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# PHARMACOLOGICAL PROPERTIES OF ACETYLCHOLINE-INDUCED EXCITATION OF SUBTHALAMIC NUCLEUS NEURONES

JEAN FEGER, CONSTANCE HAMMOND* & BÉATRICE ROUZAIRE-DUBOIS*

Université René Descartes, Paris and *Laboratoire de Physiologie des Centres nerveux, Université Pierre et Marie Curie, 4, place Jussieu, 75230 PARIS, Cédex 05, FRANCE

**1** In 15 rats anaesthetized with ketamine, microiontophoretically applied acetylcholine (ACh) excited all 58 cells studied in the subthalamic nucleus (STN).

**2** The ACh-evoked excitation was slow in onset and outlasted the ACh application. There was no sign of desensitization when the ACh application was prolonged or repeated. The excitation was prolonged by a concomitant application of physostigmine.

**3** Acetyl-β-methyl choline and oxotremorine were effective cholinomimetics. Nicotine had no effect.

**4** The ACh excitation was antagonized by atropine and scopolamine but not by mecamylamine.

**5** It was concluded that STN ACh receptors are muscarinic in character.

**6** Since large microiontophoretic applications of $Mg^{2+}$ did not suppress ACh-evoked excitation, it is suggested that ACh acts postsynaptically.

**7** The excitatory response of STN cells to striatal or pallidal stimulation was unaffected by atropine administered either microiontophoretically to single cells or intravenously (3 mg/kg) to the whole animal.

## Introduction

In the rat, the subthalamic nucleus (STN) is a well defined nucleus which lies immediately rostral to the substantia nigra (SN). The STN receives its main afferent connections from the globus pallidus (GP) (Carpenter & Strominger, 1967; Carpenter, Fraser & Shriver 1968; Nauta & Cole 1978). Olivier, Parent, Simard & Poirier, (1970) have shown the STN of the cat and monkey to stain intensely for acetylcholinesterase (AChE). The present paper describes the effects of microiontophoretically applied acetylcholine (ACh) and drugs affecting its action on single neurones of the STN. The effects of atropine on excitations evoked by electrical stimulation of the caudate-putamen and GP have also been investigated.

## Methods

The experiments were performed on 15 rats anaesthetized with ketamine (80 mg/kg, i.m.). Two experiments were performed with three barrel micropipettes (tip diameter 2.3 μm). In the other 13 experiments, a single glass micropipette (tip diameter 0.8 μm) was glued to a 3 or 4 barrel micropipette (tip diameter 10 to 15 μm). The two electrodes were glued with the recording barrel protruding 5 μm. The drug solutions used were: acetylcholine chloride (0.5 M, pH 4.5.), acetyl-β-methyl choline (0.8 M, pH 5.5.), oxotremorine (0.5 M, pH 5, Aldrich chemical company), atropine sulphate ($5 \times 10^{-2}$ M), scopolamine (0.2 M, pH 5), nicotine (0.25 M, pH 5, Sigma), mecamylamine ($2 \times 10^{-2}$ M, pH 6), glutamate (1 M, pH 7.5), physostigmine (eserine, 0.5 M, pH 6.0), NaCl (0.5 M, pH 4) and $MgCl_2$ (1 M). Recording barrels were filled with pontamine sky blue in 1 M NaCl. Electrode tip location was verified histologically following the ejection of pontamine (15 μA for 15 min). Two bipolar concentric stimulating electrodes were implanted stereotaxically (Albe-Fessard, Stutinsky & Libouban, 1966) in the caudate-putamen and GP. During the recording session, STN cells were identified by their typical response to striatal stimulation (Ohye, Le Guyader & Feger, 1976). The position of the tip of the microelectrodes was marked by deposition of iron and brain sections were subsequently stained by the ferri-ferrocyanide reaction. The activity of single STN neurones was counted by a ratemeter (time constants 0.1 to 2 s) and displayed on a pen recorder. Microiontophoretic ejection currents were measured in the current return path.

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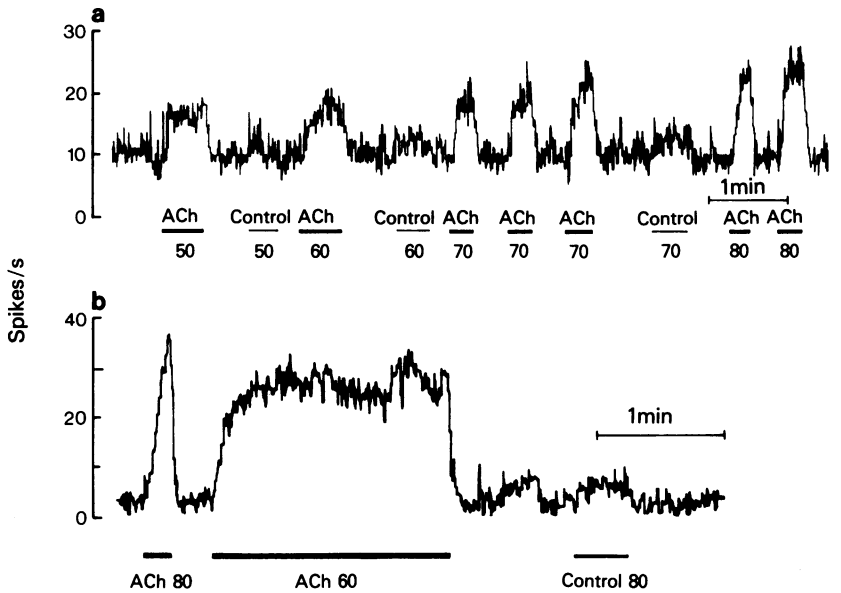


**Figure 1** (a) The excitation of subthalamic nucleus (STN) neurones by acetylcholine (ACh) and the lack of effect of control applications (NaCl). In (b) note the absence of desensitization when the ACh application was prolonged. The recordings in this figure were obtained with a single triple barrelled micropipette. In this and subsequent figures the duration of the current ejections is indicated by horizontal bars and current values are given in nA.

## Results

All 58 STN cells tested were excited by iontophoretically applied ACh. The excitation was slow in onset with a latency to firing of about 4 s. Maximal firing was attained within 7 to 20 s. Following the termination of the electrophoretic ejection, firing returned to the spontaneous levels in 5 to 10 s (Figure 1a). There was no sign of desensitization when the ACh ejection was prolonged or repeated (Figure 1a, b). Occasionally the onset of firing was preceded by a short inhibitory phase (10 s).

The ACh-induced excitation was prolonged by a concomitant application of physostigmine for the very few STN cells tested. Acetyl-$\beta$-methyl choline (14 cells) and oxotremorine (7 cells) were effective excitants of STN cells but did not reproduce exactly the excitation evoked by ACh (Figure 2). Moreover, when compared with ACh, oxotremorine was a weak excitant even with high ejecting currents (300 nA). With currents up to 300 nA, nicotine never excited STN neurones (14 cells) (Figure 2).

When ejected with currents of up to 100 nA, atropine antagonized the action of ACh within 2 min (19 cells) and had only a slight effect on that of glutamate (Figure 3). Nevertheless, when atropine was ejected for a long time (more than 1 min), it depressed the spontaneous activity of STN neurones. Scopolamine ejected with currents up to 100 nA (15 cells) behaved like atropine but was a weaker antagonist of ACh-evoked excitations. On 10 STN cells, the nicotinic antagonist mecamylamine (with currents up to 300 nA) had no effect on excitations evoked by ACh and acetyl-$\beta$-methyl choline (Figure 3).

In an attempt to differentiate presynaptic from postsynaptic effects of ACh, the action of iontophoretic $Mg^{2+}$ was tested on 10 cells. Even with high currents (500 nA) $Mg^{2+}$ did not affect the ACh-evoked excitations (Figure 4). With currents greater than 80 nA, $Mg^{2+}$ increased the firing of STN neurones.

Striatal stimulation excited all 58 STN cells tested. The excitatory response had a latency of 7 to 15 ms (Figure 5) and was composed of 1 to 7 spikes. This response, first described in the monkey (Ohye, *et al.*, 1976) can also be obtained by pallidal stimulation in the rat. Since no direct projection from the striatum to the STN has been revealed with recent anatomical techniques (Grofova, Rinvik, Deniau, Feger & Hammond-Le Guyader, 1978), this excitatory response was thought to be elicited via GP neurones. Atropine administered either microiontophoretically (14 cells) or systemically (3 mg/kg i.v., 2 cells)

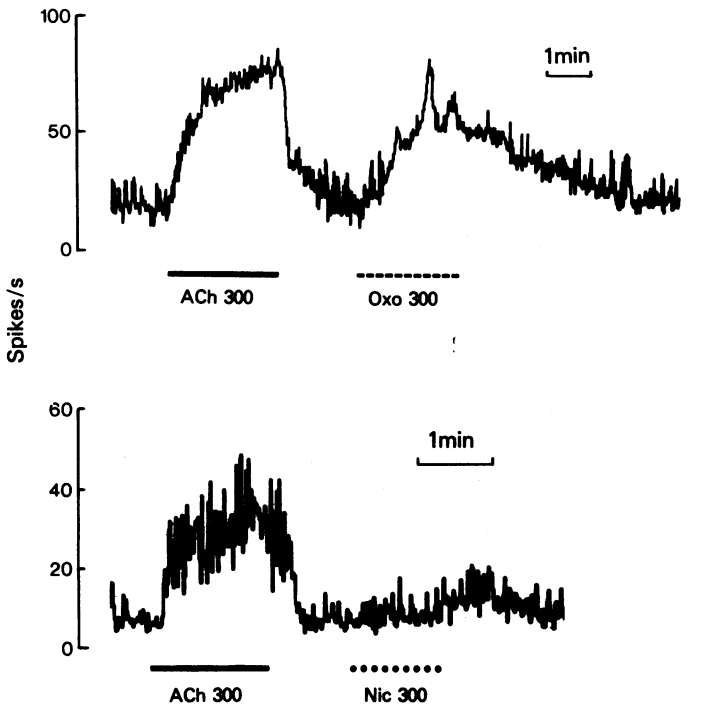


**Figure 2** The response of subthalamic nucleus neurones to acetylcholine (ACh, 300 nA) oxotremorine (Oxo, 300 nA) and nicotine (Nic, 300 nA). In this and subsequent figures, the recordings were obtained from a single micropipette glued to a four barrelled drug containing micropipette.

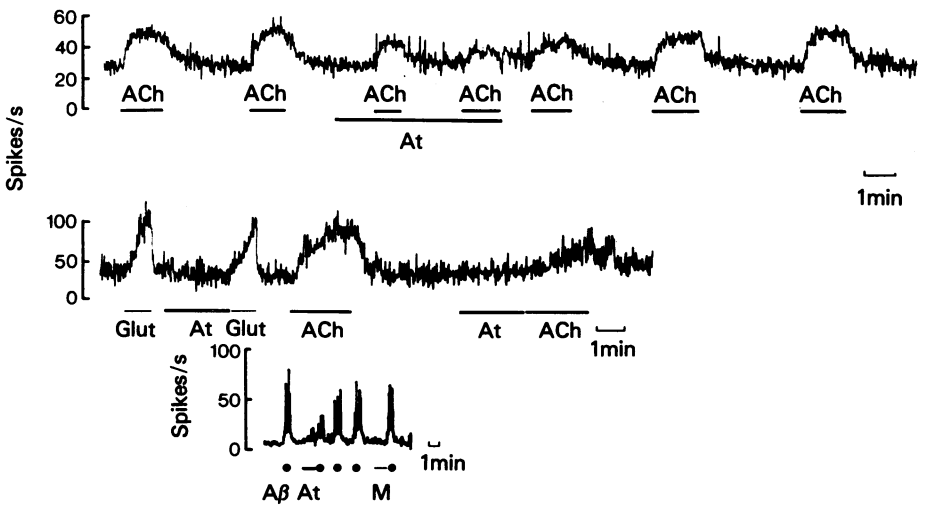


**Figure 3** Excitatory responses of subthalamic nucleus neurones to applications of acetylcholine (ACh, 150 nA), acetyl-β-methyl choline (Aβ, 200 nA) and glutamate (Glut, 80 nA); specific antagonism of ACh-induced excitations by an atropine (At) application of 80 nA and the lack of effect of mecamylamine (M, 150 nA) are shown.

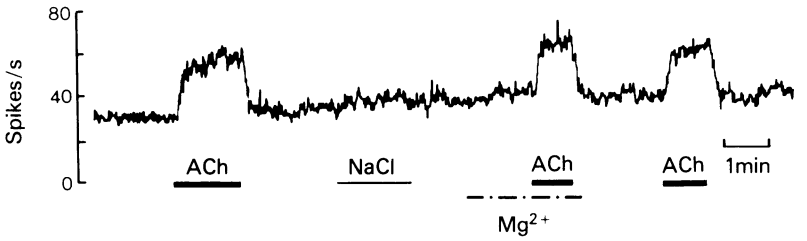


**Figure 4** The response of a subthalamic nucleus neurone excited by acetylcholine (ACh, 200 nA) to an ejection of $Mg^{2+}$ (100 nA). Note the lack of effect of control applications (NaCl, 200 nA).

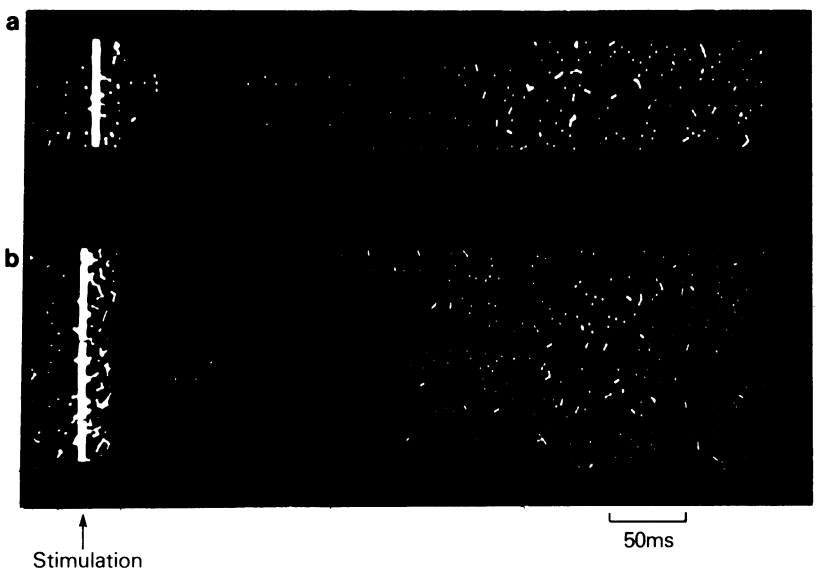


**Figure 5** Response of a subthalamic nucleus cell to striatal stimulation (raster displays): excitation (latency 7 ms) is followed by inhibition (300 ms duration). (a) Control; (b) 10 min after intravenous injection of atropine (this injection blocked acetylcholine-evoked excitation).

had no effect on the excitatory response although given in sufficient amounts to block ACh-induced excitations (Figure 5).

## Discussion

The excitatory action of acetyl-β-methyl choline and oxotremorine and the ineffectiveness of nicotine strongly suggest the existence in the STN of cholinoceptors with a well defined muscarinic character. Moreover, the antagonism of ACh-evoked excitations by atropine and scopolamine and not by mecamylamine support this suggestion. ACh receptors in the STN are thus similar to those found by Krnjević & Phillis (1963) in the cerebral cortex of the cat and different from those studied by Andersen & Curtis, (1964) in the thalamus of the cat. Though the action of atropine has sometimes been considered as relatively unspecific (Curtis & Phillis, 1960), we found that small doses of atropine blocked the excitant action of ACh without interfering significantly with the firing evoked by an excitatory amino acid. Nevertheless, with higher doses, atropine and scopolamine depressed completely the spontaneous activity of STN neurones. In a recent study McLennan & Hicks, (1978) have reported that central cholinoceptive neurones in the rat, particularly in the cerebral cortex, ventrobasal thalamus and for Renshaw cells, cannot be readily described as being clearly muscarinic or nicotinic. In our experiments, the excitatory action induced by ACh on ventrobasal thalamic neurones

was mimicked by both nicotinic and muscarinic agents. However, in the same microelectrode descent, units of the STN had clear muscarinic characteristics thus suggesting some anatomical specificity of the latter effects. In microiontophoretic studies, the ACh-evoked excitation could be exerted directly (that is postsynaptically) or indirectly (that is either on presynaptic terminals or on nearby excitatory interneurones). Indeed, when the drug micropipette is glued too far away from the tip of the recording microelectrode, an indirect drug action may be considered more probable. For these reasons, in some experiments we recorded with multibarrel micropipettes and in others tested the effect of $Mg^{2+}$. Both tests suggested that the excitatory response may be due to a direct action.

Another point which needs discussion is the range of current necessary to observe an effect of drug applications with glued micropipettes. When the recording barrel protruded approximately 10 μm from the drug containing pipettes and was separated by 15 μm before it was introduced in the brain, excitatory effects of ACh applications were readily obtained with ejection currents between 80 to 150 nA. However, it is difficult to evaluate the exact significance of these parameters *in situ* since at the end of many experiments the lateral distance was found to increase by a factor of approximately two. Thus the relatively high ejection currents employed in these experiments could be explained by a further separation of the two electrodes after they were inserted into the brain.

In the rat, Jacobowitz & Palkovitz, (1974) found no AChE containing cell bodies within the STN and showed only a few AChE containing fibres entered the nucleus. Furthermore, Kobayashi, Brownstein, Saavedra & Palkovits (1975) reported that the acetylcholine transferase activity found in the STN of the rat was relatively low when compared with that in the striatum. These findings contrast with those of Olivier *et al.*, (1970) who showed a particularly rich AChE staining in the STN of the cat and monkey. Therefore, a difference between those species might exist. Since the main projection to the STN comes from GP where a small number of AChE containing cell bodies have been found (Olivier *et al.*, 1970; Jacobowitz & Palkovitz, 1974), the lack of effect of atropine on the STN cells response to GP is of interest. Thus it seems unlikely that a cholinergic input from GP to STN exists in the rat. Another possible explanation of the ACh-sensitive cells in the STN of the rat is suggested by the work of Shute & Lewis, (1967) who have described a direct cholinergic fibre bundle to the STN of the rat from the ventral tegmental area.

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