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Magnocellular and parvocellular divisions of pigeon nucleus isthmi differentially modulate visual responses in the tectum

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Abstract The electrophysiological responses of 162 tectal cells to computer-generated visual stimuli were extracellularly recorded from 24 homing pigeons before and after injecting either lidocaine or *N*-methyl-D-aspartate (NMDA) into the nucleus isthmi pars magnocellularis (Imc) or the nucleus isthmi pars parvocellularis (Ipc). Micro-injections of lidocaine into Imc resulted in a significant reduction of firing rate in 80% of tectal cells, whose excitatory receptive fields (ERFs) were localized within the ERF of the Imc cell where the lidocaine was injected. In contrast, when lidocaine was injected into Ipc under identical circumstances it had no effect on the visually driven activity of 68% of tectal cells. However, when the excitatory amino acid NMDA was injected into Ipc it produced a significant reduction in the visually driven firing of 75% of tectal neurons when their ERFs were within the isthmic ERF, while similar application of NMDA into Imc had no effect on the visually driven response of 94% of tectal neurons. When the ERFs of tectal cells were localized outside the ERF of the isthmic cell where the chemical was injected, Imc-injected lidocaine had no effect in 9 out of 10 tectal cells, whereas Ipc-injected NMDA increased firing in 7 out of 17 tectal cells. Therefore, it is suggested that the Imc-tectal fibers participate in a positive feedback pathway and the Ipc-tectal fibers are involved in a negative feedback pathway.

Key words Optic tectum · Nucleus isthmi (Imc, Ipc) · Visual responses · Lidocaine · *N*-Methyl-D-aspartate (NMDA) · Pigeon

Introduction

Electrophysiological studies have shown that the nucleus isthmi (NI) in teleostean fish (Williams et al. 1983; Northmore 1991) and amphibians (Vinogradova and Manteifel 1977, 1979; Glasser and Ingle 1978; Gruberg and Lettvin 1980; Wang et al. 1981a, b, 1982; Felix et al. 1985; Wiggers and Roth, 1991), the nucleus isthmi pars magnocellularis (Imc) in reptiles (S.R. Wang et al. 1983), and the nucleus isthmi pars parvocellularis (Ipc) and its magnocellular partner in birds (Yan and Wang 1986; Wang and Frost 1991), as well as its mammalian analogue, the parabigeminal nucleus (Sherk 1978, 1979a, b) are all visual nuclei containing a retinotopic map. This nuclear complex receives its visual information from the ipsilateral tectum or superior colliculus by means of the tectoisthmic pathway or the colliculoparabigeminal pathway. These neuronal pathways are excitatory and possibly use acetylcholine (Hunt et al. 1976; Felix et al. 1985; Wang et al. 1985, 1986a; Li et al. 1987) and/or glutamate (Wang et al. 1985) as their physiological neurotransmitters.

This nucleus, or its subdivisions, primarily projects back to the tectum ipsilaterally in teleosts (Ito et al. 1981, 1982; Sakamoto et al. 1981; Sas and Maler 1986) and birds (Hunt and Künzle 1976; Hunt et al. 1977), or bilaterally in amphibians (Grobstein et al. 1978; Gruberg and Udin 1978; Gruberg and Lettvin 1980; Udin and Keating 1981; Y.T. Wang et al. 1983; Wiggers and Roth 1991), reptiles (S.R. Wang et al. 1983; Künzle and Schnyder 1984), and mammals (Graybiel 1978; Baley-dier and Magnin 1979; Watanabe and Kawana 1979; Mendez-Otero et al. 1980; Linden and Perry 1983; Roldan et al. 1983; Jen et al. 1984; Künzle and Schnyder 1984). It also has neural connections with the nucleus pretectalis (Ito et al. 1981, 1982) and the torus semicircularis (Sas and Maler 1986) in teleosts and with the anterodorsal tegmental nucleus in amphibians (Udin 1987), with the nucleus profundus mesencephali in reptiles (S.R. Wang et al. 1983), and with the dorsal lateral geniculate nucleus and the pulvinar nucleus in mammals

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(Graybiel 1978; Harting et al. 1986; Hashikawa et al. 1986; Diamond et al. 1992). However, very little is known about the physiological function of the isthmic efferents, especially those constituting the isthmotectal pathway.

It has been shown that the NI is the main source of cholinergic activity in the optic tectum or the superior colliculus (Ricciuti and Gruberg 1985; Wang et al. 1986a, b; Mufson et al. 1986; Desan et al. 1987; Li et al. 1987; Zottoli et al. 1988; Wallace et al. 1990), suggesting that the nucleus could modulate tectal activity via the cholinergic isthmotectal pathway. However, electrophysiological data about the functional role of acetylcholine in the tectum are in disagreement (Stevens 1973; Hock 1983; Fite and Wang 1986; Wang et al. 1986b). Behavioral tests with bilateral lesions of the amphibian NI have produced somewhat contradictory results (Collett and Udin 1983; Caine and Gruberg 1985; Gruberg et al. 1991). Furthermore, electrolytic lesions of the NI led to disinhibition of tectal cells, implying that the isthmotectal pathway is inhibitory (Glasser and Ingle 1978). This is consistent with the finding that the isthmotectal pathway may also contain inhibitory GABAergic and glycinergic fibers (Hunt and Künzle 1976; Reubi and Cuenod 1976; Hunt et al. 1977; Künzle and Schnyder 1984). Recently, Wang and Matsumoto (1990) have shown using intracellular recording and staining techniques, the electrical stimulation of the NI exerts inhibition on nearly 70%, and excitation on about 30% of the tectal cells recorded from bullfrogs. Therefore, it has been suggested that there exist at least two feedback loops between the tectum and the NI: a positive one which could provide an augmentation of certain activated loci, and a negative one that may inhibit other regions within the tectum (Serenio and Ulinski 1987; Wang and Frost 1991). Avian species may provide a useful model system to understand isthmic functioning because morphologically distinct neurons are segregated into separate nuclei.

In birds, there exist reciprocal projections between the tectum and the Ipc (Hunt and Künzle 1976; Hunt et al. 1977), but the Ipc circuitry is less clear. Although it is known that this latter subnucleus is visually responsive (Yan and Wang 1986) and projects to the ipsilateral tectum (Wang and Wang 1990), its afferent input is not known. Recently, Nealey et al. (1991) have shown that microinjection of lidocaine is a useful way to selectively block specific neuronal pathways in the visual system and thus determine their specific physiological role. In order to reveal the functional action of the Ipc and the Imc on tectal cells in pigeons, we studied visual responses of tectal cells before and after injecting lidocaine into either the Ipc or the Imc. Since lidocaine produced a significant decrease in visual responsiveness in tectal cells only when applied to Imc, the potent excitatory amino acid *N*-methyl-D-aspartate (NMDA) was used to reveal the differential role of Imc and Ipc on tectal responsiveness to visual stimuli.

Material and methods

The experiments were performed on 24 adult homing pigeons (*Columba livia*) anaesthetized with ketamine hydrochloride (initially 40 mg/kg then 20 mg/kg per h) and xylazine (initially 4 mg/kg then 2 mg/kg per h). The bird was placed in a stereotaxic apparatus and its body temperature maintained at 41°C by a heated water pad. The left optic tectum was surgically exposed and the dura removed. The pigeon was rotated so that it faced a large tangent screen 40 cm distant from the its right eye. The pecten was plotted onto tracing paper attached to the screen by the reversing ophthalmoscope technique, and the midline projection of the bird's beak and the horizon were marked as well.

Using coordinates from the atlas of the pigeon's brain (Karten and Hodos 1967) a tungsten-in-glass microelectrode (exposed tip 7–10 µm, impedance 4–5 MΩ) was advanced into either the Ipc or the Imc and a single visual cell was isolated. From our previous work (Wang and Frost 1991), we were able to recognise the characteristic response patterns and receptive field (RF) characteristics of Imc and Ipc units. The excitatory receptive field (ERF) of the isthmic cell was then precisely plotted on the tracing paper screen. The metal electrode was then replaced by a micropipette (20–30 µm tip diameter) filled with either lidocaine hydrochloride (2%) or NMDA (50 mM, pH 7.0). In some experiments, the micropipettes were loaded first with a saturated solution of Fast Green dye, then a thin layer of liquid paraffin was used to separate the dye from the lidocaine or NMDA located in the electrode tip. The micropipette was connected to a Pico-injector (Medical Systems Corp), so that the chemicals to be tested could be injected into the isthmic site where the visual unit was located, in volumes ranging from 10 to 50 nl. In several cases a normal saline solution was used as a control. At the conclusion of some experiments, Fast Green dye was injected for histological confirmation of the injection site.

Tungsten-in-glass microelectrodes were also used for isolating tectal cells whose visual ERFs were located within the larger elliptical ERF of the isthmic cell, and then their RFs were also mapped on the tracing paper screen. The visual stimulus used to characterize and quantify the tectal cell's response was a 2° black spot projected on the screen by an Electrohome ECP 4000 High Resolution projector controlled by a Silicon Graphics IRIS 4D/310 GTX graphics computer. This spot was moved through the ERF of the isolated tectal cell at a velocity of 10–15°/s and randomly presented in eight directions (0, 45, 90, 135, 180, 225, 270 and 315 degrees relative to the temporonasal direction). An interval of 3 s occurred between consecutive presentations of the visual stimulus. Three stimuli were presented for each direction in a randomly interleaved sequence. The random presentation of directions in combination with the 3-s inter-trial interval was sufficient to prevent habituation of the tectal cell. Electrophysiological responses were fed through a microelectrode AC amplifier (A-M systems, Model 1800) into a digital storage oscilloscope (Dynascan Corp, Model 2520) and a window discriminator (Neuro Feedback Instruments) connected with a custom-made data-collecting system which transferred the interspike intervals to the IRIS graphics computer after each stimulus presentation, and these accumulated responses were used to produce peri-stimulus-time histograms, and directional tuning curves.

At the end of some experiments, the Fast Green dye was pressure-injected into the isthmic site where the pharmacological agents had been applied. The brain was immediately removed from the skull, fixed in formaldehyde (10%) overnight and then immersed in 30% sucrose solution for several hours. Frozen sections of the brain blocks were cut at 80 µm thickness and counterstained with neutral red to histologically verify the position of micropipette tips in the isthmic area.

Results

A total of 180 units were extracellularly isolated in the optic tectum of 24 pigeons. Of these units, 162 were

Table 1 Classification of effects on tectal cells (*n* number of cells)

Site	Drug	Effect		
		Increase in activity <i>n</i> (%)	Decrease in activity <i>n</i> (%)	No effect <i>n</i> (%)
Imc	Lidocaine	2 (4)	41 (80)	8 (16)
		1 (10) ^a	0	9 (90) ^a
	NMDA	1 (6)	0	16 (94)
Ipc	Lidocaine	0	0	6 (100) ^a
		3 (12)	5 (20%)	17 (68)
	NMDA	0	27 (75)	9 (25)
		9 (53) ^a	1 (6) ^a	7 (41) ^a

^a The number of cells whose receptive fields were located outside of the receptive field of the isthmic cell where the drug injections were made

used to test application of drugs to the NI, 8 were for control observations, and 10 were used to establish criteria for altered responsiveness. Recording depths ranged from 45 μm to 1100 μm below the tectal surface, with two-thirds of the recorded cells having recording depths of less than 500 μm based on the micro-manipulator readings. To set a criterion for determining whether firing rate was increased, decreased or not affected in experimental observations, we collected data from 10 tectal units in four pigeons under conditions where they received no injections of the chemicals in either the Imc or the Ipc. The firing rates of these visual units for the eight directions of motion through their ERFs were measured. The statistics from the 10 visual units in these four pigeons showed that the least variable parameter was the

Fig. 1 A The histograms show the visual response of a typical tectal cell during control, administration of *N*-methyl-D-aspartate (NMDA) into nucleus isthmi pars parvocellularis (*Ipc*), and recovery (10 min after injection). Arrows underneath the figure indicate the directions of a 2° black spot moving through the excitatory receptive field (ERF) of the cell. Note that the visual responses were almost totally inhibited by microinjection of 10 nl NMDA (50 nM, pH 7.0) into *Ipc*. U, D, N, T represent up, down, nasal and temporal directions, respectively. B A total of 36 tectal cells were recorded whose ERFs were contained within the ERFs of *Ipc* cells where the NMDA was applied. Responsivities of these tectal cells during NMDA administration and recovery (*Recov*) are plotted against the control level (pre-drug application) firing rate. It is clear that these tectal cells decreased their firing rate by an average of 80% (slope=0.20) following injection and recovered to 89% (slope=0.89) of the control firing rate after 10 min. However, administration of NMDA into nucleus isthmi pars magnocellularis (*Imc*) had no effect on 17 tectal cells, as shown in C

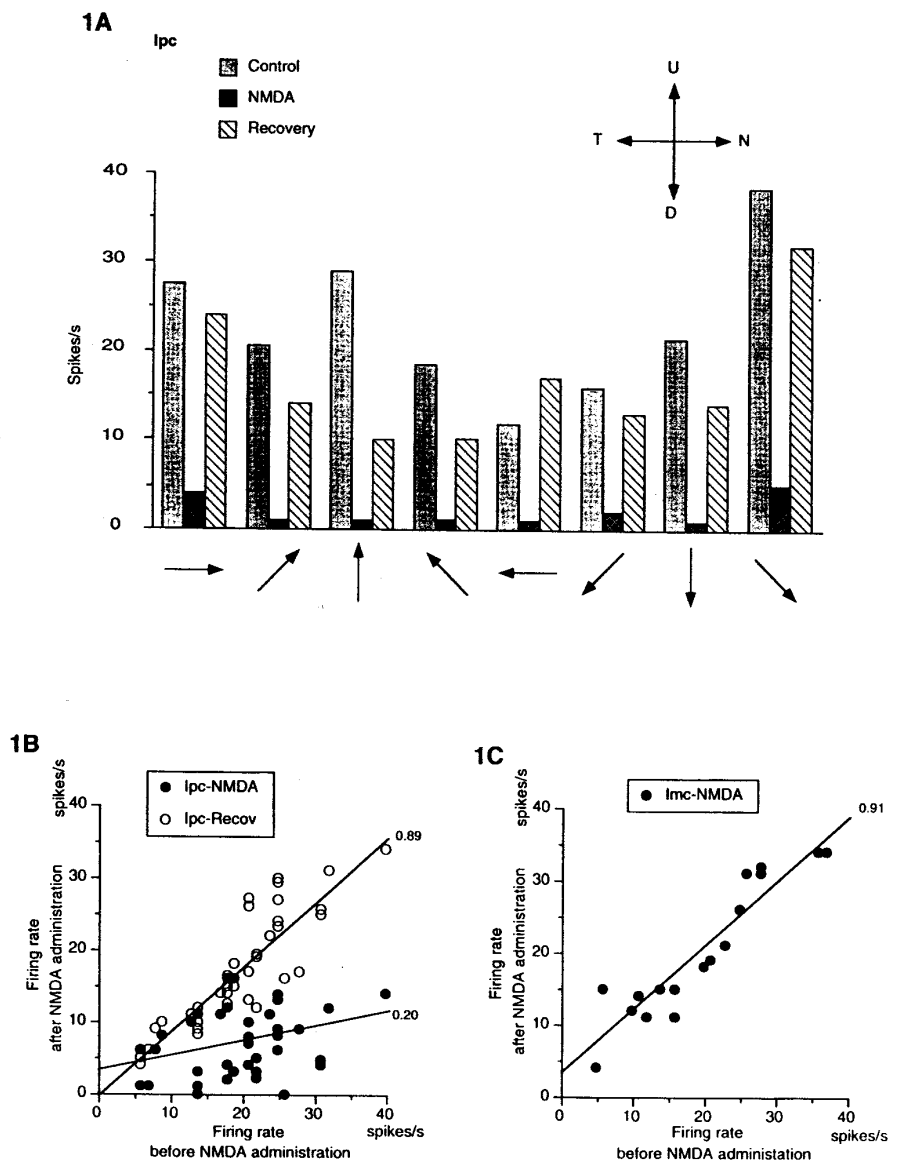
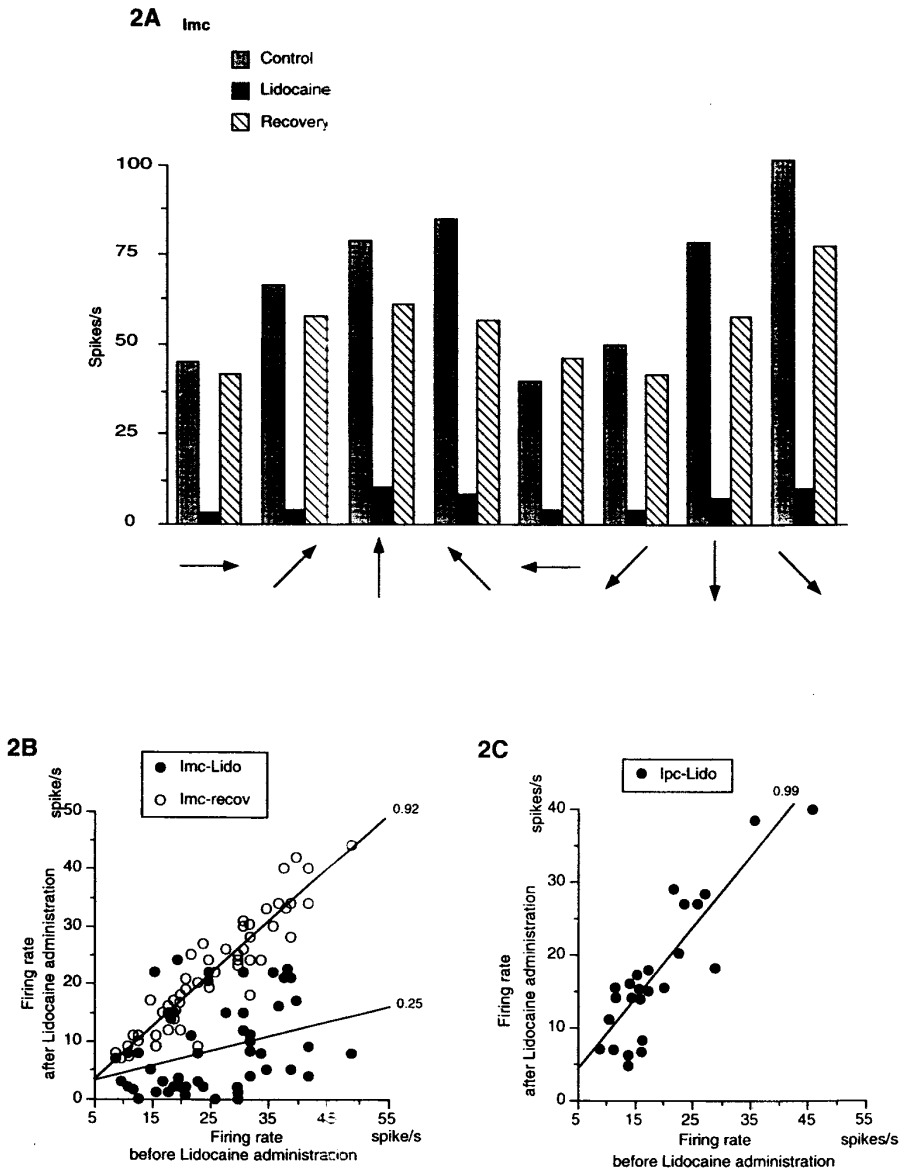


Fig. 2 **A** The visual response of a tectal cell during control, administration of lidocaine into Ipc, and recovery (10 min after injection). Note again that the visual responses are dramatically reduced following microinjections of 10 nl lidocaine hydrochloride (2%) into Ipc. **B** A total of 51 tectal cells were recorded whose ERFs were located within the ERFs of Ipc cells where the lidocaine was applied. Note that the visual response of these tectal cells decreased by 75% (slope=0.25) following injection, and recovered to 92% (slope=0.92) of control level after 10 min (*lido* lidocaine, *recov* recovery) Injection of lidocaine into Ipc had no effect on 25 tectal cells, as shown in **C**



percentage change of total number of spikes; the average percentage change was 14%, with a maximum of 25%. Therefore, we used a criterion of 30% change in total spike number to represent an index of change in a unit's activity.

It was our initial intention to selectively inactivate neurons in either Ipc or Ipc by the local injection of lidocaine. Preliminary observations revealed that while lidocaine produced measurable changes in tectal unit activity when applied to Ipc, it produced very little or no change in tectal activity when applied to Ipc. Consequently NMDA, a potent excitatory amino acid, was applied to Ipc and it was found that NMDA alone produced dramatic changes in tectal activity. The following experiments systematically explored this double dissociation

effect on tectal cells, between microinjections of lidocaine and NMDA, into either Ipc or Ipc.

Modulation of tectal activity by Ipc

The effects of Ipc injections of lidocaine (six pigeons) and NMDA (six pigeons) on 78 visual units in the optic tectum were examined (Table 1). Of 36 tectal units whose ERFs were located within the isthmus ERF, 27 units (75%) decreased their firing rate by an average of 80% following injections of NMDA into the Ipc.

In some units, the response to the visual target moving through the ERF in all eight directions was completely abolished (Fig. 1A). The firing rate of these units

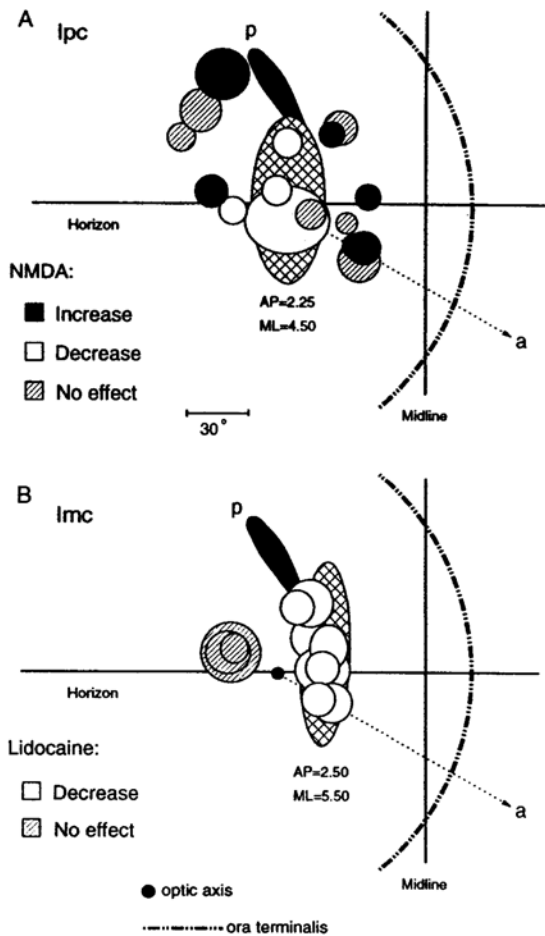


Fig. 3A–B The feedback effects were related to the topographic relation between ERFs of tectal cells and NI ERFs. **A** The results from an experiment on one bird where NMDA was injected into Ipc. Excitation of Ipc-tectal feedback by NMDA strongly inhibited the visual response of tectal neurons whose ERFs were located within the Ipc cells' ERFs; otherwise, there were either no effects or even slight increases in the responsiveness of tectal cells. **B** The results from another pigeon where lidocaine was injected into Imc. Removal of the tectal feedback pathway from Imc by lidocaine, on the other hand, significantly reduced the excitability of tectal cells. This figure shows that the effects were again topographically related. There was no effect on those cells whose ERFs were located outside of the Imc cells' ERFs. P, pecten; a, optic axis; the hatched ellipses symbolize the excitatory receptive field of the isthmic cells

returned to 89% of the original level 10–15 min after injection. A few tectal units completely recovered from the depression produced by the NMDA injection into Ipc. Another nine units (25%) did not respond to the Ipc injection of NMDA, suggesting that they received no input from the injected region. Figure 1B shows the effect of NMDA on the visually driven response of all 36 tectal cells whose ERFs were located within the isthmic ERF, and their subsequent recovery. The effect of NMDA on 17 tectal units whose ERFs were located outside the isthmic unit ERF was varied; nine units increased their firing

rate by an average of 45% (range 31% to 78%); seven units showed no obvious change; and one decreased its firing rate. The positions of micropipettes used to deliver NMDA were marked in the final penetration in five of six pigeons, and all were located within the Ipc (Fig. 4). Figure 1C shows that, in contrast, NMDA injected into Imc had little effect. Figure 3A shows the results from an experiment on one bird where NMDA injected into Ipc decreased the activity of tectal cells whose ERFs were within, or close to, the Ipc ERF, but for those tectal cells that were outside the ERF it had either no effect or increased their activity.

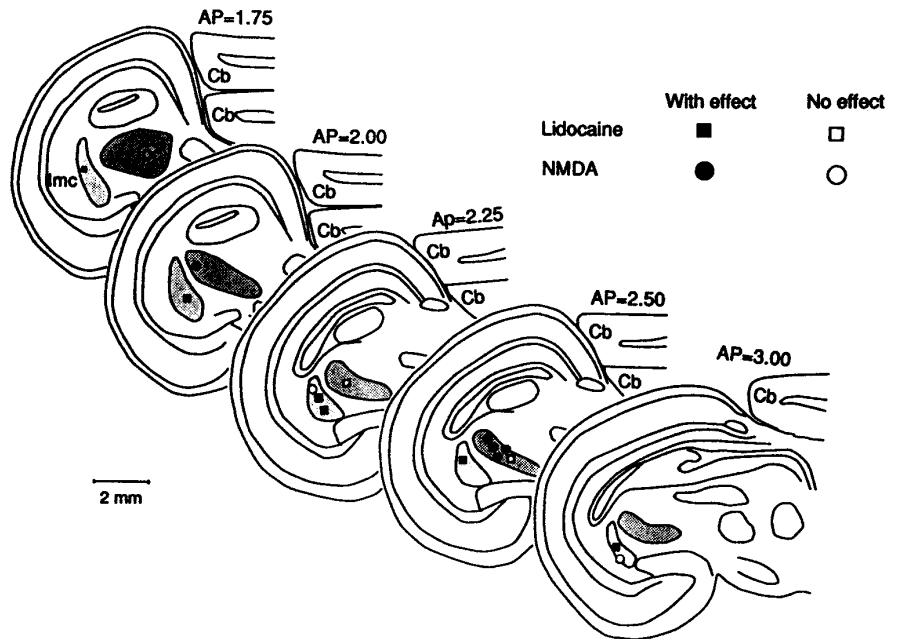
When lidocaine injections were made into the Ipc under identical conditions to those described above, 17 tectal units (68%) showed no responses to lidocaine injections. Five units (20%) decreased firing rate to the visual stimuli by an average of 54% (range 37–69%), while three units (12%) increased their firing rate by an average of 88% (range 48–112%). In two pigeons where Fast Green was injected at the final site investigated, the dye marking showed that the lidocaine-injected micropipette tips were located within Ipc (Fig. 4).

Modulation of visual activity by Imc

Eighty-four tectal units were tested for changes in their visual responsivity after lidocaine or NMDA injections into the Imc (eight pigeons) (Table 1). Of 51 tectal units whose ERFs were located within the ERF of the isthmic unit, 41 units (80%) decreased their visual responses by an average of 75% within 10–20 s after the injection of lidocaine. In some units, visual responses to all directions of target motion were completely abolished (Fig. 2A). These tectal units recovered their excitability by 92% (range 40–110%) 10–15 min after injection. Figure 3B shows the results from an experiment on one bird where lidocaine was injected into Imc. When the tectal ERFs were located within the Imc ERF, all cells showed a dramatic decrease in their firing rate, but when the tectal ERFs were outside the Imc ERF no change was noticed.

Some units took a shorter time (5 min) and others a longer time (45 min) to return to about 92% of their control firing level. Eight units (16%) showed no change to lidocaine injected in the Imc, suggesting that these units did not receive any input from the injected part of Imc. Two units (4%) increased their visually driven firing rate by 60% and 110%, respectively. Data for the effect of lidocaine on all 51 tectal units whose ERFs were located within the Imc ERF are presented in Fig. 2B. Of 10 tectal units whose ERFs were located outside the isthmic ERF, nine units were not affected by Imc lidocaine injections. The remaining unit increased its firing by 50%, but this unit was spontaneously bursting. Figure 2C shows that in contrast, lidocaine injections into Ipc have minimal effect. Fast Green markings in five pigeons whose tectal units were examined for the effects of lidocaine showed that the micropipette tips were all positioned within the Imc (Fig. 4).

Fig. 4 Histological confirmation of some injection sites in the Ipc and Imc where lidocaine (*squares*) and NMDA (*circles*) were applied (AP anterior-posterior, Cb cerebellum)



A series of control observations were made in one pigeon to determine whether the pressure injection itself into either Ipc or Imc might modify the tectal neurons' responses. Whereas the visually elicited firing rate of the first three tectal units encountered was depressed by an average 90% after lidocaine was injected into the topologically overlapping area of Imc, when a saline solution (0.9%) was injected through the same micropipette, at the same site, no modulation of visually driven tectal firing rate was found. For example, the number of visual spikes produced by one of these units was decreased significantly, by 98%, following the injection of 10 nl of lidocaine into Imc, but this unit was not affected at all by 50 nl of saline injected at the same site. Similar saline injections made in the Imc produced no effects on another five (well-isolated) tectal units in three penetrations. This indicates that pressure itself did not produce the effects reported here. The position of the micropipette tip for injecting both lidocaine and the saline solution was marked by Fast Green and histologically verified to be located within the Imc at AP 3.00 and ML 5.5 mm (Fig. 4).

The visual responsivity of 23 tectal units in three pigeons was studied following NMDA microinjections into the Imc. Of 17 units whose ERFs were located within the ERF of the isthmus unit, 16 units (94%) were not affected by the drug. One unit (6%) slightly increased its firing rate by 34%. These data are presented in Fig. 1C for comparison. Six other units, whose ERFs were located outside the isthmus unit ERF, did not respond to the application of NMDA to the Imc. The dye markings made in two of these pigeons showed that the injection sites were localized in the Imc (Fig. 4).

Discussion

The present study shows that microinjections of lidocaine in the Imc and of NMDA in the Ipc potently depress visual responses of tectal units, which receive their input from the same retinotopic area of Imc or Ipc. In contrast, microinjections of lidocaine into Ipc and NMDA into Imc under identical conditions had little or no effect on the visually driven responses of tectal neurons. Collectively, these results lend strong support to the notion that Imc provides positive feedback to the tectum while Ipc provides negative feedback to the tectum. These effects are not due to hydraulic pressure or diffusion of the injected drugs. Firstly, the control experiments showed no effects (on tectal units) of saline solution injected at even five times the volume of the drugs. Secondly, latencies (10–15 s) were too short for the drugs to diffuse from the injection site to the recording site, which was located at least 3000–3800 μm away. There was also no dye visible on the tectal surface near the entry point of the micropipette, or at the even more remote tectal recording sites, when Fast Green was injected into Imc or Ipc in much larger volumes. Thirdly, there was a strong retinotopic relationship between the receptive fields at the NI injection sites and the location of tectal units responding to microinjection of drugs, even though these sites were very remote from each other. Generally speaking, the tectal units whose ERFs were outside the isthmus ERF showed no effect or an opposite response, implying that the action of the drugs was restricted to a very circumscribed region around the injection site.

The specificity, reproducibility and reversibility of the effects of these drugs on visually elicited responses lend

very strong support to the notion that these drug actions are pharmacological and not toxicological. It is known that lidocaine is a local anaesthetic which operates as a Na⁺ channel blocker, and NMDA is a more potent excitant than L-glutamate in the nervous system. This study shows that lidocaine has a dramatic effect on the tectal cells when injected into the spatially corresponding visual area in the Imc but not in the Ipc, while NMDA functions as an excitatory amino acid in the Ipc but not in the Imc. The most plausible explanation why NMDA is effective only in the Ipc, is the possible existence of NMDA receptors on the isthmotectal projection neurons and/or interneurons intrinsic to the Ipc. A recent study lends strong support to this suggestion by showing that 96% of the recorded Ipc cells in urethane-anaesthetised pigeons were sensitive to iontophoretically applied NMDA, whereas the proportion in Imc was only 9% (Wu et al. 1994).

The most plausible explanation for observing no effect on the visual responsiveness of tectal cells when lidocaine is injected into Ipc is that Ipc-tectal connections are inhibitory and therefore lidocaine blockage of nerve conduction results in no observable effect when tectal cells are visually stimulated. However, lidocaine blockade of the excitatory Imc-tectal pathway, in contrast, results in the reduction of visually driven activity in tectal cells. This is somewhat analogous to the finding that lidocaine blockade of the lateral geniculate nucleus (LGN) reduces cortical responses by 28–42%, suggesting that cortical elements receive substantial excitatory drive from the LGN (Nealey et al. 1991).

One question that arises from this study is why the anaesthetic ketamine, one of the non-competitive NMDA antagonists, did not block NMDA receptors in Ipc and render our local microinjections ineffective. One possible reason is that NMDA receptors in this nucleus might be particularly resistant to blockage by the anaesthetic, while another explanation could be that repeated applications of NMDA might accelerate the recovery of responses from ketamine blockage (for review see Kemp et al. 1987). In any event, the fact that similar results to those reported here have been obtained in pigeons under urethane anaesthesia (Felix et al. 1994) suggests that they are unlikely to be the result of the anaesthetic agent or its differential effect on these two structures.

The main result of this study suggests that the majority of visual neurons in the avian optic tectum may receive an excitatory input from the Imc and an inhibitory input from the Ipc. When the excitatory Imc-tectal pathway is *blocked* by lidocaine, it reduces the visual responsiveness of many tectal cells. In contrast, when the inhibitory Ipc-tectal pathway is blocked by lidocaine, tectal cells retain their visual responsiveness. On the other hand, when the Ipc-tectal pathway is *activated* by the application of NMDA, there is a reduction of visual responsiveness of tectal neurons. It is known that in birds, the Ipc receives its input from the ipsilateral tectum and projects back ipsilaterally (Hunt and Künzle 1976; Hunt et al. 1977), while the Imc projects its output to the

deeper layers of the ipsilateral tectum (Wang and Wang 1990); however, the pathway afferent to the Imc is still not clear. It appears from our data that the Imc efferent fibers are directly or indirectly connected with tectal neurons or optic terminals. Several electrophysiological studies (Yan and Wang 1986; Wang and Frost 1991) have indicated excitatory input from the tectum to both the Imc and the Ipc. Therefore, our data lend considerable support to the suggestion that there exist both a positive feedback loop and a negative feedback loop between the optic tectum and NI (Sereno and Ulinski 1987; Wang and Frost 1991). This hypothesis is also consistent with the findings that the isthmotectal pathway may contain inhibitory GABAergic and glycinergic fibers (Hunt and Künzle 1976; Reubi and Guenod 1976; Hunt et al. 1977; Künzle and Schnyder 1984), as well as excitatory cholinergic fibers (Ricciuti and Gruberg 1985; Fite and Wang 1986; Mufson et al. 1986; Wang et al. 1986a, b; Desan et al. 1987; Li et al. 1987; Wallace et al. 1990) and glutamatergic fibers (Stoop et al. 1989). Wang and Matsumoto (1990) have recently demonstrated the existence of both excitatory and inhibitory pathways from the NI to the optic tectum in bullfrogs. Interestingly, recent studies on pigeons have shown that electrical stimulation of Ipc exerts inhibition on tectal cells, which can be blocked by iontophoretically applied bicuculline, a GABA antagonist, while activation of tectal cells by Imc stimulation can be blocked by either CPP (NMDA antagonist) or atropine (acetylcholine antagonist) (Felix et al. 1994).

It has been proposed that the NI may play a critical modulatory role on visual processing within the optic tectum, possibly involving delayed inhibition effects (Bagnoli et al. 1979) or delayed, prolonged excitation (see Sereno and Ulinski 1987). It has also been suggested that interaction between the retinotectal and the isthmotectal visual map could be used to calculate the three-dimensional trajectories of moving objects, such as prey (Wiggers and Roth 1991). The present study shows that the two subnuclei of the avian NI, the Ipc and the Imc, have differential modulation effects on tectal cells. These findings are quite consistent with the view that the positive feedback loop and the negative feedback loop may work together in a "winner-take-all" network to permit the bird to orient ("attend") to only one of several competing moving visual stimuli simultaneously present in the visual field (Sereno and Ulinski 1987; Wang and Frost 1991). This notion should be further examined by electrophysiological recordings and behavioral tests.

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References

- Bagnoli P, Francesconi W, Magni F (1979) Interaction of optic tract and visual wulst impulses on single units of the pigeon's optic tectum. *Brain Behav Evol* 16:19-37
- Baleyrier C, Magnin M (1979) Afferent and efferent connections of the parabigeminal nucleus in cat revealed by retrograde axonal transport of horseradish peroxidase. *Brain Res* 161:187-198
- Caine HS, Gruberg ER (1985) Ablation of nucleus isthmi leads to loss of specific visually elicited behaviors in the frog *Rana pipiens*. *Neurosci Lett* 54:307-312
- Collett TS, Udin SB (1983) The role of the toad's nucleus isthmi in prey-catching behavior. In Lara R, Arbib MA (eds) *Proceedings of the Second Workshop on Visuomotor Coordination in Frog and Toad: models and experiments*. COINS Tech Rep 83-19, pp 117-135
- Desan PH, Gruberg ER, Grewell KM, Eckenstein F (1987) Cholinergic innervation of the optic tectum in the frog *Rana pipiens*. *Brain Res* 413:344-349
- Diamond IT, Fitzpatrick D, Conley M (1992) A projection from the parabigeminal nucleus to the pulvinar nucleus in *Galago*. *J Comp Neurol* 316:375-382
- Felix D, Wang SR, Yan K, Wang YT (1985) The effect of acetylcholine on neurons of the amphibian nucleus isthmi. *Brain Res* 326:313-316
- Felix D, Wu GY, Wang SR (1994) GABA as an inhibitory transmitter in the pigeon isthmo-tectal pathway. *Neurosci Lett* 169:212-214
- Fite KV, Wang SR (1986) Microiontophoresis and single-unit analysis of cholinergic drugs in the optic tectum of frog. *Brain Behav Evol* 28:198-206
- Glasser S, Ingle DJ (1978) The role of the nucleus isthmi in the ipsilateral visual projection to the frog's optic tectum. *Soc Neurosci Abstr* 4:630
- Graybiel AM (1978) A satellite system of the superior colliculus: the parabigeminal nucleus and its projections to the superficial collicular layers. *Brain Res* 145:365-374
- Grobstein P, Comer C, Hollyday M, Archer SM (1978) A crossed isthmo-tectal projection in *Rana pipiens* and its involvement in the ipsilateral visuotectal projection. *Brain Res* 156:117-123
- Gruberg ER, Lettvin JY (1980) Anatomy and physiology of a binocular system in the frog *Rana pipiens*. *Brain Res* 192:313-325
- Gruberg ER, Udin SB (1978) Topographic projections between the nucleus isthmi and the tectum of the frog *Rana pipiens*. *J Comp Neurol* 179:487-500
- Gruberg ER, Wallace MT, Caine HS, Mote MI (1991) Behavioral and physiological consequences of unilateral ablation of the nucleus isthmi in the leopard frog. *Brain Behav Evol* 37:92-103
- Harting JK, Hashikawa T, Van Lieshout D (1986) Laminar distribution of tectal, parabigeminal and pretectal inputs to the primate dorsal lateral geniculate nucleus: connectional studies in *Galago crassicaudatus*. *Brain Res* 366:358-363
- Hashikawa T, Van Lieshout D, Harting JK (1986) Projections from the parabigeminal nucleus to the dorsal lateral geniculate nucleus in the tree shrew *Tupaia glis*. *J Comp Neurol* 246:382-394
- Hock FJ (1983) Cholinergic system in the toad's (*Bufo bufo* L.) visual system. *Behav Neural Biol* 38:313-316
- Hunt SP, Künzle H (1976) Observations on the projections and intrinsic organization of the pigeon optic tectum: an autoradiographic study based on anterograde and retrograde, axonal and dendritic flow. *J Comp Neurol* 170:153-172
- Hunt SP, Henke H, Künzle H, Reubi JC, Schenker T, Streit P, Felix D, Cuenod M (1976) Biochemical neuroanatomy of the pigeon optic tectum. *Exp Brain Res Suppl* 1:521-525
- Hunt SP, Streit P, Künzle H, Cuenod M (1977) Characterization of the pigeon isthmo-tectal pathway by selective uptake and retrograde movement of radioactive compounds and by Golgi-like horseradish peroxidase labelling. *Brain Res* 129:197-212
- Ito H, Tanaka H, Sakamoto N, Morita Y (1981) Isthmic afferent neurons identified by the retrograde HRP method in a teleost, *Navodon modestus*. *Brain Res* 207:163-169
- Ito H, Sakamoto N, Takatsuji K (1982) Cytoarchitecture, fiber connections, and ultrastructure of nucleus isthmi in a teleost (*Navodon modestus*) with a special reference to degenerating isthmic afferents from optic tectum and nucleus pretectalis. *J Comp Neurol* 205:299-311
- Jen LS, Dai ZG, So KF (1984) The connections between the parabigeminal nucleus and the superior colliculus in the golden hamster. *Neurosci Lett* 51:189-194
- Karten HJ, Hodos W (1967) A stereotaxic atlas of the brain of the pigeon (*Columba livia*). Johns Hopkins Press, Baltimore, Md
- Kemp JA, Foster AC, Wong EHF (1987) Non-competitive antagonists of excitatory amino acid receptors. *Trends Neurosci* 10:294-298
- Künzle H, Schnyder H (1984) The isthmus-tegmentum complex in the turtle and rat: a comparative analysis of its interconnections with the optic tectum. *Exp Brain Res* 56:509-522
- Li Z, Wang SR, Xu HY, Yan K (1987) Acetylcholinesterase staining patterns of the tectum-nucleus isthmi systems in frogs and pigeons. *Acta Anat Sin* 18:42-47
- Linden R, Perry VH (1983) Retrograde and anterograde-transneuronal degeneration in the parabigeminal nucleus following tectal lesions in developing rats. *J Comp Neurol* 218:270-281
- Mendez-Otero R, Rocha-Miranda CE, Perry VH (1980) The organization of the parabigemino-tectal projections in the opossum. *Brain Res* 198:183-189
- Mufson EJ, Martin TL, Mash DC, Wainer BH, Mesulam MM (1986) Cholinergic projections from the parabigeminal nucleus (Ch 8) to the superior colliculus in the mouse: a combined analysis of horseradish peroxidase transport and choline acetyltransferase immunohistochemistry. *Brain Res* 370:144-148
- Nealey TA, Ferrera VP, Maunsell JHR (1991) Magnocellular and parvocellular contributions to the ventral extrastriate cortical processing stream. *Soc Neurosci Abstr* 17:525
- Northmore DPM (1991) Visual responses of nucleus isthmi in a teleost fish (*Lepomis macrochirus*). *Vision Res* 31:525-535
- Reubi JC, Cuenod M (1967) Release of exogenous glycine in the pigeon optic tectum during stimulation of a midbrain nucleus. *Brain Res* 112:347-361
- Ricciuti AJ, Gruberg ER (1985) Nucleus isthmi provides most tectal choline acetyltransferase in the frog *Rana pipiens*. *Brain Res* 341:339-402
- Roldan M, Reinoso-Suarez F, Tortelly A (1983) Parabigeminal projections to the superior colliculus in the cat. *Brain Res* 280:1-13
- Sakamoto N, Ito H, Ueda S (1981) Topographic projections between the nucleus isthmi and the optic tectum in a teleost, *Navodon modestus*. *Brain Res* 224:225-234
- Sas E, Maler L (1986) Identification of a nucleus isthmi in the weakly electric fish *Apteronotus leptorhynchus* (Gymnotiformes). *Brain Behav Evol* 28:170-185
- Sereno MI, Ulinski PS (1987) Caudal topographic nucleus isthmi and the rostral nontopographic nucleus isthmi in the turtle, *Pseudemys scripta*. *J Comp Neurol* 261:319-346
- Sherk H (1978) Visual response properties and visual field topography in the cat's parabigeminal nucleus. *Brain Res* 145:375-379
- Sherk H (1979a) A comparison of visual-response properties in cat's parabigeminal nucleus and superior colliculus. *J Neurophysiol* 42:1640-1655
- Sherk H (1979b) Connections and visual-field mapping in cat's tectoparabigeminal circuit. *J Neurophysiol* 42:1656-1668
- Stevens RJ (1973) A cholinergic inhibitory system in the frog optic tectum: its role in visual electrical responses and feeding behavior. *Brain Res* 49:309-321
- Stoop R, Wang SR, Zhu YY, Xu HY (1989) Sensitivity of avian mesencephalic neurons to amino acids. *Chin Sci Bull* 34:1736-1739
- Udin SB (1985) The role of visual experience in the formation of binocular projections in frogs. *Cell Mol Neurobiol* 5:85-102

- Udin SB (1987) A projection from the mesencephalic tegmentum to the nucleus isthmi in the frogs, *Rana pipiens* and *Acris crepitans*. *Neuroscience* 21:631-638
- Udin SB, Keating MJ (1981) Plasticity in a central nervous pathway in *Xenopus*: Anatomical changes in the isthmotectal projection after larval eye rotation. *J Comp Neurol* 203:575-594
- Vinogradova VM, Manteifel TB (1977) Neuronal responses in the nucleus isthmi area of the frog to optic nerve stimulation (in Russian) *Neurophysiol* 9:33-40
- Vinogradova VM, Manteifel TB (1979) Neuronal reactions of the isthmi nucleus area of the frog *Rana temporaria* to visual stimulation (in Russian) *J Evol Biochem Physiol* 15:172-178
- Wallace MT, Ricciuti AJ, Gruberg ER (1990) Nucleus isthmi: its contribution to tectal acetylcholinesterase and choline acetyltransferase in the frog *Rana pipiens*. *Neuroscience* 35:627-636
- Wang SR, Matsumoto N (1990) Postsynaptic potentials and morphology of tectal cells responding to electrical stimulation of the bullfrog nucleus isthmi. *Vis Neurosci* 5:479-488
- Wang SR, Yan K, Wang YT (1981a) Visual field topography in the frog's nucleus isthmi. *Neurosci Lett* 35:37-41
- Wang SR, Yan K, Wang YT (1981b) Visual field topography and binocular responses in the frog's nucleus isthmi. *Sci Sin* 24:1292-1301
- Wang SR, Yan K, Wang YT (1982) Nucleus isthmus of toad is secondary visual center. *Sci Sin B* 25:1172-1178
- Wang SR, Yan K, Wang YT, Jiang SY, Wang XS (1983) Neuroanatomy and electrophysiology of the lacertilian nucleus isthmi. *Brain Res* 275:355-360
- Wang SR, Yan K, Xu HY (1985) Chemosensitivity of the frog isthmic neurons to acetylcholine, glutamate and glycine. *Kexue Tongbao* 30:1681-1683
- Wang SR, Wang YT, Wang XS (1986a) The distribution of acetylcholinesterase in the nucleus isthmi of amphibians and reptiles. *Kexue Tongbao* 31:700-702
- Wang SR, Li Z, Xu HY (1986b) Muscarinic action of acetylcholine in the pigeon optic tectum. *Exp Neurol* 94:436-440
- Wang YC, Frost BJ (1991) Visual response characteristics of neurons in the nucleus isthmi parvocellularis of pigeons. *Exp Brain Res* 87:624-633
- Wang YT, Wang SR (1990) Applications of a carbocyanine fluorescent dye to tracing neuronal connections of the pigeon tectum with some mesencephalic nuclei (in Chinese). *Acta Biophys Sin* 6:112-116
- Wang YT, Yan K, Wang SR (1983) Reciprocal topography between the toad's tectum and nucleus isthmi and cell classification. *Kexue Tongbao* 28:1681-1684
- Watanabe K, Kawana E (1979) Efferent projections of the parabigeminal nucleus in rats: a horseradish peroxidase (HRP) study. *Brain Res* 168:1-11
- Wiggers W, Roth G (1991) Anatomy, neurophysiology and functional aspects of the nucleus isthmi in salamanders of the family Plethodontidae. *J Comp Physiol [A]* 169:165-176
- Williams B, Hernandez N, Vanegas H (1983) Electrophysiological analysis of the teleostean nucleus isthmi and its relationships with the optic tectum. *J Comp Physiol* 152:545-554
- Wu GY, Wang SR, Felix D (1994) Effect of acetylcholine and NMDA on neurones of avian tectum and nucleus isthmi. *Nuereport* 5:850-852
- Yan K, Wang SR (1986) Visual responses of neurons in the avian nucleus isthmi. *Neurosci Lett* 64:340-344
- Zottoli SJ, Rhodes KJ, Corrodi JG, Mufson EJ (1988) Putative cholinergic projections from the nucleus isthmi and the nucleus reticularis mesencephali to the optic tectum in the goldfish (*Carassius auratus*). *J Comp Neurol* 273:385-398