NA application. Involvement of alpha$_1$ receptors in the responses cannot be excluded, but a better insight into the role of each type of noradrenergic receptor could be given by in vitro recordings.

The noradrenergic agonists tested on VA-VL neurons were unable to mimic LLI evoked by NA. In thalamic nuclei, the organization of noradrenergic terminations

and NA-containing boutons extends not only to the soma but also to distal and proximal regions of the dendritic tree (16, 36, 71). This organization may contribute to long-lasting effects and again, a possible involvement of alpha$_1$ noradrenergic receptors cannot be excluded either by a direct hyperpolarizing action or an indirect depolarization of interneurons.

Regardless of the mechanism, the responses to NA had larger magnitudes and appeared more specific (SLI and firing modifications) in VL than in VA. As cerebellar and pallidal projections to thalamic motor nuclei are at least partially segregated to VL and VA respectively (4, 63, 70) with only a limited overlap zone (50), this result could suggest a stronger noradrenergic control on the cerebello-thalamo-cortical than on the striato-pallido-thalamo-cortical motor pathway. On the other hand, the inhibitory action of NA on cerebellar dentate neurons (the main excitatory input to VL), is weak (17) and in contrast the strong excitation induced by NA on the subthalamic nucleus (3, 21) enhances the pallidal inhibitory input delivered to VA neurons through the indirect path. So, even if NA is able to modulate both cerebello thalamo-cortical and striato-pallido-thalamo-cortical motor circuits, its action on the former is stronger at the thalamic level and on the latter at the subthalamic level.

In contrast with the well accepted desynchronizing action of NA on thalamic neuronal firing, the final effect induced on motor performance by noradrenergic modulation of thalamic motor nuclei remains uncertain. A hypothesis advanced on the function of NA in thalamic nuclei (11) is that the amine could operate like a high-pass filter that enhances the high frequency effects by depressing low-frequency responses and the background firing rate. This hypothesis needs to be tested by analyzing the action of NA on the neuronal responsiveness to specific thalamic inputs.

5-HT-evoked responses

These data suggest that 5-HT was less effective than NA in modifying the firing rate of VA-VL neurons, the most frequently observed response to 5-HT being long-lasting depressions (LLI) of firing rate. Furthermore, 5-HT induced a short-lasting desynchronization in units discharging in bursts and modifications of the pattern in some single spike discharging units. The latter effect was limited to neurons that were excited or whose firing rate was unmodified during 5-HT application.

The long lasting inhibitory responses to 5-HT that we observed in a majority of motor ventral thalamic neurons are in line with results obtained in other thalamic nuclei where, with the exception of lateral geniculate, the action of 5-HT was strictly inhibitory (45), probably with two mechanisms, one direct and another involving an enhancement of GABA release by interneurons. It seems likely that the units in thalamic motor nuclei that exhibited excitatory responses to 5-HT were interneurons.

The prevalence of LLI may be explained by the loose network of serotoninergic fibers that generally make intimate but unspecialized membrane associations not only with dendrites and somata of relay cells, but also with presynaptic terminations of interneurons (28, 52, 71), thus suggesting some kind of complex modulation of

the release of other transmitters (35). In fact, in ventrobasal thalamus 5-HT modulates responses to excitatory amino acids and facilitation can be reversed to inhibition by enhancing the ejection currents (18).

Although the action of NA is more focused on VL than on VA, modulation exerted by 5-HT on neuronal firing rate appears more homogeneous in both nuclei, but to ascertain the role of this action on motor control appears problematic. Given the direct and indirect mechanisms involved, authors who studied the effect of 5-HT in various specific and non-specific thalamic nuclei concluded that "serotonergic inputs act differentially across the thalamus in a complex manner" (45). In motor thalamic nuclei, differences in response appear to be less marked. However, some differences between VA and VL can be found with regard to the receptors that mediate the effects induced by 5-HT.

The 5-HT evoked inhibitions were mostly mediated by 5-HT_{1A} receptors. However, they appeared more involved in VA, where their activation mimicked both LLI and SLI, than in VL where they only appeared to be involved in LLI. Furthermore, in VL the 5-HT_{1A} receptor agonist was ineffective in half of the tested neurons. Inhibitory responses mediated by 5-HT_{1A} receptors are usually recorded in motor structures such as cortex (15), cerebellar nuclei (17), red nucleus (33) and vestibular complex (31, 32, 34). These receptors are diffusely distributed in the thalamus (5) and are responsible for the inhibitory effects induced by 5-HT in various thalamic nuclei (45). Therefore it is possible that they mediate 5-HT induced inhibition in VA and at least some inhibitory responses to 5-HT in VL. As the effects evoked by 5-HT_{1A} receptor agonist had smaller magnitudes and durations than responses to 5-HT, involvement of other receptors in the inhibitory effects is probable.

In fact, 5-HT_2 receptors are implicated in both inhibitory and excitatory responses to 5-HT in both VA and VL. In motor structures, these receptors mediate increases of the neuronal firing rate in the vestibular complex (31, 32, 34), red nucleus (33) and facial motoneurons (58) as well as decreases in firing rate in the cerebellar nuclei (17). It is likely that inhibitions mediated by 5-HT_2 receptors are an indirect effect of the excitatory action, and therefore of dendritic GABA release, which these receptors are known to evoke in thalamic interneurons (48).

Pattern variations induced by 5-HT have various characteristics. In addition to interrupting bursting, 5-HT application modifies the discharge pattern in some cells discharging single spikes. The former effect could indicate a serotoninergic modulation related to the vigilance level, and in fact a desynchronizing influence of neuroamines has been described in studies on thalamic neurons in vitro (55).

In some units 5-HT induced an increase of the mean firing rate together with a modification of the background discharge pattern, effects that suggest a more specific short-term modulation exerted by 5-HT on the output of some thalamic neurons, perhaps interneurons.

The modifications of discharge pattern that appear after a long latency in units whose mean firing rate is uninfluenced by 5-HT are harder to interpret. In fact the long and variable latency of some of these effects raises some doubt as to the cause-effect relationship of the response. A tentative hypothesis is that the modulation of

the discharge pattern in the presence of 5-HT appears only when the amine interacts with another neurotransmitter, released by some specific or unspecific input. A study of the interaction of 5-HT with other neurotransmitters such as glutamate or GABA in thalamic motor nuclei could provide useful information. In any case, this type of modulation at long latency was never observed following NA application.

On the whole, the two amines, although they induce similar desynchronizing effects on the bursting pattern, show quite different actions on the firing rate and discharge pattern of VA and VL neurons, suggesting that NA and 5-HT exert differential modulation of motor activity by acting on single spike neuronal firing in thalamic motor nuclei.

SUMMARY

The effects induced on neuronal firing by microiontophoretic application of the biological amines noradrenaline (NA) and 5-hydroxytryptamine (5-HT) were studied "*in vivo*" in ventral-anterior (VA) and ventrolateral (VL) thalamic motor nuclei of anaesthetized rats. In both nuclei the amines had a mostly depressive action on neuronal firing rate, the percentage of units responsive to NA application (88%) being higher than to 5-HT (72%). Short-lasting (less than 2 min) and long lasting (up to 20 min) inhibitory responses were recorded, the former mostly evoked by NA and the latter by 5-HT ejection. In some cases 5-HT application had no effect on the firing rate but modified the firing pattern. NA-evoked responses were significantly more intense in VL than in VA neurons.

Short-lasting inhibitory responses similar to NA-induced effects were evoked by the alpha$_2$ adrenergic receptor agonist clonidine and to a lesser extent by the beta adrenergic receptor agonist isoproterenol. Inhibitory responses to 5-HT were partially mimicked by application of the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) and of the 5-HT$_2$ receptor agonist alpha-methyl-5-hydroxytryptamine (ALPHA-MET-5-HT). The latter evoked excitatory responses in some cases. Both 5-HT agonists were more effective on VA than on VL neurons. The effects evoked by agonists were at least partially blocked by respective antagonists.

These results suggest that although both 5-HT and NA depress neuronal firing rate, their effects differ in time course and in the amount of inhibition; besides aminergic modulation is differently exerted on VA and VL.

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