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Directional responses of basal optic neurons are modulated by the nucleus lentiformis mesencephali in pigeons

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Abstract

The nucleus lentiformis mesencephali (nLM) and the nucleus of the basal optic root (nBOR) in pigeons are both involved in optokinetic nystagmus. They are reciprocally connected and thus may interact with each other. The present study injected lidocaine into nLM and then examined the effects of nLM blockade on visual responses of nBOR neurons to target motion. The results indicate that nLM could modulate nBOR activity in two ways. First, nLM enhances visual responses of 70% of nBOR cells to motion in the preferred directions, sharpening their directional tuning. Second, nLM reduces visual responses of 13% of nBOR cells to motion in the preferred directions, broadening their directional tuning. The remaining 17% of nBOR cells are not affected by drug application. Taken together with the previous results that nBOR could modulate nLM activity (Gu et al., Neuroscience, 104 (2001) 153), it suggests that both nuclei can mutually modulate each other in generating optokinetic nystagmus.

Keywords: Accessory optic system; Directional selectivity; Optokinetic nystagmus; Pretectum; Receptive field

The nucleus lentiformis mesencephali (nLM) in the pretectum and the nucleus of the basal optic root (nBOR) of the accessory optic system in nonmammals are both involved in generating optokinetic nystagmus, which stabilizes images on the retina by compensatory movement of the eyes. In the pigeon, the receptive field of optokinetic neurons is composed of excitatory and inhibitory regions, both of which have opposite directionalities and may spatially overlap or occupy different regions of the visual field [7,8,21,24,27]. Most directional cells in the pigeon nLM prefer temporonasal motion [2,7,8,21,23,25], whereas those in the pigeon nBOR are predominantly sensitive to motion in the dorsoventral, ventrodorsal and nasotemporal directions [4,11,18,22,24,27]. It appears that both nuclei may play different [21] but complementary roles in generating the optokinetic reflex.

Anatomical studies [1,3,10,16,26] have verified the existence of neuronal connections between nLM and nBOR in birds, implying functional interactions between both optokinetic nuclei. It was shown that visual responses of nLM neurons can be modulated by nBOR activity [2,12] in a direction-dependent way, and that the pigeon nLM mainly excites nBOR units with temporonasal directionality and inhibits those with nasotemporal preference [17]. However, the effects of nLM on nBOR were obtained by examining changes in the spontaneous activity of nBOR cells following electrical stimulation of nLM. To further reveal the physiological action of pretectal activity on the visual responses and directional selectivity of nBOR cells, the present study was undertaken to quantitatively analyze changes in the visual responses and directional selectivity of nBOR neurons during blockade of nLM by lidocaine, which is a useful tool for studying functional interactions between visual structures [6,20].

Seventeen pigeons *(Columba livia)* having body weights of 300–420 g were used following the policy on the use of animals in neuroscience research approved by the Society for Neuroscience. The pigeons were anesthetized with urethane (20%, 1 ml/100 g), and then placed in a stereotaxic apparatus. The rostral tectum and caudal forebrain on the left side were exposed, and the dura mater overlying nLM and nBOR was excised. The right eye was kept open and the left covered. A screen of 130×140 ° was placed 40 cm away from the viewing eye. The horizontal and vertical meridians of the visual field on the screen were rotated clockwise by 38° [4,7] to meet the pigeon's normal conditions [5]. The receptive field of nBOR neurons was plotted with a hand-held target. A black bar of 6° in width and 130°

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in length was generated with a workstation (Silicon Graphics Indigo 2) and back-projected onto the screen with a projector (Electrohome ECP4). The bar was always oriented perpendicular to its direction of motion, and moved at velocities of 6–45°/s in eight directions (spaced by 45° with nasal 0°) randomly to determine the preferred direction of a given nBOR cell. The optimal velocity of the cell was then determined in a velocity range of 6–45°/s in the preferred direction.

The accessory optic nucleus and the pretectal nucleus were stereotaxically approached according to the pigeon brain atlas [14]. Visual responses of nBOR cells were recorded with a pipette (1–3 μm tip diameter) filled with 2M sodium acetate and 2% pontamine-skyblue [13], amplified and fed into the workstation for on-line analysis. A two-barrel pipette (5–10 μm) was filled with 2 M sodium acetate and 2% pontamine-skyblue in a recording channel and 2% lidocaine hydrochloride in a drug application channel. The recording channel was used for further electrophysiological localization of nLM and marking pipette tip sites, and the drug channel was connected to a pneumatic picopump (PV800, Medical Systems Corp.) for applying drug. Lidocaine was pressure-injected into nLM in volumes of 40–120 nl to block nLM-nBOR transmission. Before, during and after lidocaine application, the total number of spikes was accumulated and an average firing rate obtained for three sweeps in each of four orthogonal directions including the preferred direction. Paired *t*-tests were statistically made between the control and experimental values.

At the end of some experiments, the recording sites in nBOR and drug sites in nLM were marked with dye by using negative current pulses of 10–20 μA in intensity and 0.5 s in duration at 1 Hz for 10–15 min. Under deep anesthesia, the brain was removed from the skull and fixed in 4% paraformaldehyde for 6–12 h and soaked in 30% sucrose solution in a refrigerator overnight. Frozen sections were cut at 100 μm thickness and counterstained with cresyl violet. Sections were dehydrated and covered for subsequent microscopic observation of the recording sites of nBOR cells and of lidocaine administration sites within the pretectal nucleus.

Thirty visual neurons were recorded from nBOR and all identified as directional cells. Twenty-five of these were spontaneously discharging at an average rate of 13.5 ± 8.6 spikes/s (mean ± SD). Following an injection of lidocaine into nLM, spontaneous rates in four cells were decreased to 74.4 ± 12.4% and those in three cells increased to 133 ± 18.6% of the control values. Eighteen others did not show a significant change in spontaneous rates during lidocaine ($t = 0.06$, $n = 18$, $P > 0.01$). Histological markings verified that lidocaine-injection sites were in nLM and recording sites in nBOR (Fig. 1).

Effects of nLM blockade on visual responses of nBOR cells started 0.5 min after lidocaine injection (40–120 nl) and completely recovered 1.5–7 min after stopping drug application. Among 30 nBOR cells examined, 21 cells (70%), including 17 cells preferring dorsoventral or ventrodorsal motion and four cells preferring nasotemporal motion, decreased firing rates to 80.2 ± 15.8% of the control values in the preferred directions during lidocaine ($t = 5.67$, $n = 21$, $P < 0.01$) (Fig. 2A). Nineteen of these cells did not show significant changes in firing rates in all other directions (pairs of data = 3 × 19) ($t = 1.49$, $n = 57$, $P > 0.01$).

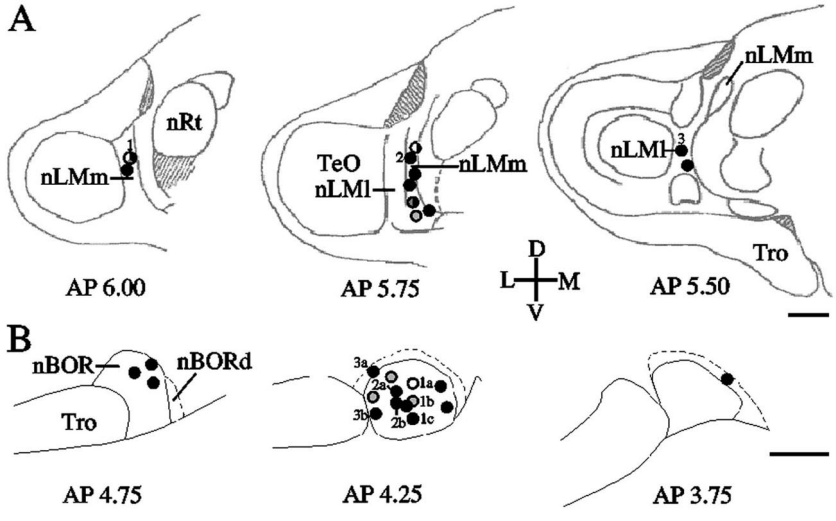

Fig. 1. Cross-sections of the pigeon brain showing topographic distributions of lidocaine injection sites in nLM (A) and recording sites in nBOR (B). Solid, empty and stippled symbols represent nLM sites (A) where lidocaine produces a decrease, increase or no change in visual responses of nBOR cells, some of which are localized in nBOR with the corresponding symbols (B). Numerals 1–3 marking drug sites in (A) correspond to those marking recording sites in (B), with nBOR cells in the same penetration labeled with alphabetic letters. AP, anterior-posterior levels according to the pigeon brain atlas [14]. D, L, V and M represent dorsal, lateral, ventral and medial, respectively. Other abbreviations: nLMm, nLM pars medialis; nLMl, nLM pars lateralis; TeO, optic tectum; Tro, optic tract; nRt, nucleus rotundus; nBORd, nBOR dorsalis. Scales: 1 mm.

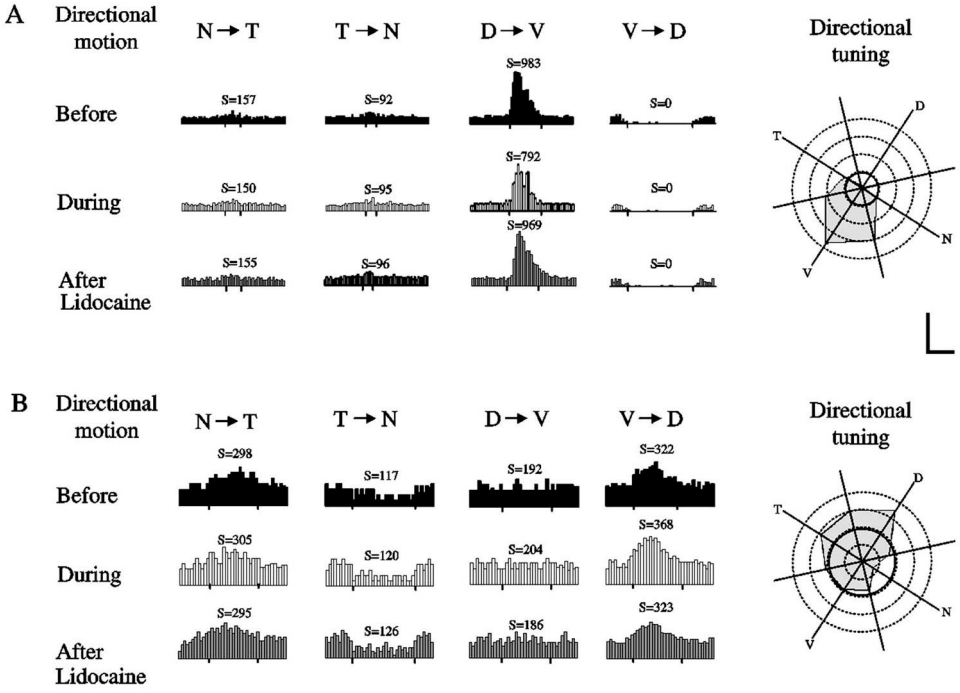

Fig. 2. Histograms showing effects of lidocaine application in nLM on visual responses of two nBOR cells (A,B) to motion (arrows). Cell (A) preferred dorsoventral motion at 13°/s and spontaneously fired at 23 spikes/s, and its responses in the preferred direction were reduced by lidocaine (80 nl) in nLM. Cell (B) preferred ventrodorsal motion at 14°/s and spontaneously fired at 19 spikes/s, and its responses were enhanced by lidocaine (80 nl) in nLM. Solid, empty, and stippled histograms represent responses before, during and after lidocaine application, respectively. S, total number of visual spikes accumulated in three sweeps. Vertical lines beneath histograms mark the start and end of visual stimulation. Stippled polygons in polar coordinates are directional tuning curves measured in eight directions as controls. Equal firing-rate circles are spaced by 25 spikes/s in (A) and 10 spikes/s in (B), and solid-line circles represent spontaneous activity levels. The recording sites of cells (A) and (B) are shown with 1c and 1a in Fig. 1B, and their drug-site is marked with 1 in Fig. 1A. Abbreviations N, D, T, and V respectively represent nasal, dorsal, temporal and ventral in the visual field, whose horizontal and vertical meridians were rotated clockwise by 38° to meet the pigeon normal conditions. Scales: 80 spikes /200ms, and 3.2 s in (A); 20 spikes/200 ms, and 2 s in (B).

Two others reduced firing rates to 60–90% of the control values not only in the preferred ventrodorsal but also in the temporonasal or nasotemporal directions. Four of 30 cells (13%), preferring ventrodorsal or nasotemporal motion, increased average firing rates to $117.2 \pm 7.0\%$ of the control values in the preferred directions ($t = 4.92, n = 4, P < 0.01$) (Fig. 2B). Visual responses in all other directions (pairs of data $= 3 \times 4$) were not changed ($t = 4.92$, $n = 12$, $P > 0.01$). Five others (17%), including four cells preferring dorsoventral or ventrodorsal motion and one cell preferring nasotemporal motion, did not change visual firing rates in all directions examined (pairs of data $= 4 \times 5$) ($t = 1.36$, $n = 20$, $P > 0.01$) during lidocaine application (40–200 nl) in nLM.

Eleven lidocaine sites marked with dye were all within nLM, with nine in the nLM pars medialis (nLMm) and two in the nLM pars lateralis (nLMl) [9] (Fig. 1A). Sixteen recording sites marked were all within nBOR, including 14 in the nBOR proper and 2 in the nBOR dorsalis [3] (Fig. 1B). No significant differences were observed in changes in visual responses of nBOR cells between nLMm and nLMl injections. In ten cases, more than two nBOR cells were each examined for effects of lidocaine injected at the same nLM site. Visual responses in nBOR cells recorded in the same or various penetrations were differentially affected by lidocaine injected at the same nLM site. An example is given in Fig. 1 showing that visual responses of nBOR cells 1a–c recorded in the same penetration were increased, decreased or not affected by lidocaine injected at the same nLM site 1. Based on the recording sites and directional selectivity of nBOR cells, it appears that ventrodorsal-, dorsoventral- and nasotemporal-preferring cells are distributed along the dorsoventral extent of nBOR [24]. However, no apparent correlation was observed between effects of lidocaine administration in nLM on visual responsiveness of nBOR cells and their locations within nBOR.

Several studies [6,15,19,20] have shown that lidocaine is a useful tool for investigating the functional interaction between visual structures, due to the specificity and reversibility of its effects on firing activity in a cell that receives input from the site where lidocaine is applied. However, it is worth noting that a decrease (increase) in firing rate of a nBOR cell following lidocaine administration in nLM implies an excitatory (inhibitory) connection of the cell

with nLM. The present study indicates for the first time that nLM modulates the visual responses and directional selectivity of nBOR neurons in a direction-dependent way in two modes: (1) to enhance visual responses in the preferred directions, sharpening direction-tuning in most (70%) directional cells; (2) to reduce visual responses in the preferred directions, broadening direction-tuning in a small portion (13%) of directional cells. The remaining 17% of nBOR cells are not affected by drug injections, probably they do not receive input at least from the drug sites within nLM. The present results indicate that most dorsoventral- and ventrodorsal-preferring nBOR cells are affected by lidocaine administration in nLM, whereas Nogueira and Britto [17] show that these cells are affected by wulst stimulation but not by nLM stimulation. This discrepancy may be due to different methodologies, e.g. electrical stimulation vs. lidocaine blockade, and/or measurement of spontaneous activity vs. directional visual responses. Different sampling of nBOR cells and nLM sites might also be a factor contributing to this discrepancy. Taken together with the previous results that nBOR cells are predominantly sensitive to motion in the dorsoventral, ventrodorsal and nasotemporal directions [4,11,18,22,24,27] whereas nLM cells mostly prefer temporonasal motion [2,7,8,21,23,25], and that reversible blockade of nBOR could change visual responsiveness of nLM neurons in a direction-dependent manner [12], it suggests that both optokinetic nuclei work together to generate optokinetic nystagmus. The directional preferences of the nuclei are complementary and they mutually modulate directional responses of optokinetic neurons.

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