

RESEARCH NOTE

Muscarinic Action of Acetylcholine in the Pigeon Optic Tectum

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Received March 20, 1986; revision received June 3, 1986

Eighty visual units were extracellularly recorded from the pigeon optic tectum, and the effects of iontophoretically applied acetylcholine and its antagonists, atropine and tubocurarine, on these units were examined. The results showed that acetylcholine in the avian tectum functions as an excitatory transmitter or modulator and acts predominantly through a muscarinic mode of action. © 1986 Academic Press, Inc.

The avian tectum contains three prominent bands stained for acetylcholinesterase (AChE), two of them corresponding to the region having the most retinal terminations (3, 4, 18). It is also a rich source of both nicotinic (1, 15, 24) and muscarinic receptors (2, 10, 19). Therefore, it appears that acetylcholine (ACh) may function as a transmitter or modulator in the tectum.

Very few electrophysiological studies, however, have been carried out on the functional role of ACh in the avian tectum. It was reported briefly that ACh caused an increase of firing in one-quarter of the tectal cells tested, with slow onset of ACh-induced responses (7). This was supported by data showing that neurotoxin obviously diminished the postsynaptic excitatory currents in dendrites of the radial cells in the tectum (11). Fite and Wang (9) recently found that ACh may play a role as a neurotransmitter or modulator in the amphibian tectum. From a comparative viewpoint, we undertook the present study to provide some new data concerning chemical transmission or modulation in the avian tectum.

Abbreviations: ACh, AChE—acetylcholine, esterase; Ipc—nucleus isthmi pars parvocellularis; GABA— γ -aminobutyric acid.

The experiments were conducted on pigeons, *Columba livia*, weighing 250 to 400 g. The pigeons were anesthetized with pentobarbital sodium, paralyzed with gallamine triethiodide, artificially ventilated using an O₂-CO₂ mixture, and the body temperature was maintained at 41°C by a heating pad (26). The left tectum was exposed and dura opened. Visual and auditory stimulation were carried out as before (25). Extracellular recordings of spikes were made throughout the exposed tectum with a four-barrel micropipet assembly containing a recording pipet filled with 4 M NaCl (4.5- to 5- μ m tip diameter, 5 to 15 M Ω resistance). The other barrels contained the following compounds to be ejected: ACh (0.5 M, pH 3.5), atropine sulfate (10 mM, pH 5.7) and *d*-tubocurarine chloride (10 mM, pH 5.7). Spontaneous and evoked activity were displayed on a storage oscilloscope and photographed on Polaroid film.

A total of 80 visual units was recorded from 100- to 1400- μ m depths corresponding to tectal layers II and III. Most units were spontaneously active, and responded to onset and offset of a stationary spot of light. The size of the receptive field averaged 15°, ranging from 4° to 30°. All units tested did not respond to auditory stimulation.

The effects of iontophoretically applied ACh, atropine, or curare on the tectal units are summarized in Fig. 1 and Table 1. Current intensities for ejecting these chemicals ranged from 50 to 200 nA. In 73% of the units, after ACh ejection visual responses were enhanced as indicated by an increase in the frequency and duration of discharge to a moving black disc, often accompanied by an evoked spontaneity-like firing (Fig. 1A2). In some units, ACh alone produced a vigorous, continuous discharge, lasting for several minutes after cessation of ACh ejection (Fig. 1Bb, c). Visual responses of these units were also significantly enhanced (Fig. 1Bd). On the other hand, ACh had an inhibitory action in 12% of the units, and no effect on 15% of the units (Table 1). In nine units, the smaller dosage of ACh produced an excitatory effect, whereas the larger dosage was inhibitory. In most cases (62% units), atropine suppressed both spontaneous activity and evoked responses to visual stimulation (Fig. 1A4). Atropine suppression and ACh enhancement were often restored to normal activity in less than 10 min after cessation of drug application. Both the reversibility and replicability of the effects produced by ACh and atropine on the units suggested that these pharmacological effects were not due to neurotoxicity or random factors not related to drug application. Curare, in addition to producing neuronal suppression, could also enhance the visual responses accompanied by a spontaneity-like discharge (Fig. 1A6), or induce vigorously continuous firing (Fig. 1A7). However, curare-induced changes usually took 30 to 60 min to disappear. For these cholinergic drugs, the onset of induced responses was slow, often exceeding 30 s after commencing iontophoresis.

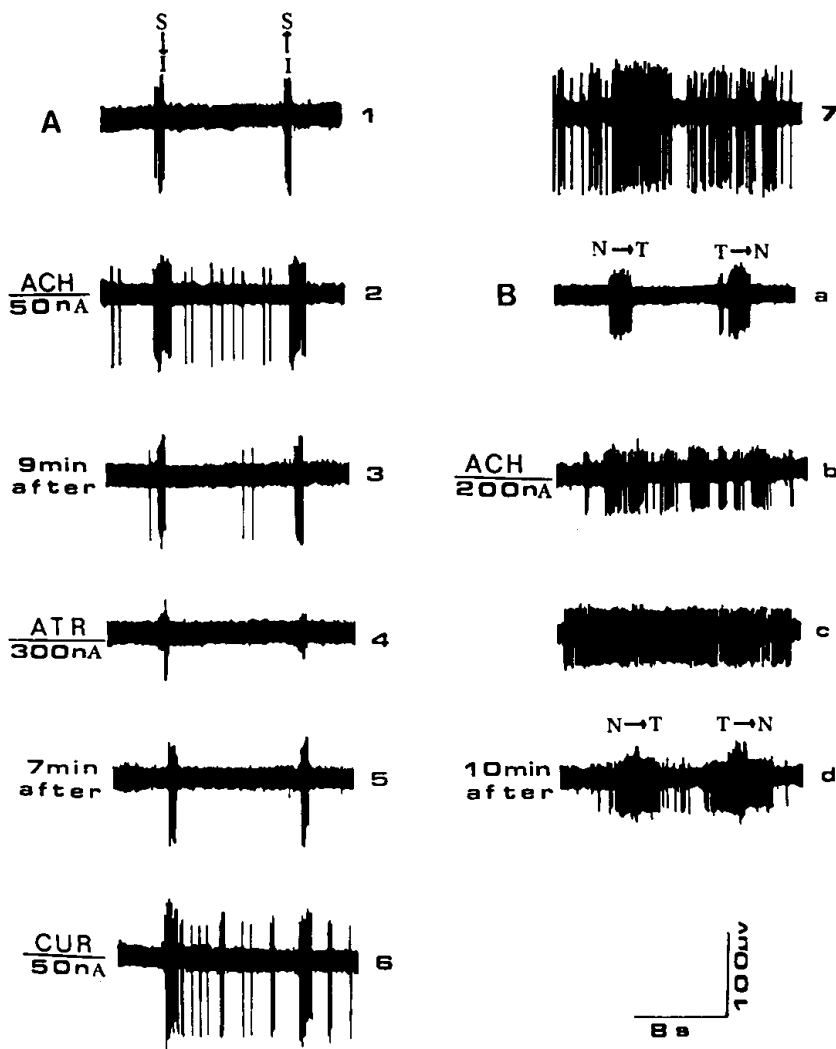


FIG. 1. Effects of acetylcholine (ACH) and its antagonists, atropine (ATR) and curare (CUR) on the discharge patterns of two (A, B) tectal units. In A1-6, visual responses were increased by ACH and CUR, and suppressed by ATR microiontophoretically applied (doses in nA). A7 shows continuous firing induced by CUR. In B, ACH first produced continuous firing for several minutes (b, c), and then visual responses were significantly increased (d). The visual stimulus used in A and B was a 7° black disk moved manually through the receptive field of a unit in the superior-inferior (S-I) and I-S directions (A), or in the nasotemporal (N-T) and T-N directions (B).

Our results show that ACh can excite most visually tectal units in birds and act predominantly via muscarinic receptors, characterized by slow onset, long-lasting duration, and blockage by atropine (12, 16). This is in good agreement with our earlier findings obtained on the amphibian tectum (9). Our recent study using AChE pharmacohistochemical procedures indicates

TABLE 1
Effects of Cholinergic Chemicals on Visual Responses of the Tectal Units^a

Response	ACh	Atropine	Curare
Excitation	58/80 (73)	14/77 (18)	51/74 (69)
Suppression	10/80 (12)	48/77 (62)	16/74 (22)
No effect	12/80 (15)	15/77 (20)	7/74 (9)

^a Numbers in parentheses are percentage of cells tested.

that neurons in the nucleus isthmi pars parvocellularis (Ipc) in pigeons contain high activities of AChE, suggesting that a fraction of the Ipc-tectal fibers may be cholinergic (18), because high AChE activity is a necessary but not sufficient characteristic for identifying the cholinergic neuron (17). This notion is supported by the fact that the nucleus isthmi is the principal source of cholinergic input to the amphibian tectum (21). ACh may also be a muscarinic transmitter in this nucleus, which receives the ipsilateral tectal input (8). Thus, the nucleus isthmi appears to be a mediary in the muscarinic-cholinergic modulation of tectal function. In addition, there is some evidence that muscarinic-cholinergic mechanisms are related to intrinsic tectal circuits which might be involved in the modulation of tectal activity (3).

The peculiar excitatory effect of curare on the tectal units in one study could be explained by the findings of Curtis *et al.* (5) that curare can block the inhibitory actions of both glycine and γ -aminobutyric acid (GABA), two putative inhibitory transmitters. In fact, the pigeon optic tectum receives glycinergic and GABAergic inputs from the Ipc (13, 20, 23). Hunt and Künzle (14) described three GABA-inhibitory networks within the pigeon tectum. Electrophysiological studies favor the possibility that GABA and/or glycine may be important synaptic transmitters in inhibitory mechanisms in the avian tectum (6). Both GABA- and glycine-sensitive neurons are localized at the depths from which our unit recordings were obtained. The excitatory effect of curare on pigeon tectal units is also supported by the evidence that curare has a facilitatory effect upon the electrical activity of tectal cells and releases feeding behavior in the frog (22).

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