

## RESEARCH REPORT

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### TEGMENTAL INHIBITION ON ISTHMIC NEURONS IS MEDIATED BY THE DECUSSATIO VELI IN AMPHIBIANS

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*Summary:* This *in vitro* study on toads (*Bufo bufo gargarizans*) provides the first electrophysiological evidence that electrical stimulation of the decussatio veli in the midbrain tegmentum predominantly produced inhibition on neurons extracellularly recorded from the nucleus isthmi (NI). These cells were distributed throughout the nucleus. The present study lends strong support to the suggestion that the extratectal input to NI is mainly inhibitory and gives a good explanation for the previously reported inhibition of isthmic neurons by the contralateral optic nerve stimulation (Wu and Wang, 1995a).

*Key words:* brain slice, nucleus isthmi, tegmentum, decussatio veli, inhibition, amphibian

### INTRODUCTION

It has been generally accepted that the nonmalian nucleus isthmi (NI), a prominent midbrain visual structure, receives its input from the ipsilateral tectum, and projects back ipsilaterally in teleosts and birds, or bilaterally in amphibians and reptiles. It also has extratectal afferents from the nucleus pretectalis and the torus semicircularis in teleosts, from the anterodorsal tegmental nucleus (AD) in amphibians, and from the nucleus profundus mesencephali (NPM) in reptiles (for review see Wang, 1988). Recently, we have found that, in addition to AD reported by Udin (1987), NPM and the superficial isthmal reticular nucleus also project bilaterally to NI, and the contralateral projection crosses the midline through the decussatio veli (DV, Wu and Wang, 1995b). Moreover, AD and NPM in amphibians have been shown to be responsive to visual stimulation (Yan et al., 1983). Therefore,

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DV could transfer visual information from these tegmental nuclei to the contralateral NI. On the other hand, Wu and Wang (1995a) have pointed out that electrical stimulation of the optic nerve produces inhibitory postsynaptic potentials in neurons of the contralateral NI, and suggested that this inhibition may be indirectly evoked by a crossed pathway presumably consisting of the tectum, anterodorsal tegmental nucleus and the decussatio veli. To further examine this hypothesis in particular, and study the function of extratectal input to NI in general, we used an *in vitro* preparation of the amphibian midbrain to extracellularly record isthmic responses to electrical stimulation applied at the decussation, because in brain slices it is much easier to place both stimulating and recording electrodes at the correct sites.

## MATERIALS AND METHODS

The experiments were performed on the Chinese toads (*Bufo bufo gargarizans*) having body length of 7.0-10 cm from snout to vent. The animal was anesthetized with MS222 and decapitated, and the brain removed from the skull and placed in cold (4 C°) Ringer solution oxygenated with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The brain block was cut at 300-500 μm thickness with Vibroslice (Campden Instruments 752M). The cutting angle was so adjusted that slices could contain both NI and the decussation. The slices were placed into the recording chamber (Medical System Corp.), which was filled with Ringer solution bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, and overflowed with this gas mixture saturated with water vapor. The Ringer solution was made by dissolving 6.5 g NaCl, 0.14 g KCl, 0.12 g CaCl<sub>2</sub>, 0.20 g NaHCO<sub>3</sub>, 0.01 g NaH<sub>2</sub>PO<sub>4</sub>, and 2 g glucose in 1000 ml of distilled water.

The extracellular electrode was a micropipette (2-3 μm tip diameter) filled with 2M NaCl solution, which was advanced into NI under visual control with a hydraulic microdrive. A metal bipolar electrode with poles 400 μm apart was placed at the decussatio veli (Fig. 1) for delivering electrical stimulation with rectangular pulses of 0.3-0.5 mA intensity, 0.1 ms duration, and 0.1-0.05 Hz (WPI Pulsemaster A360). After isolating an isthmic neuron by its spontaneous activity, the effect of the decussation stimulation on neuronal activity was examined and recorded on magnetic tape (TEAC, RD-135T), and then off-line analysed with a computer. Neuronal discharge was superimposed for up to 40 sweeps to show clear effects.

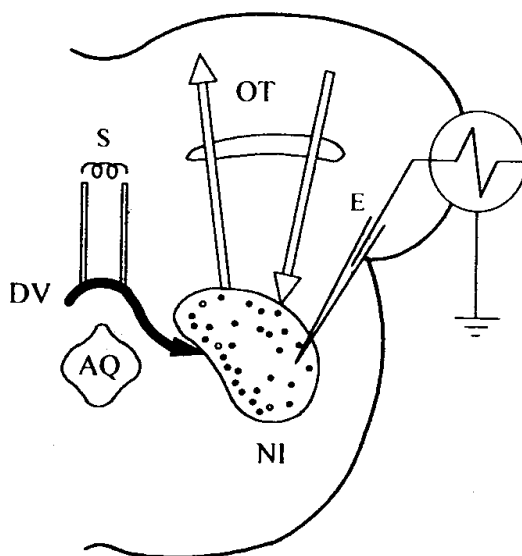


Fig. 1. Schematic cross-section showing experimental set-up and distribution of 30 recording sites. Empty arrows represent neuronal connections between the nucleus isthmi (NI) and the ipsilateral optic tectum (OT). Solid arrow denotes the decussatio veli (DV). Other abbreviations: AQ, Aqueduct; E, Recording electrode; S, Stimulating bipolar electrode. Symbols of recording sites: solid dots, units inhibited by electrical stimulation; circles, units excited by electrical stimulation

## RESULTS

In the present study, 77 cells were isolated throughout the whole nucleus by their spontaneous activity (0.1–6 spikes/sec), which was superimposed for up to 40 sweeps to show the effects of the decussation stimulation on the isthmic cells (Fig. 1). Among them, 27 cells (35%) produced a clear inhibitory period characterized by a short pause of their spontaneous activity (Fig. 2A). According to the latency and duration of inhibition evoked by electrical stimulation of the decussation, these cells could be grouped into two. The first group contained 21 cells, which had short latency ranging from 6 to 20 ms, with an average of  $11.0 \pm 9.1$  ms (mean  $\pm$  SD), and short duration of inhibition lasting 30–100ms and averaging  $70.3 \pm 25.1$  ms. They were mainly distributed in the peripheral cortex (Fig. 1). In the second group were 6 cells having long latency of  $58.3 \pm 15.5$  ms, and long-lasting inhibition of  $124.0 \pm 40.9$  ms. These cells were all localized in the medioventral region. Three cells (4%) were found to be sensitive to electrical shocks at the decussation by producing excitatory responses. Three to five shocks were enough to display a clear excitation, characterized by higher-frequency firings following electrical shocks (Fig. 2B). They were

spontaneously discharging 0.7, 1.8 and 1.9 spikes/sec, respectively. Their excitation latency ranged from 6 to 20 ms ( $11.3 \pm 6.2$  ms) and the excitation lasted 20–76 ms ( $48.6 \pm 22.8$  ms). These were localized in the medioventral and mediodorsal regions (Fig. 1). Forty-seven others (61%) did not respond at all to electrical shocks at the decussation, because no apparent excitation or inhibition occurred following up to 40 superimpositions (excitation only needed 3–5 sweeps to appear).

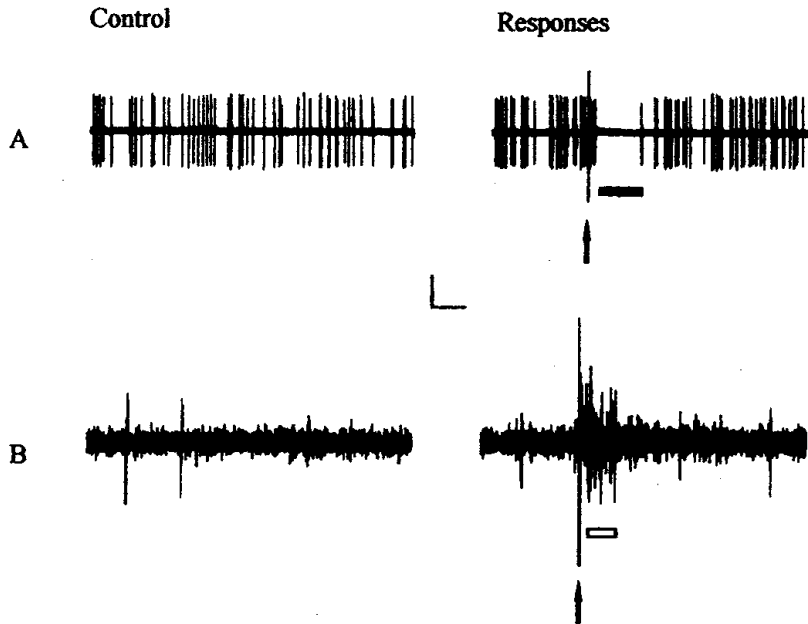


Fig. 2. Inhibitory (A) and excitatory (B) effects of electrical stimulation applied at the decussation on firings of isthmic neurons, superimposing 20 (A) or 3 (B) sweeps. Black and white bars represent inhibition and excitation, respectively; Arrows point to electrical stimulation artifacts. Scales: 0.2 mV, 200 ms in (A) and 0.05 mV, 50 ms in (B)

Therefore, most of them might have been inhibited by the decussation stimulation, but this inhibition was not clearly shown by even superimposing 40 sweeps, due to their very weak spontaneous activity. Considering this probable bias, it is reasonable to conclude that the decussatio veli may mainly exerts an inhibitory influence on isthmic cells.

## DISCUSSION

This study provides the first electrophysiological evidence that the decussatio veli may mainly inhibit isthmic cells. Anatomical studies have shown that the anterodorsal tegmental nucleus (Udin, 1987), as well as the nucleus

profundus mesencephali and the superficial isthmal reticular nucleus in amphibians (Wu and Wang, 1995b) project to the contralateral nucleus isthmi through the decussatio veli. These tegmento-isthmic fibers enter NI and therein give rise to ramifications, which make synaptic contacts with isthmic cells (Wu and Wang, 1995b). Similarly, Ito et al. (1982) have indicated that the nucleus pretectalis in the filefish projects to NI and the pretectoisthmic terminals contain flattened synaptic vesicles, and suggested that they may comprise an inhibitory input to NI. King and Schmidt (1993) also have proposed that the lateral thalamic nucleus in the goldfish provides a non-tectal inhibition to NI. This extratectal inhibition mediated by the nucleus profundus mesencephali has been suggested to explain so-called "in-out" visual responses recorded from the nucleus isthmi pars magnocellularis in reptiles (Wang et al., 1983; 1985). Electrophysiological and immunocytochemical studies have shown that  $\gamma$ -aminobutyric acid (GABA) inhibits isthmic neurons (Stoop et al., 1989), and there exists a dense plexus of GABAergic terminals in NI (Pollak et al., 1997). It is probable that at least some of GABAergic terminals may originate from the tegmental nuclei (Pollak et al., 1997). Our preliminary results (unpublished data) show that the inhibition induced by decussation stimulation could be blocked by bicuculline, a GABA<sub>A</sub> antagonist, but not by strychnine, a glycine antagonist, suggesting that the tegmento-isthmic fibers may be mainly GABAergic. Occasionally, some isthmic cells could be excited by the decussation stimulation. This action may result from direct excitation or disinhibitory effect by intervening interneurons. In fact, the decussatio veli contains a variety of fibers originating from different tegmental nuclei (Wu and Wang, 1995b), some of which may directly exert excitatory influence on isthmic cells. Intracellular recordings from the isthmic cells responding to tegmental stimulation are needed to further clarify the synaptic action of different tegmental nuclei on isthmic cells through the decussatio veli.

This study lends a considerable support to our previous suggestion that the optic tectum exerts inhibitory action on the contralateral NI by means of the anterodorsal tegmental nucleus and the decussatio veli (Wu and Wang, 1995a). This is based on the facts that the tectum projects to AD (Lázár et al., 1983; Masino et al., 1990), which is mainly related to visual information processing (Yan et al., 1983) and, in turn, also projects to the contralateral NI through the decussatio veli (Udin, 1987; Wu and Wang, 1995b). Moreover, tectal stimulation predominantly inhibits the contralateral NI (Wu and Wang, 1995a). The present study has confirmed that the tectotegmento-isthmic pathway is mainly inhibitory. The function of this kind of feedforward inhibition is suggested to limit reverberatory activity between the tectum and the nucleus isthmi (King and Schmidt, 1993), or fulfil an

indirect interaction between the two tectal hemispheres by the mediation of the nucleus isthmi.

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