bioelectric processes were measured from the excised superior cervical ganglion of the rat, mounted on glass microelectrodes, by means of an oscilloscope (Tektronix, 561, 3A3). If immersed in Krebs' solution, the excised ganglion retained its bioelectric functions intact over 12 h. If immersed in glucose-free Krebs' solution, presynaptic functions and synaptic transmission vanished within 4 h, but postsynaptic spike generation remained intact for about 24 h. Presynaptic shocks of various intensities, generated by a transistor based stimulator were delivered across a stimulus isolation unit to presynaptic platinum microelectrodes at varying time intervals. Postsynaptic spikes were generated in preparations immersed in glucose-free Krebs' solution after inactivation of the presynaptic neurons, by acetylcholine (0.01–1 µg/ml of bathing solution). All preparations were presensitized with physostigmine salicylate (5 µg/ml bathing solution). Statistical significance of the observed differences of correlation coefficients were ascertained by Student's t-test.

Results. 2 supramaximal presynaptic shocks, delivered at 50-msec-intervals, generated maximum homosynaptic potentiation. The length of potentiation exceeded, at times 1 sec. Protein (peptides, glycopeptides) extracts of cholinergic vesicle contents of either the rat brain or the electric organ increased the amplitude and the length of potentiation (table, figure 1). In preparation with inactivated presynaptic neurons, these extracts (added to the bathing fluid) increased the amplitude of postsynaptic spikes (generated by acetylcholine), and significantly increased the length of the generated postsynaptic spike trains in a dose-dependent manner (table, figure 2). Heat denatured extracts lost their ability to affect postsynaptic spike generation by both endogenous (presynaptic stimulation) and exogenous acetylcholine. The latency of the first postsynaptic spike was about 10 msec shorter than the onset of potentiation by the extracts. In the concentration used, the extracts alone did not initiate postsynaptic spikes.

Discussion. Presynaptic cholinergic vesicles contained an agent that possessed the minimum necessary properties of a 'modulator'[13] of acetylcholine effects, e.g.: 1. In absence of acetylcholine the extract did not generate postsynaptic spiking. 2. The extract potentiated the effect of both exogenous and endogenous (released during presynaptic stimulation of cholinergic neurons) acetylcholine. 3. Postsynaptic effects outlasted presynaptic stimulation (release of acetylcholine). 4. Postsynaptic cholinergic effects were smoothened (spike amplitude decreased gradually). 5. This madulator effect was dose-dependent. 6. The modulator was either a protein or its derivative (peptides, glycopeptides).

The modulator effect seemed to be multidetermined, e.g.: 1. Adsorption on this protein may facilitate transsynaptic acetylcholine transport, and may delay its hydrolysis. 2. Gradual release may smoothen the postsynaptic effects of acetylcholine. 3. Since the latency of the onset of modulation exceeded the latency of the first postsynaptic spike, postsynaptic processes seemed also to contribute. 4. Because of Ca^{2+}-transport proteins also occur in the vesicle content[26], promotion of transsynaptic Ca^{2+}-transport may facilitate the effects of Ca^{2+} on pre- and postsynaptic de- and repolarization[27]. It has not yet been ascertained which of the 22 known proteins contained in cholinergic vesicles are responsible for the modulator effect. Soller et al.[18] suggested that the acidic lipoproteins are carriers binding the putative neurotransmitter. Transport properties were also attributed to glycoproteins[22]. Even though glycoproteins prevail in membranes, glycopeptides may also occur in the vesicle fluid[22]. During presynaptic stimulation cholinergic vesicle walls is believed to fuse with the terminal membrane of the presynaptic neuron, and verse part of their content into the synaptic cleft. Musick and Hubbard[28] did collect proteins from the effluent of stimulated mouse phrenic nerve. In addition to acetylcholine, the fluid ejected from these vesicles contained proteins (or their derivatives [peptides, glycopeptides]) with modulator effects. Generation of homosynaptic (posttetanic) potentiation is inherent in the different latency-requirements of acetylcholine and the modulator to initiate postsynaptic effects. The longer latency of the onset of modulator effect prevents the modulator from changing the first (or early) postsynaptic spikes.

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Monosynaptic inhibition of thalamic neurons produced by stimulation of the substantia nigra

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Summary. Electrical stimulation to the substantia nigra (Pars reticulata) produced a monosynaptic inhibition of the neurons of the ventromedial nucleus of the thalamus in anesthetized cats.

One of the major outputs of the basal ganglia is the pallidothalamic pathway. Uno and Yoshida[2] have demonstrated that neurons in the rostroventral portion of the thalamic nucleus ventralis lateralis (VL) receive monosynaptic inhibition from the pallidal nucleus of the cat. Nigro-thalamic fibres form another output system from the basal ganglia[3–8]. In this paper we show that stimulation of the substantia nigra monosynaptically produces IPSPs (inhibitory postsynaptic potentials) in neurons of the ventromedial nucleus (VM) of the thalamus.

Materials and methods. Cats were anesthetized with sodium pentobarbital (30 mg/kg). Platinum-iridium needles of 0.2 mm diameter, insulated except for the very tip, were used as stimulating electrodes. 6 needles were combined and inserted stereotaxically into the SN[9]. Stimulating pulses were applied between 2 neighboring SN-

electrodes, and the contralateral brachium conjunctivum (BC) was also stimulated through a pair of electrodes. Stimulating positions were marked by electrolytic lesions. Recording glass microelectrodes were inserted into the thalamus from a lateral angle of 30° after suction removal of the overlying cortical structures. Recording sites were confirmed in every experiment by determining the histological location of spots made by dye electrophoresis from recording pipettes filled with 2 M potassium acetate saturated with Fast Green FCF.

Results and discussion. In the present study, 75 cells were recorded intracellularly or extracellularly in the region of VL and VM of the thalamus. When microelectrodes penetrated thalamic cells which were histologically confirmed to be located in VL, BC stimulation produced short latency EPSPs (excitatory postsynaptic potentials) as illustrated in the 2nd shock of figure 1A. The EPSP was confirmed to be evoked monosynaptically, and the thalamic cells were VL relay cells of the cerebello-thalamo-cortical pathway, as described in the previous paper[10]. Neither EPSPs nor IPSPs were evoked in these cells by SN stimulation with stimulus intensities up to 3 mA (1st shock in figure 1A).

On the other hand, very weak SN stimulation (less than 0.3 mA) produced short latency hyperpolarizing potentials in thalamic cells located ventral to BC-VL relay cells (figure 1C). BC stimulation produced no EPSPs in these cells (figure 1B and also 2nd shock in C). The SN-evoked hyperpolarization was of simple configuration with a time-to-peak of 3–4 msec and a duration greater than 80 msec. Because the hyperpolarizing potentials could easily be reversed to depolarizations by injecting chloride ions electrophoretically into the cells through the microelectrode (figures 1E and F), they were identified as IPSPs. The inhibitory nature of this hyperpolarization could readily be demonstrated by suppression of spontaneous discharges of the thalamic neurons (figure 1D).

The latency of the IPSP was measured to the apparent onset of the negative-going phase by superimposing juxtacellularly recorded field potentials. The latency thus measured was as short as 1.2–2.0 msec (mean, 1.58 msec) in 25 neurons. When the nigrothalamic fibre was stimulated by the electrode placed in the middle between the SN and the recording site, the latency of IPSP became apparently shorter (2nd shocks in figures 1E, F and G). The plot of latency of the IPSPs versus stimulating-recording distance is made in 5 cases and illustrated in figure 1H. When the straight line is extrapolated to 0 distance in each case (the recording point), the Y-intercept

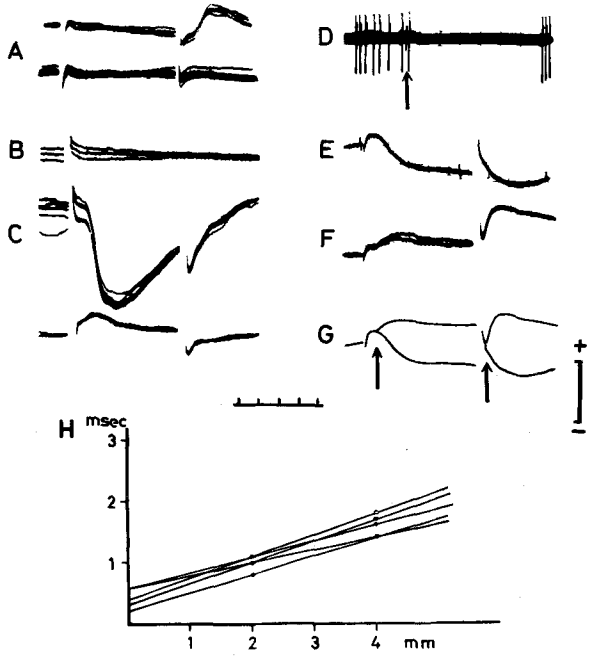

Fig. 1. Responses of VL and VM thalamic neurons. *A* Monosynaptic EPSPs evoked in a VL-neuron by stimulation of BC (2nd shock). The first shock was to the SN. *B* Intracellular recording of a VM-neuron. BC stimulation did not evoke any response in this cell. *C* SN-evoked IPSPs (1st shock) in the same cell as in *B*. Second shock indicates BC stimulation, producing no transsynaptic potentials. Lower traces in *A* and *C*: juxtacellular field potentials. *D* Inhibition of extracellularly recorded spike discharges of a VM-neuron following SN stimulation. Upward arrow indicates stimulus artifact. *E* IPSPs produced by stimulations to SN (1st shock) and the midpoint (2nd shock) between SN and recording site. *F* Same as in *E*, but IPSPs were reversed to depolarizing potentials by iontophoretic injection of Cl^- into the cell through the recording electrode. *G* Superimposed tracing of *E* and *F*. Upward arrows indicate onset of IPSPs. *H* Latencies of IPSPs (ordinate) plotted against distance (abscissa) between stimulating and recording sites in 5 cells. Voltage scales are 1 mV for *A–C*, 0.5 mV for *D* and 2 mV for *E–G*. Time scales are 2 msec for *A–C* and *E–G*, and 10 msec for *D*.

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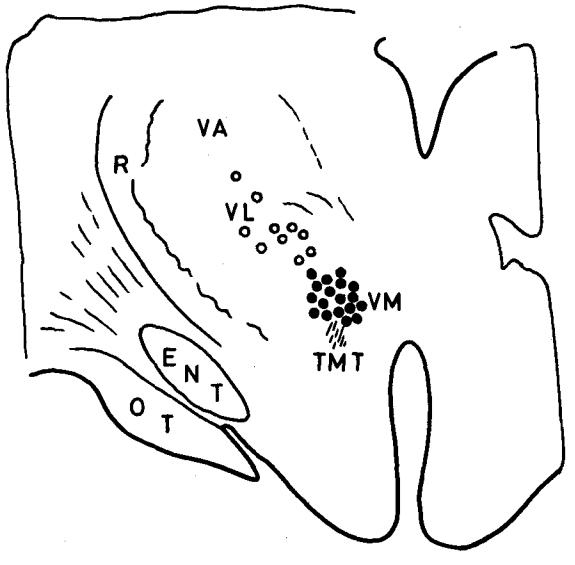

Fig. 2. Distribution of thalamic neurons in VL-VM region. The diagram represents a frontal section of the thalamus. Open circles (○) show the location of neurons in which monosynaptic EPSPs were produced by stimulation of the contralateral BC, but there were no short latency responses to SN-stimulation. Filled circles (●) show the location of neurons in which monosynaptic IPSPs were produced by stimulation of SN, but which were not activated by BC-stimulation. *VA* nucleus ventralis anterior; *VL* nucleus ventralis lateralis; *VM* nucleus ventralis medialis; *R* nucleus reticularis; *TMT* tractus mamillothalamicus; *ENT* entopeduncular nucleus; *OT* optic tract.

is 0.2–0.6 msec. The value is in good agreement with that obtained in other monosynaptic pathways[11]. It is, therefore, concluded that the SN-evoked IPSP in the thalamic cells were produced monosynaptically.

The threshold stimulus intensities for producing IPSPs in the cells studies were measured at various stimulating sites in and around SN. The positions with the lowest threshold, which ranged from 0.05 to 0.3 mA, were distributed in a localized area from the middle to the lateral part of SN; this corresponded approximately to the pars reticulata of the substantia nigra.

The histological locations of the impaled neurons were determined on serial frontal sections of the thalamus and summarized on the representative plane illustrated in figure 2. 10 cells which received monosynaptic EPSPs from BC, but without SN-evoked IPSPs, were located dorsally within the thalamus a location corresponding to VL. On the other hand, 18 cells which received monosynaptic IPSPs from SN and were not activated by BC stimulation, were located in an area ventral to VL, corresponding to the ventromedial (VM) nucleus of the thalamus.

It is agreed by many authors that the nigrothalamic pathway originates in the pars reticulata of SN[4,5,7]. That VM is an area in which the nigrothalamic projection terminates has been suggested with reservation by Rinvik[7] and more firmly by Mehler (personal communication). Our results show that the nigro-VM-pathway is inhibitory in nature and that VM-neurons which receive the inhibition from SN do not receive converging inputs from the cerebellum, indicating that cerebellar and nigral influences do not converge directly on single thalamic neurons[12].

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Response time constants in snail neurones[1]

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Summary. Neurones of *Helix aspersa* were excited and strength/duration curves plotted for an active and a silent cell. Experimental response latencies were longer than predicted by the theoretical relationship at low currents. The time constant of excitation was longer in the silent than the active cell.

The time constant of the molluscan neuronal soma, as calculated by measuring the electrotonic response of the cell to the application of a square current pulse[2–5], varies between 20 and 250 msec. This is 3–50 times longer than the time constants of mammalian neurones of different kinds measured in the same way. The time constant value, τ, calculated from strength/duration curves, obtained when motor neurones are depolarized and excited, is smaller than expected because of the effect of a subliminal local response[6–8]. When plots of strength/response time were made in snail neurones, we found that much longer, rather than shorter, response time constants were obtained. Furthermore, there was a deviation of the experimental values from the predicted curve.

Isolated brains from *Helix aspersa*, the common snail, were perfused with a physiological saline containing 80 mM NaCl, 4 mM KCl, 5 mM $MgCl_2$, 7 mM $CaCl_2$, 5 mM Tris-Cl corrected to pH 7.8, 10% glucose, 50 units/l each penicillin and streptomycin (Gibco), 0.4 ml/l 10× conc. amino acids for MEM Eagle and 0.2 ml/l 100× conc. vitamins for MEM Eagle (Gibco). 2 cells were chosen, one of which was active and the other silent. These were penetrated with glass microelectrodes containing 1 M potassium acetate at pH 6.8 with tip resistance of 5–30 MΩ. Signals were recorded via a conventional cathode follower and Wheatstone bridge circuit through which graded square-wave current pulses could be passed. Current amplitudes of 0–4 nA were injected into the cell soma, and the response time of action potentials was recorded. The membrane potential of each cell was adjusted to 5 ± 0.3 mV below the threshold of firing. The capacitative properties of the recording system gave it an inherent time constant of about 0.5 msec.

At low currents the response time of the cell tends towards infinity[9]. The minimal current required for a response, obtained by extrapolating the lower limits of a strength/duration curve, is the Rheobase (I_0). Values, as measured from curves for the active and silent cells, are given in the table. The current applied, I, is obtained from the equation

$$I = I_0 \cdot \frac{1}{1 - \exp(t/k)} \qquad (1)$$

where t is the duration of depolarization and k is a factor of dimension [T] The value of k cannot be derived directly from the results of this experiment, since it depends on a number of properties of the cell membrane, including its capacitance and space constant[9]. However, k can be obtained from equation 1. If I becomes $2I_0$, the equation simplifies to

$$k = \frac{t}{\ln \cdot 2}. \qquad (2)$$

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