

In the bird, biochemical and histochemical data suggest that the neurotransmitter between nucleus isthmi pars magnocellularis (Imc) and tectum is either acetylcholine or glutamate. There are, however, discrepancies regarding the functional role of acetylcholine. In the present study we investigated the action of acetylcholine and glutamate and their specific antagonists on excitatory isthmo–tectal synaptic transmission using electrophysiological and microiontophoretic techniques. The results show two different population of cells: (1) excitatory cholinergic input, blocked by atropine sulphate but not by glutamate antagonist; (2) excitatory glutamatergic input of NMDA or non-NMDA receptor type, which is blocked or reduced by CPP or CNQX but not by atropine sulphate.

**Key words:** Pigeon; Optic tectum; Nucleus isthmi; Microiontophoresis; Acetylcholine; NMDA receptors; CPP

## Avian Imc-tectal projection is mediated by acetylcholine and glutamate

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### Introduction

The nucleus isthmi, a prominent mesencephalic structure found in all classes of vertebrates except cyclostomes and mammals, is considered to be mainly a visual centre (for review see Ref. 1). The most significant finding in recent years seems to be that the two main divisions of the nucleus, pars magnocellularis (Imc) and pars parvocellularis (Ipc) are functionally independent structures.<sup>2,3</sup> The visual information comes from the ipsilateral tectum by means of an excitatory tecto–isthmic pathway and projects back to the tectum. Using intracellular recordings, Wang and Matsumoto<sup>4</sup> have shown that electrical stimulation of the nucleus isthmi inhibits about 70% of tectal neurones and excites the remainder. It has been proposed that there exist at least two feedback loops between the nucleus isthmi and the tectum. The fact that Imc and Ipc differentially modulate visual responses in the tectum has led to the conclusion that a positive loop originating from Imc and a negative loop from Ipc work together in a ‘winner-take-all’ network to make the animals orient to the most interesting object.<sup>2,5,6</sup>

The chemical nature of neurotransmitters involved in these pathways is not clear and is somewhat contradictory (for review see Ref. 7). There is evidence that these pathways involve glycine and GABA as inhibitory substances<sup>8–10</sup> and acetylcholine (ACh)<sup>11–14</sup> (see however Ref. 15) or glutamate<sup>16</sup> as excitatory neurotransmitters. Our recent study on neurones of the tectum and nucleus isthmi suggest that feedback loops can be modulated at the isthmic level by N-methyl-D-aspartate (NMDA) and/or ACh.<sup>17</sup> We therefore attempted to test the action of possible excitatory

neurotransmitter candidates and their specific antagonists on the excitatory Imc–tectal pathways.

### Materials and Methods

Experiments were carried out on 11 adult homing pigeons (*Columba livia*) with body weights of 280–350 g. All birds were anaesthetized with a 20% solution of urethane (1 ml 100 g<sup>-1</sup> body weight). Pigeons were placed in a stereotaxic apparatus and the surface of the optic tectum was exposed. Extracellular recording of action potentials from tectal cells was obtained using a 2 M NaCl and 100 mM cobalt chloride filled barrel of a five-barrelled micropipette (tip diameter of approximately 4  $\mu$ m and 5–15 M $\Omega$  resistance). The other channels contained the substances to be ejected microiontophoretically by appropriate anionic or cationic currents: ACh (Sigma, 0.5 M, pH 3.5), NMDA (Sigma, 0.05 M, pH 7.5), AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole, Tocris Neuramin, 0.005 M, pH 7.5) atropine sulphate (Sigma, 0.01 M, pH 5.7) CPP (3-rs-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid, Tocris Neuramin, 0.01 M, pH 7.5) and CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 0.01 M, pH 8).

Electrical stimuli were applied to the Imc through a concentric or parallel bipolar glass insulated tungsten electrode. Square pulses of 100–200  $\mu$ s and variable voltages (3–10 V, 0.1–0.5  $\mu$ A) were given at frequencies of 0.2–0.5 Hz. The stimulating electrode was placed according to the atlas of Karten and Hodos.<sup>18</sup>

At the end of each experiment, the location of recording and stimulating electrodes were examined histologically following iontophoretic ejection of cobalt

ions (recording site) or by lesioning with constant current pulses of 40  $\mu$ A (stimulation electrode).

## Results

Extracellular recordings were made from a total of 39 tectal neurones. Histological verification showed that the majority (32) of cells were located in layer III.<sup>19</sup> Five cells were found in adjacent area IIj and the remaining two in layer IV. Following stimulation of Imc the neurones were excited with relatively short latencies between 1.4 and 2.4 ms. The majority of these cells could be further excited by iontophoretic applications of glutamate or glutamate analogues NMDA and AMPA or by ACh. The distance between recording and terminal sites close enough for chemical diffusion has been estimated as 50  $\mu$ m. Of 31 tectal neurones tested, 11 were excited by NMDA or AMPA only and nine by ACh only. The remaining 11 cells responded to both glutamate agonists and ACh.

When the stimulus strength was kept at threshold (average discharge rate 0.9 spikes per sweep) an antagonistic effect on synaptic excitation could be demonstrated by the glutamate and ACh antagonists, CPP and atropine sulphate. On ACh-selective cells the responses were completely abolished by atropine but not

influenced by CPP (Fig. 1). Responses of NMDA-selective cells were either abolished (Fig. 2A) or, as shown in a post-stimulus time histogram, reduced (Fig. 2B) when tested with CPP. A similar reduction of synaptic excitation could be observed by the non-NMDA antagonist CNQX.

It was of interest to determine whether the cholinergic or the glutamatergic type of excitatory Imc-tectal pathway would show a topographic difference in location. The pattern on the tectal surface showed no preferences in any direction for the two types of neurones and the distribution within the tectal layers mapped on the basis of histological verification did not differ.

## Discussion

The present study shows that the Imc-tectal projections are mediated by two different neurotransmitter systems, a cholinergic projection which is specifically blocked by atropine and a glutamatergic projection which is reduced or blocked by CPP or CNQX, suggesting that both NMDA and non-NMDA receptor type are involved. Our data clearly demonstrate that in the investigated isthmo-tectal projections both substances are excitatory in nature. Many reports in the literature support the role of both substances as

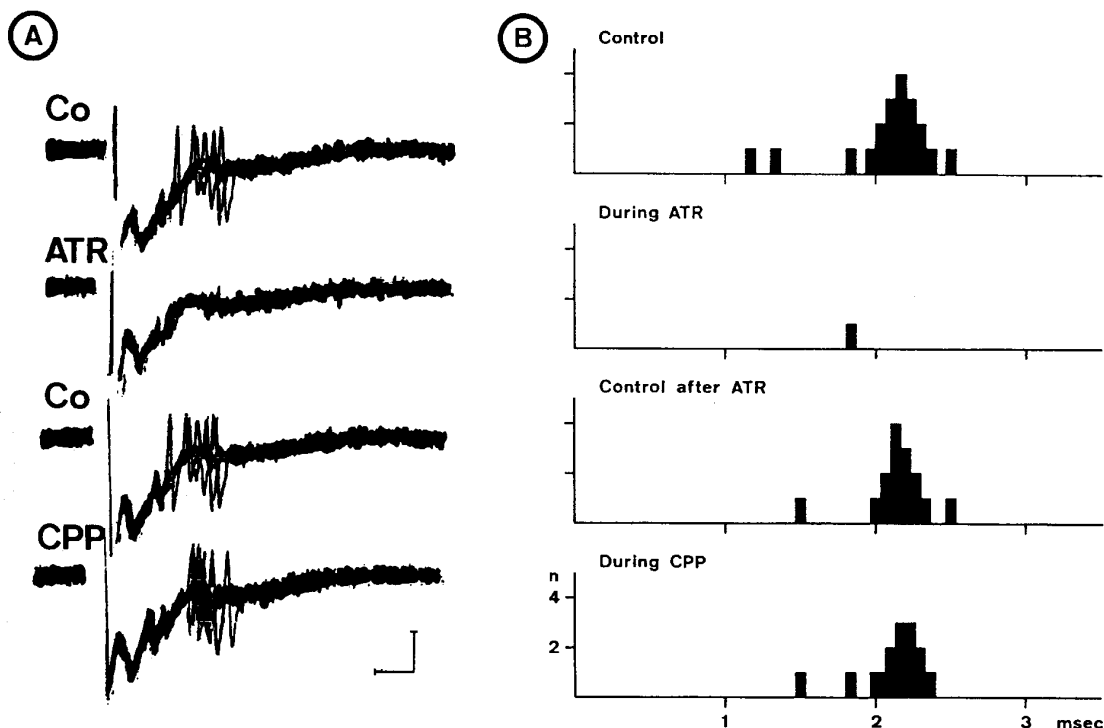


FIG. 1. (A) Effect of iontophoretically ejected atropine sulphate (ATR 25 nA) and CPP (50 nA) on an ACh-selective cell following isthmo-tectal synaptic activation (six superimposed sweeps), Co control, All traces 1 ms, 0.5 mV cm<sup>-1</sup>. (B) Effect of ATR and CPP illustrated on a post-stimulus time histogram (PST, 50 sweeps).

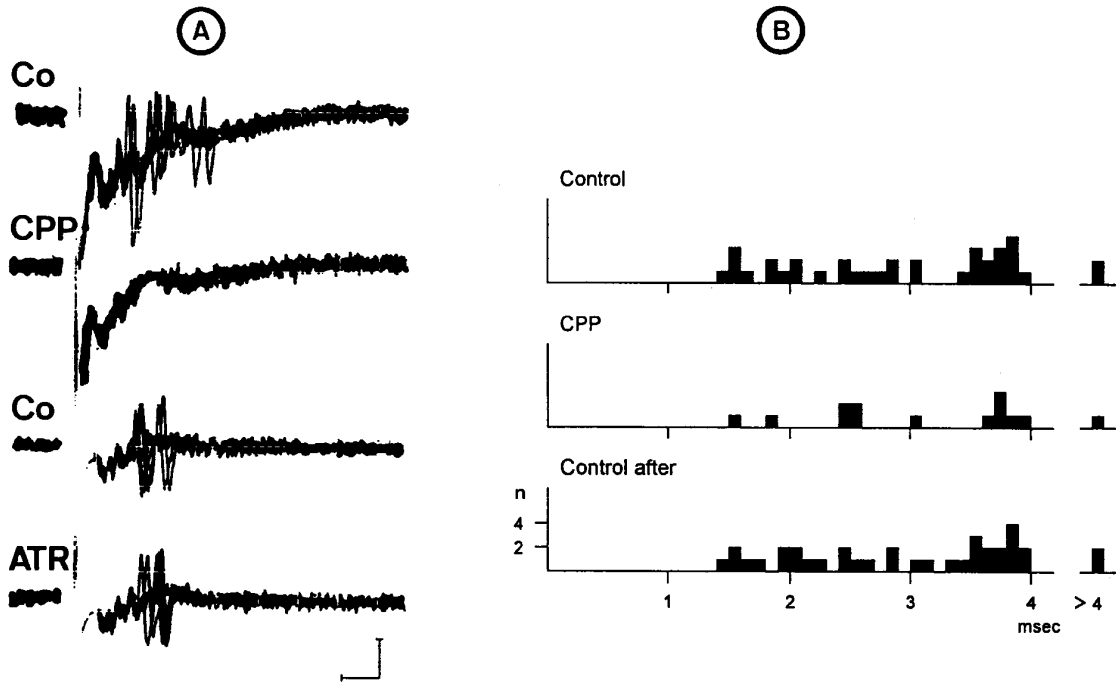


FIG. 2. (A) Effect of atropine sulphate (50 nA) and CPP (25 nA) on a cell selectively excited by NMDA (six superimposed sweeps). Abbreviations and scales as in Fig. 1(A). (B) Reduction of synaptic activation by CPP illustrated on a PST-histogram (40 sweeps).

possible transmitter candidates in the optic tectum and the nucleus isthmi. On one hand, the nucleus isthmi is the main source of cholinergic activity in the optic tectum,<sup>11,13,14,20</sup> suggesting that the nucleus could modulate tectal activity via the cholinergic pathway. Distribution of choline acetyltransferase correlates with staining of acetylcholinesterase<sup>21</sup> and lesions of the optic tectum stop acetylcholinesterase activity in the nucleus isthmi.<sup>9</sup> On the other hand, high affinity uptake of glutamate in the tectal synaptosomal preparation of pigeons has been reported with similar  $K_m$  and  $V_{max}$  values.<sup>22</sup> A large number of tectal neurones is excited by glutamate<sup>23</sup> and synaptic excitation of tectal neurones by the stimulation of the optic nerve can be blocked by glutamate antagonist.<sup>24</sup>

The mosaic-like appearance of the cholinergic and glutamatergic projection to the tectum is somewhat intriguing. Although care was taken in the placement of the stimulation electrode, as well as keeping the stimulus strength at threshold level, the possibility cannot be ruled out that the two populations merging in the optic tectum have different sources and that the stimulation of a relatively large area activates the two systems. However, the fact that Imc stimulation excites tectal neurones only, as shown in the present investigation, whereas stimulation of Ipc activates predominantly inhibitor pathways<sup>4,25</sup> supports the idea of two

parallel cholinergic and glutamatergic isthmo–tectal pathways. Furthermore, our data support the existence of positive feedback loops originating in Imc and projecting into the deeper layers of the tectum.<sup>2,5,9,26</sup> On the basis of intracellular recording of short latency EPSPs in tectal neurones<sup>4,27</sup> it was postulated that there are direct isthmo–tectal projections. Judging from the latency with large variances, the activation of the tectal neurones following Imc stimulation could be polysynaptic. The effects of the antagonists of ACh and glutamate, therefore, could be caused by indirect effects on tectal intrinsic neurones.

Taken together, the nucleus isthmi pars magnocellular projection to the tectum is mediated by two separate neurotransmitter systems, a cholinergic and a glutamatergic one which appear in a mosaic-like tectal distribution pattern.

## Conclusion

The results of this study indicate that the nucleus isthmi pars magnocellular projection to the tectum is mediated by a cholinergic and a glutamatergic transmission of both, NMDA- and non NMDA-receptor type. The results extend our previous observations on the role of ACh and glutamate in Imc–tectal projection.

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## General Summary

One of the main interests of Professor Wang Shu-Rong's research group at the Institute of Biophysics, Beijing, China, is the role of the nuclei isthmi as an important visual centre in amphibians and reptiles. In recent years they have extended this investigation to the pigeon brain, where they have shown that visual and auditory projections are differentially located in the isthmic area of the midbrain. In the present study we investigated the role of neurotransmitter candidates in the excitatory pathway from the magnocellular portion of the nucleus isthmi to the tectum. We could show that these projections are mediated by two neurotransmitter systems, a cholinergic and a glutamatergic one which are blocked by the appropriate antagonists atropine and CPP.