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视觉信息加工开放研究实验室  
论 文 汇 编

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## 前 言

本论文汇编是中国科学院生物物理研究所视觉信息加工开放研究实验室编辑的第四本论文集。它收录了1993年至1994年3月份发表的研究论文及部分综述,计44篇。若将四本论文集作一动态比较,可以反映出本开放实验室科研工作的概貌及进展。与前几年相比,本实验室取得的成果在横向拓宽、纵深发展、跻身于世界先进科学之林等方面又上了一个新台阶。国际合作研究不断扩大,说明本实验室对国外同行的吸引力正在加强。此外,以青年科学工作者为第一作者的论文数量大增,约占论文的二分之一,这一可喜的现象标志着本开放实验室在人才培养方面结出了丰硕的果实。从论文汇编能看到视觉信息加工开放研究实验室在艰苦的科研道路上走过来的一个个坚实的脚印。它说明,视觉信息加工开放研究实验室为我国的神经科学发展贡献了一份力量,今后还将贡献更大的力量。

中国科学院视觉信息加工  
开放研究实验室

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# EFFECT OF ACETYLCHOLINE AND NMDA ON NEURONES OF AVIAN TECTUM AND NUCLEUS ISTHMI

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## **Abstract**

In the present study the effects of microiontophoretically applied acetylcholine and NMDA were investigated on neurones of the avian nucleus isthmi. Neurones in the parvocellular portion (lpc) of the nucleus isthmi were more sensitive to the external application of NMDA than those in the magnocellular portion (lmc). The acetylcholine-induced excitation did not differ in the two isthmic parts. The NMDA-induced firing was specifically blocked by the antagonist CPP. Our results support earlier findings that isthmo-tectal feedback loops can be modulated by distinct mechanisms in separate divisions of nucleus isthmi.

**Key words:** Pigeon, optic tectum, nucleus isthmi, microiontophoresis, acetylcholine, NMDA receptors, CPP

## Introduction

The electrophysiology and neuroanatomy of the avian nucleus isthmi, which is a complex of several cellular aggregates<sup>1</sup> have been extensively studied<sup>2</sup>. Although there are some discrepancy, it is now generally accepted that the two main portion of the nucleus, pars magnocellularis (Imc) and pars parvocellularis (Ipc) are related to visual information processing<sup>3,4</sup>. In birds, the Ipc receives its input from the ipsilateral tectum and projects back ipsilaterally to superficial tectal layers<sup>5</sup>, and the Imc projects to deeper layers of the ipsilateral tectum<sup>6</sup>.

The search for the chemical nature of synaptic interconnection within the nucleus isthmi has included autoradiographic, biochemical, histochemical and electrophysiological data<sup>7,8,9</sup>. The excitatory tecto-isthmi pathway possibly use acetylcholine<sup>10,11</sup> and/or glutamate<sup>12</sup> as neurotransmitters. There is evidence that isthmo-tectal pathways uses glycine and GABA as inhibitory substances<sup>13,14</sup> and acetylcholine<sup>15,16,17</sup> or glutamate<sup>18</sup> as excitatory transmitters. In the central nervous system there is strong evidence, that the excitatory amino acid neurotransmitter glutamate is mediated by distinct receptor types, e.g., N-methyl-D-aspartate (NMDA), quisqualate and kainate<sup>19</sup>. Furthermore, NMDA receptors might play an important role in information processing<sup>20</sup>.

Recently, Wang and coworkers<sup>21</sup> have shown that microinjection of NMDA into Ipc reduces visual responsivity of neurones in the tectum by activating Ipc-tectal pathway, whereas injection into Imc was not effective. In order to elucidate whether different receptor mechanism are involved in starting this feedback pathways, we tested NMDA, acetylcholine and its specific antagonists in the two parts of the nucleus isthmi.

## Methods

Our experiments were carried out on 13 adult pigeons (*Columba livia*) having body weights of 280-340g. The animals were anesthetized with Urethane (1ml/100g body weight, 20% solution) and placed in a stereotaxic apparatus. The exposition of the surface of the optic tectum was done in a conventional manner. Multibarreled micropipettes with an overall tip diameter of approximately 4 $\mu$ m were inserted through the tectal layers and aimed at the nucleus isthmi using coordinates from the atlas of the pigeon brain<sup>22</sup>.

Extracellular action potentials were obtained trough a 2M NaCl- and 100mM Cobalt chloride-filled barrel of the five-barrel glass micropipettes and the neuronal discharge was recorded continuously and stored on the magnetic tape of a data recorder (KS-609, Sony) or displayed on an UV-recorder (Bell & Howell. 5-137). The

other barrels of the micropipette were used for microiontophoretic application of the compounds being tested. Each substance was ejected from the electrode with the appropriate anionic or cationic current. Substances tested included acetylcholine chloride (ACh, Sigma, 0.5M, pH 3.5), NMDA (N-methyl-D-aspartic acid, Sigma 0.05M, pH 7.5), Sulphate atropine (Sigma, 0.01M, pH 5.7) and CPP (3-*rs*-2-carboxypiperazin-4-yl-propyl-1-phosphonic acid, Tocris Neuramin, 0.01M, pH 7.5).

At the end of each experiment, cobalt ions were ejected microiontophoretically and subsequently the brain was processed for histological verification of the recording location.

## Results

Extracellular recordings were made from a total of 97 cells, 51 lying in the isthmus nucleus area and the remainder in different layers of the optic tectum. Within the nucleus isthmi 23 neurones were located in the magnocellular part and 28 in the parvocellular portion. Visual stimulation was used to identify neurones. The majority of these neurones showed spontaneous activity. Neurones in the magnocellular part of the nucleus usually showed stronger spontaneous firing rate than those of the parvocellular part.

The effects of iontophoretically applied ACh and NMDA on tectal and isthmus neurones are illustrated in Table 1. In the tectum opticum the two substances tested, showed regional distribution patterns in terms of effectiveness. Whereas in superficial layers of the tectum only half of the tested neurones were excited by NMDA, the majority of cells in deeper layer responded with excitatory effects. In contrast, ACh was more effective on neurones in the superficial layers. This finding is substantiated by the fact, that in superficial areas some neurones were found to be excited by ACh only, whereas specifically NMDA-sensitive neurones were located in deeper tectal structures.

In the two parts of the nucleus isthmi a clear distinction between the effects of the substances tested could be seen, (Fig. 1A). An increase in the discharge frequency following the ejection of NMDA was predominantly confined to the parvocellular portion of the nucleus. In order to make appropriate quantitative comparison, cells were tested with the same iontophoretic currents for each drug. The minimum current used to produce an excitatory effect was 10nA. The discharge frequency of neurones showed a marked acceleration with an onset of usually less than 2 seconds. In contrast to the distinct location of NMDA-sensitive cells in *Ipc*, ACh almost equally affected neurones in both parts of the nucleus. Although the amount of current (20nA) to produce an excitatory effect was slightly higher, the average latencies for isthmus acetylcholine-induced excitation were 5.5 seconds. A

comparison of relative potencies of NMDA and ACh on lpc neurones showed, that out of 28 cells tested, 17 had stronger excitatory response to NMDA when similar amounts of current were applied, and the remaining 11 cells were equally affected by both agents.

NMDA receptors are one of the best characterized types in the central nervous system and mediate excitatory amino acid synaptic transmission. Having demonstrated that NMDA shows a highly potent excitatory action on lpc cells, we then proceeded to study the specific competitiveness of the NMDA antagonist CPP. In five experiments CPP was applied iontophoretically in doses from 70-150nA on lpc cells showing excitatory action to both NMDA and ACh. The data were analyzed with respect to changes in firing frequency, latency of onset and latency for recovery. In all cells tested, the NMDA-induced firing rate was antagonized by CPP in a reversible and specific manner, (Fig. 1B). The antagonistic effect started 1-3min and reached its maximum 3-5 min after the beginning of the application; complete recovery usually occurred 5-8 min after the termination of the ejection. The action of ACh was never blocked by CPP.

## Discussion

The electrophysiological data obtained in our investigations show striking differences in terms of neuronal sensitivity towards NMDA in magnocellular and parvocellular divisions of pigeon nucleus isthmi. This is strengthened by the fact, that ACh-induced activation did not differ in the two isthmic parts. The findings are in agreement with previously published data from Wang and coworkers<sup>21</sup> who showed that the two division of nucleus isthmi differentially modulated neuronal firing in avian optic tectum. It has been proposed, that the nucleus isthmi play an important role on visual processing, possibly involving delayed inhibitory effects<sup>23</sup> and prolonged excitation<sup>24</sup>. A positive feedback loop exists, originating in lmc and projecting into deeper layer of the tectum,<sup>10,14</sup> as well as an inhibitory feedback loop having its origin in the lpc and its terminals in superficial tectal layers,<sup>4,24</sup>. Microinjection of NMDA into the lpc resulted in a reduction of tectal firing, thus activating an inhibitory isthmo-tectal pathway<sup>21,25</sup>. The present results supports the idea that this activation might be mediated by the glutamatergic NMDA-receptor type. In contrast, the majority of lmc neurones did not respond to NMDA, suggesting that no NMDA receptor population exists in that area. Our data show, that lmc contains cholinceptive neurones. lmc projection terminates in deeper layer of the tectum<sup>6</sup>, its chemical nature of transmission is not yet known. The majority of visual neurones in the avian optic tectum receive an excitatory input from the lmc, suggesting that either an excitatory amino acid and/or acetylcholine might be

candidates as possible transmitters.

## **Conclusion**

In conclusion, the results of this study indicate that neurones in the optic tectum and the two subnuclei of the nucleus isthmi, lpc and lmc, are differentially activated by NMDA and ACh. These results extend and support previous observation and suggest that feedback loops between nucleus isthmi and the tectum can be modulated at the isthmic level by NMDA and/or ACh.

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## Figure Legends

Table 1. Summary of effects of N-methyl-D-aspartic acid (NMDA) and acetylcholine (ACh) in different areas of tectum opticum (TO) and subnuclei of nucleus isthmi (lpc, Imc).

Fig. 1. A. Effect of NMDA in the two parts of nucleus isthmi. All neurones were identified by histological techniques and mapped on the basis of their response: excitation (closed circles) or no effect (open circles). Anterior-posterior levels according to the atlas of Karten and Hodos<sup>22</sup>. Abbreviations: TO, tectum opticum; lpc, nucleus isthmi, pars parvocellularis; Imc, pars magnocellularis. B. Effect of NMDA iontophoretically applied (10 nA) and ACh (20 nA) before, during and after the ejection of the NMDA antagonist CPP (100 nA), illustrated on a continuously recorded integrated firing frequency. The ejected substances are indicated below and expressed in nanoampères.

Table 1

drugs	effects	TO superficial layers	TO deeper layers	lmc	lpc
NMDA	excitation	12	20	2	27
	no effect	12	2	21	1
ACh	excitation	16	10	23	24
	no effect	8	12	0	4
total. neuro nes		24	22	23	28