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Electrophysiological and pharmacological properties of nucleus basalis magnocellularis neurons in rats¹

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ABSTRACT

AIM: To investigate the primary electrophysiological and pharmacological properties of the nucleus basalis magnocellularis (nbM) neurons. **METHODS:** Single unit extracellular recordings from the nbM neurons were obtained with glass micropipettes in urethane-anesthetized rats. **RESULTS:** Most nbM neurons responded to noxious but not innocuous mechanical, thermal, chemical, and electrical stimuli. The receptive fields were usually very large and bilateral. Electrical stimulation applied to the frontal cortex (FCX) either activated orthodromically or antidromically the nbM neurons. The FCX stimulation-induced excitatory response in the nbM neurons could be partly blocked by intracerebroventricular (icv) injection of atropine 2.5 mmol/L or tubocurarine 0.1 mmol/L. Icv injection of ACh (1, 10, and 100 mmol/L) dose-dependently increased the spontaneous firing rate in most of the nbM neurons. Atropine (2.5, 25, and 250 mmol/L) or tubocurarine (0.1, 1, and 10 mmol/L) not only antagonized the ACh-induced excitation, but also inhibited the spontaneous firing of the nbM neurons. **CONCLUSION:** The nbM might be involved in nociception, although it was considered to play a critical role in cognitive function. Also, the nbM appears to be rich in cholinergic autoreceptors.

INTRODUCTION

The nucleus basalis magnocellularis (nbM), also termed nucleus basalis of Meynert in primate, includes cells located in the ventral and medial globus pallidus (GP) and substantia innominate (SI). The nbM contains a heterogeneous population of neurons, in that most large neurons are cholinergic. These neurons are

multipolar and have large dendritic fields. In addition to cholinergic neurons, some of the large neurons are GABAergic^[1,2]. The smaller neurons intermixed with cholinergic neurons are non-cholinergic, which include GABA, somatostatin, neuropeptide Y, neurotensin, substance P, enkephalin, and glutamate neurons^[3-5]. Anatomical studies in monkeys, cats, and rats have established that the nbM receives projections from the cortex, amygdale, the lateral hypothalamus, midline thalamus, central lateral thalamic nuclei, parabrachial, raphe, locus coeruleus, as well as periaqueductal gray (PAG)^[6-10]. The nbM neurons project to widespread structures in both forebrain and brainstem. The best-characterized projections are those to the frontoparietal cortexes. Also, this cortical region projects reciprocally back to the nbM. In addition, there are projections from the nbM to the

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olfactory bulb, the hypothalamus, interpeduncular nucleus, reticular nucleus of the thalamus, and amygdala^[2,11-14]. Despite getting a great deal of morphological data about the nbM, electrophysiological, and pharmacological data on the nbM are still limited. The present study is, therefore, designed to examine in greater detail the electrophysiological and pharmacological properties of the nbM neurons.

MATERIALS AND METHODS

Animals preparation Male Sprague-Dawley rats (220-250 g, Grade II, Certificate No 02-22-2) were used. The animals were initially anesthetized with urethane (20 %, 1.5 g/kg) for surgery. The jugular vein was catheterized to enable administration of supplemental anesthetic doses. After tracheal intubation, the rat was then placed in a stereotaxic instrument. The position of the nbM (1-1.5 mm caudal to bregma, 2.3-3 mm lateral and 6.8-7.8 mm below the cortical surface) and the lateral ventricle (1-mm caudal to bregma, 1.2-1.5 mm lateral and 3.5-3.8 mm below the cortical surface) were determined according to the atlas of Paxinos and Watson^[15] and then frontoparietal cortex was exposed to insert recording electrode, stimulation electrodes, and the guide cannula for drug administration. The physiological condition of the animals was monitored by recording the electrocardiogram and rectal temperature to ensure that these parameters remained within 330-460 beat/min and 37 °C-38 °C, respectively.

Unit recordings and cortical stimulation Single unit extracellular recordings were obtained from the nbM with glass micropipettes filled with sodium acetate acid 0.5 mol/L containing 2 % pontamine sky blue (10-20 M Ω impedance). When a single unit was isolated, spontaneous activity was recorded for 5-10 min and then single electrical pulse (500 μ s duration, 100-1000 μ A (1.5 \times threshold intensity) was delivered to the frontal cortex (FCX, 2.5-1.5 mm anterior to the bregma, 1-2 mm lateral, and 1-mm below the surface of brain) through a pair of insulated stainless steel stimulation electrodes (shank diameter 100 μ m, tip diameter 30 μ m, exposed tip 100 μ m, tip separation 1-mm). A series of 10 stimuli were applied in each trial and the recorded responses were displayed using a post stimulus histogram. The total number of spikes per ten stimuli was calculated by a computer data collection system.

Neurons were identified to be antidromic if stimulation produced evoked action potentials with the fol-

lowing criteria: fixed latency, high frequency following, and collision test.

Peripheral stimulation The cutaneous mechanical stimuli used to examine the peripheral inputs to the nbM neurons consisted of innocuous brushing, touching, light pressure, noxious heavy pressure, and pinching (with forceps). The last stimulus was noxious. Since the neurons responded only to noxious stimuli and the receptive fields were large, it was not feasible to determine the exact borders of the receptive fields. Thus stimuli were normally applied only to the following body regions: tail, all paws, ears, and nose.

The peripheral electrical stimulation was applied with a pair of needle electrode inserted into the skin in the receptive field. Short 200 Hz trains (3 pulses) of 1 ms duration pulses, delivered at 0.2-0.3 Hz were used, and the response threshold, latency, and response pattern of the nbM neurons to these stimuli were determined.

The thermal stimulation was delivered by immersion of the tail in hot water (52 °C) for 15 s. When repeated thermal stimulation was employed to the tail, an interval of at least 10 min was allowed between applications.

The chemical stimulations were KCl 3 mol/L, 2.5 % formalin, and NaCl 4 mol/L injected in volumes of 0.15-0.4 mL into the plantar surface, the muscle of one hindpaw, and abdominal cavity, respectively.

All of the recorded neurons were examined with mechanical stimulation first. Then the effect of noxious thermal, electrical and chemical stimulation was examined on those nbM neurons that responded to noxious mechanical stimulation.

Drugs pretreatment A guide cannula was stereotaxically placed at 1-mm dorsal to the lateral ventricle and then a needle was inserted via the guide cannula and protruded 1 mm to approach the lateral ventricle according to atlas coordinates. Acetylcholine chloride, atropine, and tubocurarine (Sigma) were all dissolved in 0.9 % NaCl (normal saline, NS). Drugs were injected in 2 min via the catheter at a volume of 5 μ L, and followed by 5 μ L NS for flushing. In some experiments when more than one unit were tested, the second injection was performed after at least 30 min.

Histology At the end of each experiment, pontamine sky blue was iontophoresed using cathodal current of 10-20 mA for 20 min to mark the recording site. The location of drug injection was marked by injecting 5 μ L pontamine sky blue into the same place as that of

lateral ventricle injection of drugs. The animal was perfused transcardially with NS followed by 10 % buffered formalin. The brain was removed and 60- μm coronal sections were cut with a freezing microtome, mounted, and stained with cresyl violet or AChE histochemistry. An example of the recording site in the nbM was shown in Fig 1. The recording sites were reconstructed and plotted on coronal sections modified from the Paxion and Watson's atlas. The locations of the unmarked recording sites were determined by their distance from the marked one on the basis of the microdriver's readings.

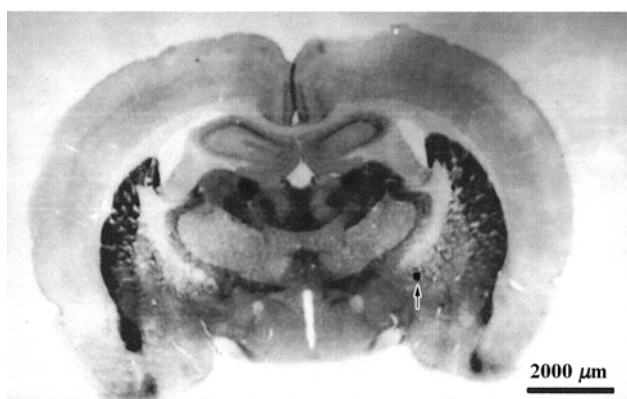


Fig 1. Histological photograph showing the location of the recording site of a nbM neuron. The arrow indicates the marked spot of Pontamine Sky Blue. AChE histochemistry stain ($\times 4$).

Data analysis All data were expressed as mean \pm SD, and analyzed for statistical significance ($P < 0.05$) by chi-square test and analysis of variance (ANOVA) followed by the Newman-Keuls test.

RESULTS

Spontaneous activity of the nbM neurons

Single unit recordings were obtained from 154 neurons located within the nbM. All but 8 of these neurons (94 %) were spontaneously active. Ninety-five of them had a strikingly regular firing pattern, the remainder a slowly irregular firing pattern (Fig 2A, 2B). The mean spontaneous firing rate was (16.6 ± 0.4) Hz.

Responses to peripheral stimuli None of the neurons in the nbM responded to innocuous mechanical stimulation (including brushing, touching, and light pressure). In contrast, noxious mechanical stimulation was found to alter the firing of 75 % of the 48 nbM

neurons. Most (78 %) of these neurons were excited, 6 were inhibited, and 2 were excited by stimulation of some parts of the body and inhibited by stimulation of other parts (excitatory and inhibitory) (Fig 2C, 2D). The excitatory and inhibitory responses of the nbM nociceptive neurons were often maintained for the entire duration of stimulation. In some neurons the stimulation elicited pronounced after-discharges lasting up to several minutes (Fig 2E).

The effects of noxious thermal stimulation were examined on 34 nbM neurons that responded to noxious mechanical stimulation. All except 2 of the neurons responded to noxious thermal stimulation (Fig 2F, 2G). Of these 26 were excited, 6 were inhibited by noxious thermal stimulation. In all cases the response (excitation or inhibition) to noxious heating was the same as the response to mechanical stimulation.

Pinching the digits of either paw or applying heavy pressure against the tibia bone could often elicit a strong response in the nbM neurons. Furthermore, squeezing the gastrocnemius muscle or the Achilles tendon with either forceps or by hand yielded similar results.

In order to further examine the effects of nociceptors located in regions other than the skin as well as the effects of noxious chemical stimuli on the nbM neurons, KCl 3 mol/L, 2.5 % formalin, and NaCl 4 mol/L were injected sc, im, and ip, respectively (Fig 2H-2J). Only 1 cell per animal was tested with these stimuli and which was performed as the last stimulus. In all cases when neurons were excited by mechanical stimulation, it was also excited by the chemical stimulation.

Some of the neurons responded to noxious mechanical stimulation was also determined their responses to electrical stimulation applied to the skin. In all of the neurons, the responses (excitation or inhibition) to electrical stimulation were the same as the response to mechanical stimulation. Fig 3A-3D showed examples of the nbM neurons excited and inhibited by noxious electrical stimulation.

The response threshold of the nbM neurons to transcutaneous electrical stimulation was (2.71 ± 0.23) mA ($n=18$). The latency of excitatory responses evoked by electrical stimulation applied to the ipsilateral hind paw was (154 ± 32) ms ($n=18$). In 2 neurons that had clear responses to electrical stimulation of the tail, the conduction velocity of the peripheral afferents producing the responses was estimated by comparing the difference in latencies of responses to the stimulation at 2

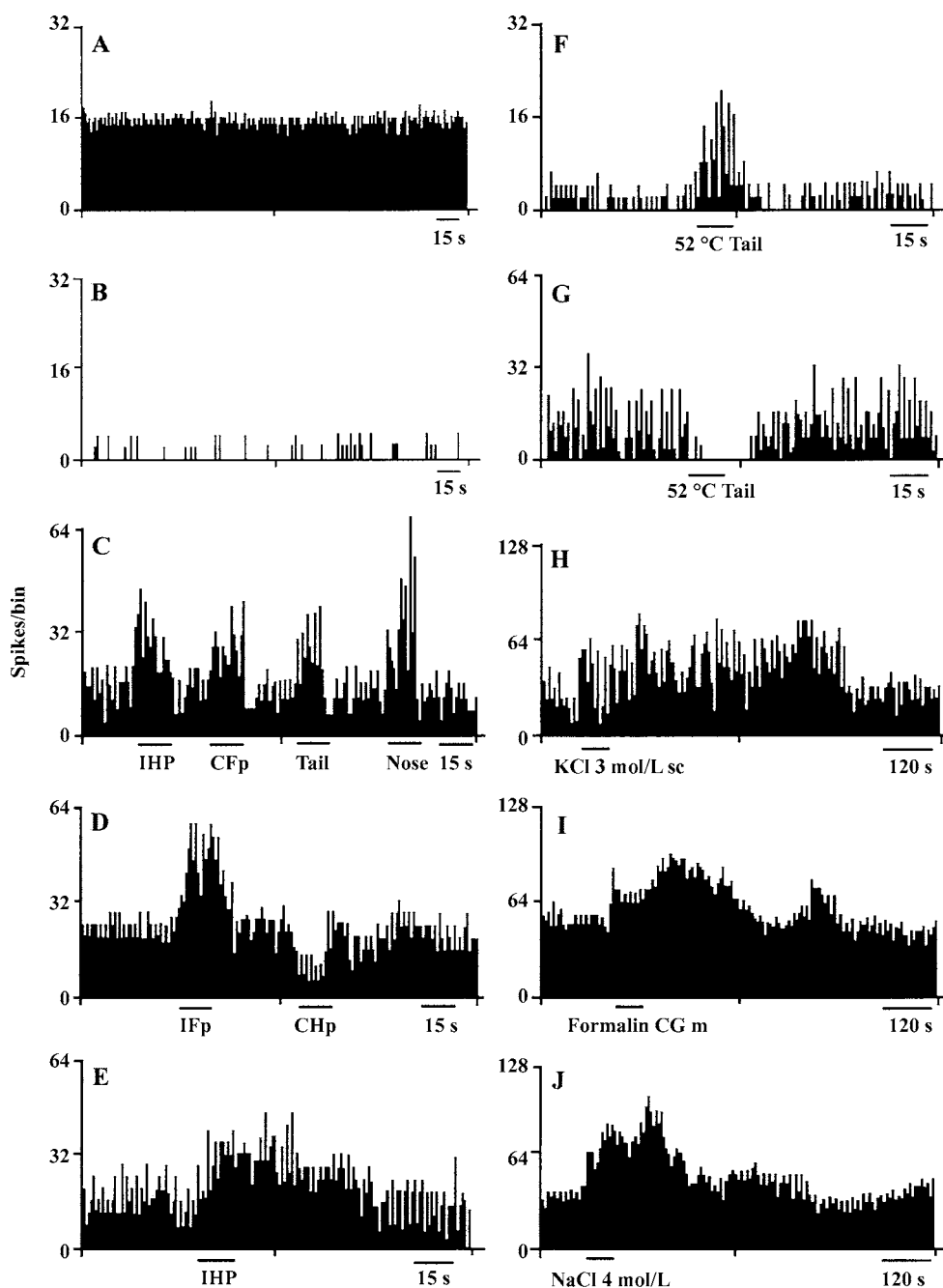


Fig 2. Examples illustrating a type of regular firing pattern (A) and slowly irregular firing pattern (B) observed in the nbM; C-J: Examples showing the responses of the nbM neurons to noxious mechanical (C-E), thermal (F, G), and chemical (H-J) stimulation, respectively. Abbreviations: IHP: ipsilateral hind paw; CFP: contralateral fore paw; IFP: ipsilateral fore paw; CHP: contralateral hind paw.

sites 100-mm apart along the tail. The large difference in latencies for stimulation at the 2 tail sites suggested that C-fiber inputs were responsible for eliciting the responses (Fig 3E, 3F).

Responses to frontal cortex stimulation Frontal cortex (FCX) stimulation was found to alter the firing of 91 % (60/66) of the nbM neurons. Most (73 %)

of 60 nbM neurons were excited and 10 inhibited. Examples of the nbM neurons excited and inhibited by FCX stimulation were shown in Fig 4.

The threshold of the excitatory or inhibitory responses of the nbM neurons to FCX stimulation was (0.56 ± 0.06) mA ($n=50$) and (0.41 ± 0.05) mA ($n=10$), respectively. The average latency of excitatory re-

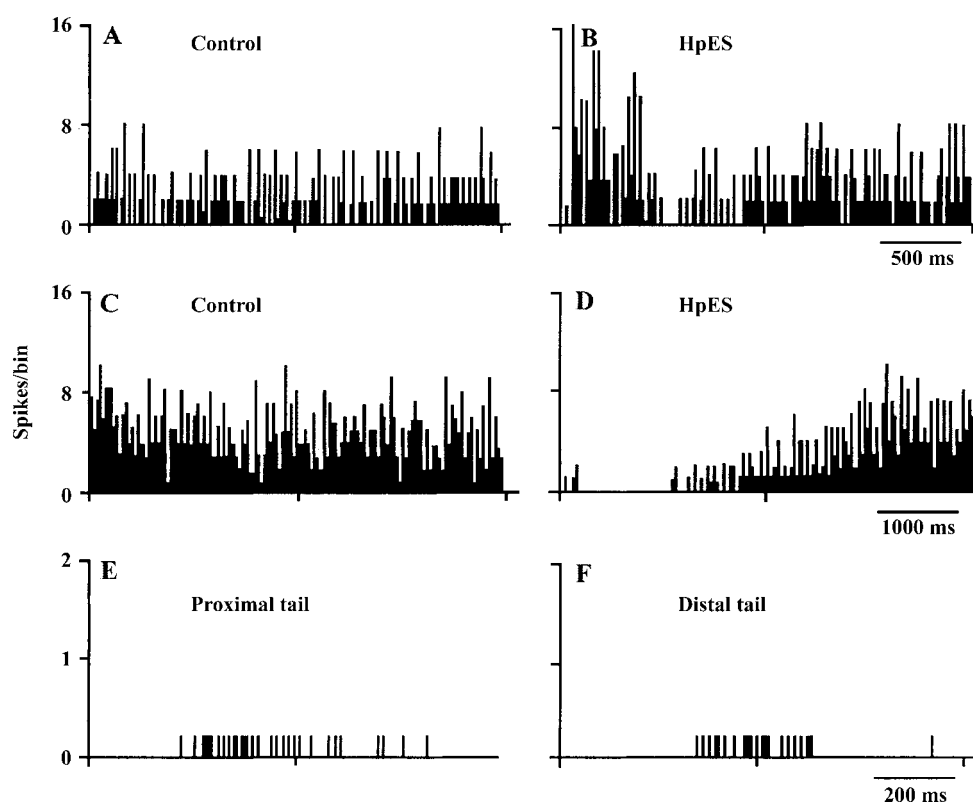


Fig 3. Examples showing the excited (A & B) and inhibited (C & D) responses of the nbM neurons to electrical stimulation of hind paw (HpES) [0.3 Hz, 5 mA, train of pulses (3 pulses, 1 ms duration, 200 Hz)]. E and F: histograms showing the responses of an nbM neuron to electrical stimulation of proximal and distal parts of the tail. Each histogram is the sum of 10 repetitions of the stimulus.

sponses evoked by FCX stimulation was (12.6 ± 0.7) ms ($n=50$).

A proportion of the nbM neurons was activated antidromically by FCX stimulation. The range of latencies of antidromic activation was 1.0-14.3 ms. The mean latency to antidromic responses was (2.5 ± 0.5) ms ($n=30$).

In 17 neurons that had clear responses to FCX stimulation (13 excited, 4 inhibited), the effects of icv injection of atropine 2.5 mmol/L or tubocurarine 0.1 mmol/L on FCX stimulation-induced responses were examined. Neither atropine 2.5 mmol/L nor tubocurarine 0.1 mmol/L had effect on the FCX stimulation-induced inhibitory response (Fig 4E-4H). In contrast, icv injection of atropine 2.5 mmol/L significantly decreased the FCX stimulation-induced excitatory responses in 11 of 13 nbM neurons (Fig 4A-4D, 5). Similarly, icv injection of tubocurarine 0.1 mmol/L also partly blocked the FCX stimulation-induced excitatory responses in 9 of 13 nbM neurons (Fig 5).

Effects of cholinergic agents on spontaneous activity of the nbM neurons ACh was applied at doses

of 1, 10, and 100 mmol/L in young rats (Fig 6A). ACh 10 mmol/L significantly increased the spontaneous firing rate in most of the nbM neurons (7/10) (Fig 7). At a dose of 100 mmol/L, ACh-induced excitation was further enhanced. No changes were found in the spontaneous firing rate of the nbM neurons after icv injection of the same volume of NS. One out of 10 neurons was inhibited and 2 out of 10 neurons were not influenced by icv injection of ACh (10 mmol/L), so they were not included in the dose-effects curve.

Atropine 2.5 mmol/L had no effect on the spontaneous firing of the nbM neurons. Whereas this dose of atropine was administered 4 min before ACh treatment, the ACh-induced excitation could be antagonized obviously (Fig 7A, 7B). Atropine 25 mmol/L markedly reduced the spontaneous firing rate in most of the nbM neurons (7/11). The inhibitory effect was further enhanced at a dose of 250 mmol/L (Fig 6B).

Tubocurarine 0.1 mmol/L had no effect on the spontaneous firing of nbM neurons, but at this dose, the ACh-induced excitation was also blocked obviously (Fig 7C, 7D). Tubocurarine 1 mmol/L and 10 mmol/L

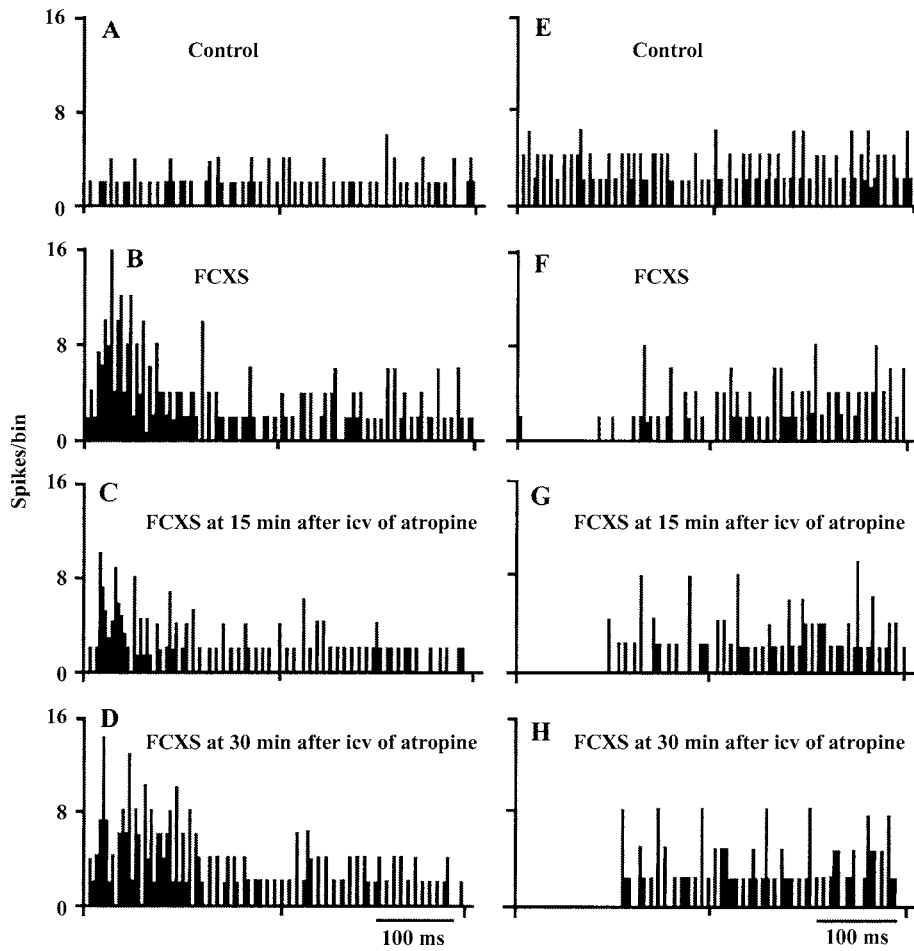


Fig 4. Examples showing the excited (A-D) and inhibited (E-H) responses of the nbM neurons to frontal cortex stimulation (FCXS) (500 μ s duration, 500 μ A intensity), and the effect of icv injection of atropine 2.5 mmol/L on FCXS-induced excitation.

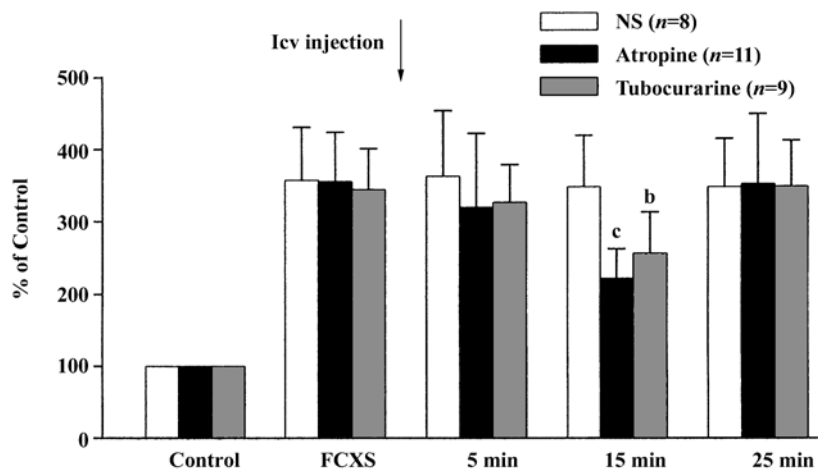


Fig 5. The effect of icv injection of atropine 2.5 mmol/L and tubocurarine 0.1 mmol/L on FCXS-induced excitation of the nbM neurons. ^b $P < 0.05$, ^c $P < 0.01$ vs NS control. FCXS: FCX stimulation; 5, 15, and 25 min: FCXS at 5, 15, and 25 min after icv injection of atropine or tubocurarine, respectively. Mean \pm SD.

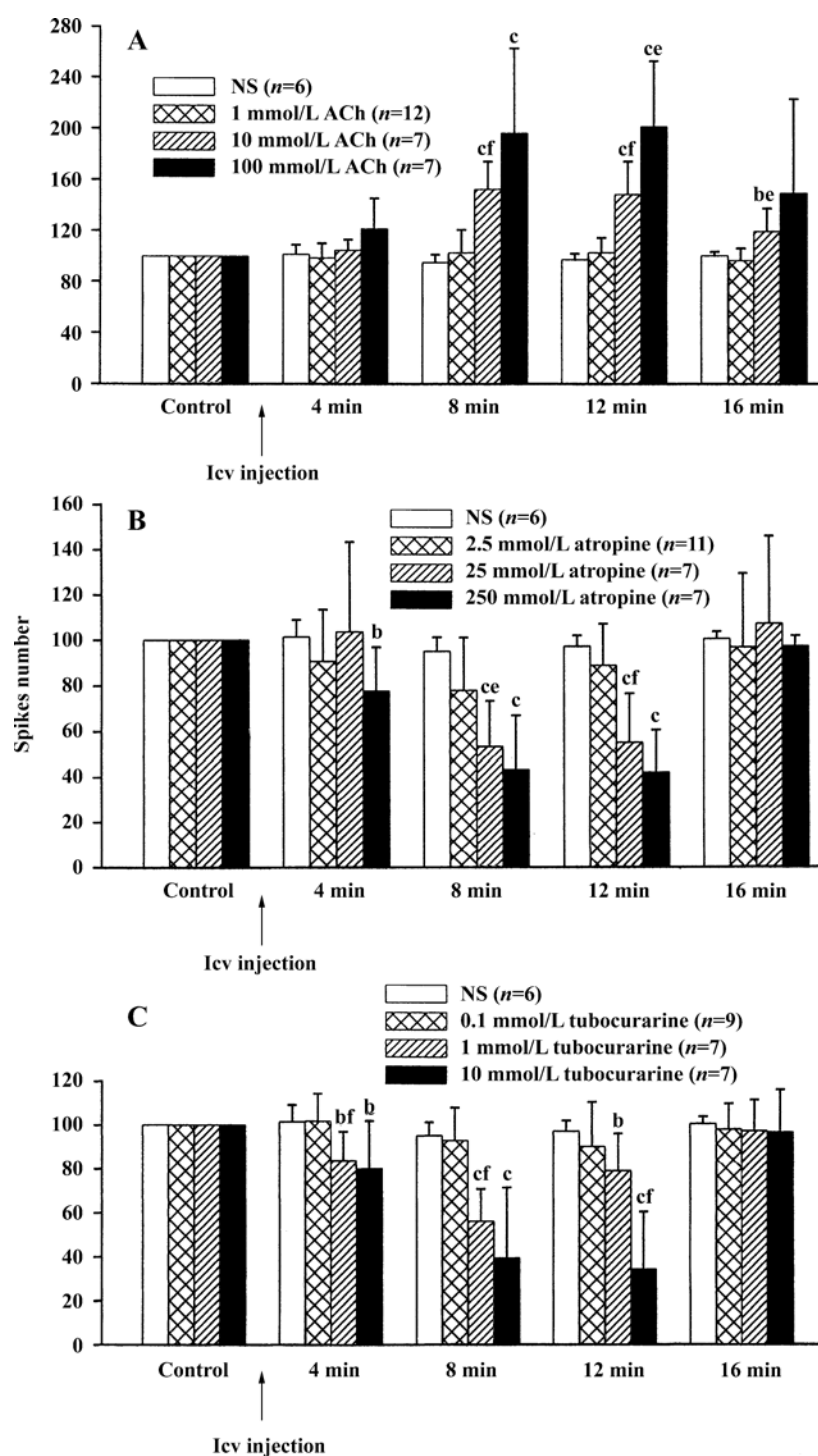


Fig 6. Effects of icv injection of ACh (A), atropine (B), and tubocurarine (C) of different doses on the spontaneous firing of the nbM neurons, respectively. The effects are expressed as percentages of the control level in term of accumulating spikes numbers within 4 min. ^b $P < 0.05$, ^c $P < 0.01$ vs NS control. ^e $P < 0.05$, ^f $P < 0.01$ vs low doses. Mean \pm SD.

significantly decreased the firing rate in most of the nbM neurons (7/9) (Fig 6C).

To identify the direct action of the drugs on the nbM neurons, in some experiments, the effects of microinjection of ACh (on two neurons), atropine (on two

neurons), and tubocurarine (on one neuron) into the nbM (0.1 μ L, the same concentration with that of lateral ventricle injection) were compared. The results showed that the nbM microinjection produced the same kind of effects as that of lateral ventricle injection, ex-

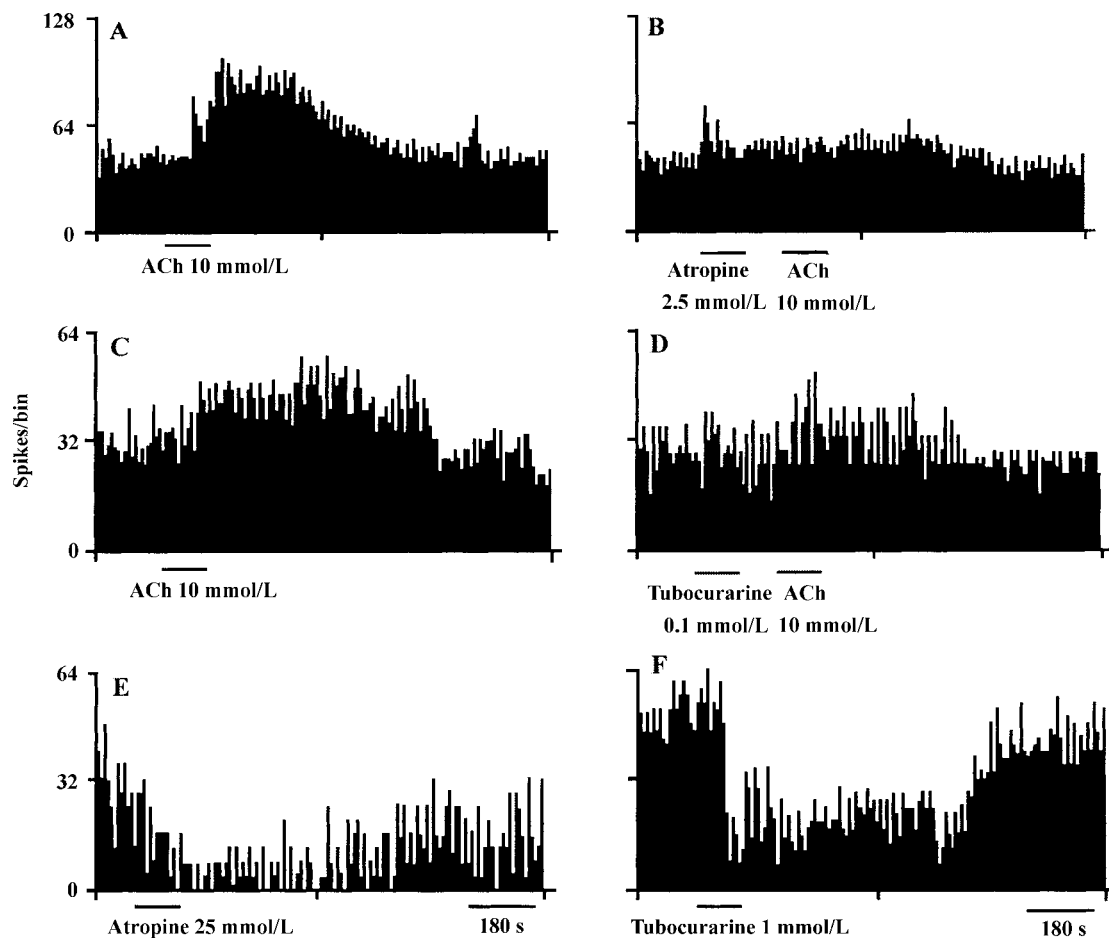


Fig 7. Histograms showing the effects of icv injection of 10 mmol/L ACh (A and C), atropine 2.5 mmol/L plus ACh (B), tubocurarine 0.1 mmol/L plus ACh (D), atropine 25 mmol/L (E), and tubocurarine 1 mmol/L (F) on the spontaneous firing of the nbM neurons, respectively.

cept that the latency was shortened.

DISCUSSION

Morphological data had demonstrated that more than 80 % of neurons in the nbM were cholinergic^[16]. The neurons studied in the present experiments quite probably belong to the Ch_4 sector of Mesulam *et al*^[17]. This group of cholinergic neurons is heterogeneous, encompassing cells from the substantia innominate (SI), the nucleus basalis and even from the preoptic magnocellular neurons or from the area bridging the gap between the nucleus of horizontal limb of the diagonal band of Broca and nucleus basalis. The nbM in the rat is not as well delineated as in the monkey. However, all the 154 neurons recorded in the present study were in the ventral and medial globus pallidus and SI, that was as part of the nbM, as defined by Lehmann and Bigl *et al*^[18,19].

The present study confirmed and extended the earlier finding from electrophysiological study that many neurons in the nbM could be activated by noxious mechanical stimulation^[20,21]. In addition, the present study presented that the nbM neurons activated by noxious mechanical stimulation could also be activated by noxious thermal, chemical, and electrical stimuli. Most of the responses were excitatory and receptive fields were very large. Generally, the nbM neurons can only be activated by extreme nociceptive stimuli. For example, responses to electrical stimulation can only be evoked by very high intensity electrical stimuli delivered in short trains and frequently the responses only become apparent after a number of repetitions. This observation suggested that recruitment of C fibers might be necessary to provide a sufficiently strong input to the nbM to elicit responses. The large receptive fields, high thresholds and multimodal responses to cutaneous mechanical, thermal, and chemical stimuli, suggested that a great

deal of convergence might occur. These results indicated that the nbM might be involved, at least in part, in relaying or modulating nociceptive information. Indeed, the nbM receives afferents from the cortex, amygdale, the lateral hypothalamus, midline thalamus, central lateral thalamic nuclei, parabrachial, raphe, locus coeruleus as well as periaqueductal gray (PAG) and projects primarily to hypothalamus, amygdale and all part of the cortex^[22-24]. The projections from thalamus and brainstem, in particular from the central lateral thalamic nuclei, parabrachial nucleus and PAG, may contribute to the responses observed.

A concern regarding the function of basal forebrain neurons that can be activated by nociceptive stimuli applied to any region of the body is that the responses are related more closely to a change in brain state and thus only indirectly to the noxious stimulation. For example, the level of arousal of an animal will be altered by a noxious stimulus. In the anesthetized animal, it is likely that innocuous stimuli will not provide a sufficiently intense input to alter such brain states but that a nociceptive input will. In order to rule out such a possibility, we monitored the electrocorticogram (EcoG) in some experiments and ensured that the responses observed were not accompanied by EcoG changes.

As mentioned above, the nbM projects primarily to frontal cortex and that the cortical region projects reciprocally back to the nbM. Correspondingly, the present study showed that the nbM neurons were not only activated orthodromically, but also antidromically by FCX stimulation. The range of latencies of antidromic activation from the present data is in accordance with the results of Lamour *et al*^[21]. Assuming a cortical projection length of 5.9-8.5 mm (depending on electrode and neurons location), conduction velocities of 0.5-7.2 m/s were calculated [mean (3.5±0.4) m/s, *n*=30]. Such values are consistent with the view that the axons of the nbM are fine myelinated axons.

A new finding in the present study was that icv injection of atropine and tubocurarine partly blocked FCX stimulation-induced excitation, but not FCX stimulation-induced inhibition. This result suggested that some of the projections from the FCX to the nbM might also be cholinergic, even though the percentage of cholinergic projection from the nbM to the FCX seems to be much higher. Morphological studies have demonstrated that cholinergic neurons in the nbM receive numerous synaptic inputs from cholinergic axon^[25]. Immunocytochemical and binding experiments had shown

that the forebrain cholinergic nuclei including the nbM were rich in muscarinic receptors, and some of them are muscarinic autoreceptors^[26,27]. The present results that icv injection of ACh excited the nbM neurons in a higher proportion (70 %) and ACh-induced excitation could be readily antagonized by atropine and tubocurarine also suggested the possibility that muscarinic and nicotinic receptors existed in the nbM. Furthermore, icv injection of atropine and tubocurarine inhibited the spontaneous firing of the nbM neurons, indicating that there might be some tonic correlation between the spontaneous activity of the nbM neurons and their own transmitter, Ach. When atropine or tubocurarine blocked the cholinergic autoreceptors in the nbM neurons, the ACh-mediated tonic activity was relaxed and thereby the spontaneous firing rate was reduced. However, with the intracerebroventricular administration, the possibility that the drugs first directly act on the neurons adjacent to the lateral ventricle and then indirectly on the nbM neurons existed in the present study. Therefore, further experiments are still required for elucidation of the functional significance of cholinergic agents-induced effects on the nbM neurons.

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