Gamma-aminobutyric acid and $GABA_A$ receptors are involved in directional selectivity of pretectal neurons in pigeons

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Abstract The present study describes the effects of gamma-aminobutyric acid (GABA) and its antagonists, bicuculline and 2-hydroxysaclofen, on visual responses of neurons in the pigeon nucleus lentiformis mesencephali (nLM). The results indicate that GABA significantly reduces both spontaneous activity and visual responsiveness, and $GABA_A$ antagonist bicuculline but not $GABA_B$ antagonist 2-hydroxysaclofen enhances visual responses of nLM cells examined. Furthermore, inhibition produced by motion in the null-direction of pretectal neurons is diminished by bicuculline but not by 2-hydroxysaclofen. It is therefore concluded that the null-direction inhibition of directional cells in the pigeon nLM is predominantly mediated by GABA and $GABA_A$ receptors. This inhibition may at least in part underlie directional asymmetry of optokinetic responses.

Keywords: directional selectivity, GABA, nucleus lentiformis mesencephali, optokinetic nystagmus, pigeon.

The pretectal nucleus lentiformis mesencephali (nLM) in lower vertebrates such as pigeons and the nucleus of the optic tract (NOT) in mammals are homologues, both of which are involved in generating optokinetic nystagmus (OKN) that stabilizes an image of the visual environment on the retina by compensatory motion of the eyes. Electrolytic or chemical lesions of these nuclei result in reduction or elimination of horizontal OKN in pigeons[1] and monkeys[2]. On the other hand, electrical stimulation of the monkey NOT causes a strong OKN[3]. In monocular vision, horizontal OKN shows a directional asymmetry, i.e. temporal-nasal motion is more efficient than nasal-temporal motion in provoking optokinetic responses[1,4].

Though it has been suggested that directional asymmetry is correlated with the lateral position of the eyes[4], it appears that this asymmetry may be related at least in part to directional selectivity of optokinetic cells. In fact, it has been shown that most neurons recorded from the pigeon nLM are direction-selective, preferring a particular (preferred) direction of motion of a stimulus to motion in the opposite (null) direction[5—7].

It has been known that the excitatory receptive field (ERF) and inhibitory receptive field (IRF) in most nLM cells in pigeons usually overlap, with ERF being maximally excited by motion in the preferred direction and IRF inhibited by motion in the null direction. Interaction between excita-

tion and inhibition induced by motion respectively through ERF in the preferred direction and through IRF in the null direction results in overall summation of excitatory and inhibitory responses[5,6]. GABA plays an important role in directional selectivity of the rat NOT neurons[8], and it is also involved in modulating directional selectivity in the pigeon nucleus of basal optic root (nBOR)[9]. However, little is known about neuropharmacological mechanisms underlying the directional selectivity of nLM neurons and the possible role of GABA in their directionality. Therefore, the present study was undertaken to examine the effects of GABA and its antagonists on visual responses extracellularly recorded from the pigeon nLM neurons by using microiontophoresis techniques.

1 Materials and methods

The experiments were performed on 19 adult pigeons (*Columba livia*) , with body weight of 350—420 g, either sex, and under guidelines regarding the use of animals in neuroscience research approved by the Society for Neuroscience. The pigeon was anesthetized with urethane (20%, 1 mL/100 g b.wt, i.m.), and then placed in a stereotaxic apparatus. Its body temperature was maintained at 41℃ by a heating pad. The caudal forebrain on the left side was surgically exposed and the dura matter overlying nLM was excised. The nictitating membrane of the right eye was removed to keep the eye open. The left eye was occluded with an opaque cover. A screen was positioned 40 cm distant from the viewing eye, and 24 deg to the midsagittal plane of the pigeon, and measured 180 cm in height and 220 cm in width. The area being visually stimulated was about 140 deg (horizontal angle)×130 deg (vertical angle). In view of the fact that the angle between the horizontal meridian of the visual field and the eye center-bill tip line of the stereotaxically fixed pigeon was 72 deg in these experiments, while it is 34 deg under normal conditions for flying, walking, standing and perching, the horizontal meridian of the visual field was therefore rotated clockwise by 38 deg to meet the normal conditions[5,6].

A random-dot pattern with 250 white dots/m^2 in density, each of which subtended 2 deg visual angles, was generated by a workstation computer (Silicon Graphics Indigo 2) and rear-projected by a three-color projector (Electrohome ECP 4101) onto the screen. These dots were moved on black background, and their luminance was 6.6 cd/m^2 and 0.1 cd/m^2, respectively. The location and extent of the receptive field of a nLM cell were plotted with a single target. The dotted pattern was moved over the field at a series of velocities (1, 2, 4, 8, ..128 deg/s) and randomly in eight directions (0 deg-nasal, 45 deg, 90 deg-dorsal, 135 deg, 180 deg-temporal, 225 deg, 270 deg-ventral, 315 deg) to measure the optimal velocity and preferred direction of the cell. The cell's response strength was maximal when the visual pattern was moved at the optimal velocity in the preferred direction. It was minimal when motion was in the opposite (null) direction. The interval between two consecutive stimulations was more than 10 s for complete recovery. The effects of pharmacological agents on visual responses of nLM cells were examined.

A 5-barrel micropipette was used for both extracellular recording and drug application. The

recording channel was filled with solution containing 2 mol/L NaCl and 100 mmol/L $CoCl_2$, and the three other channels contained the following compounds to be ejected by appropriate currents: gamma-aminobutyric acid (GABA, Fluka, 0.5 mol/L, pH 3.3), bicuculline methchloride (Sigma Chemical Co., 10 mmol/L, pH 3.3), and 2-hydroxysaclofen (RBI, Natick, MA., U.S.A., 20 mmol/L, pH 3.0). The remaining channel filled with 2 mol/L NaCl was used for minimizing current effects. The micropipette was stereotaxically advanced into either the magnocellular or parvocellular divisions of nLM, guided by the pigeon brain atlas of Karten and Hodos[10]. Neuronal responses of pretectal cells to visual stimulation were conventionally amplified and fed into the workstation for on-line processing. In some experiments, cobalt ions were ejected by positive current (5—10 μA, 0.5 s duration, 1 Hz, 5—10 min) to mark the recording sites. The pigeon was killed by overdose of the anesthetic, and the brain was removed from the skull, and then immersed in saline solution containing 10% sulfide ammonium for 30—40 min to form CoS black precipitate. The brain was fixed in 10% formaldehyde for 48 h, soaked in 30% sucrose solution for 24 h, and then trimmed and frozen-sectioned at 80 μm thickness. The sections were counterstained with cresyl violet, covered, and observed with a microscope to localize the recording sites.

2 Results

The effects of GABA and its antagonists, bicuculline and 2-hydroxysaclofen, were tested on visual responses of 82 nLM cells, 10 of which were histologically verified to be localized within the magnocellular (nLMmc, 6 cells) and parvocellular (nLMpc, 4 cells) divisions of the nucleus as expected from their recording coordinates (fig.1). According to their stereotaxic coordinates corrected by these markings, characteristic visual responses and spontaneous activity, 75 of the cells were assigned with a certainty to be in nLMmc and 7 within nLMpc.

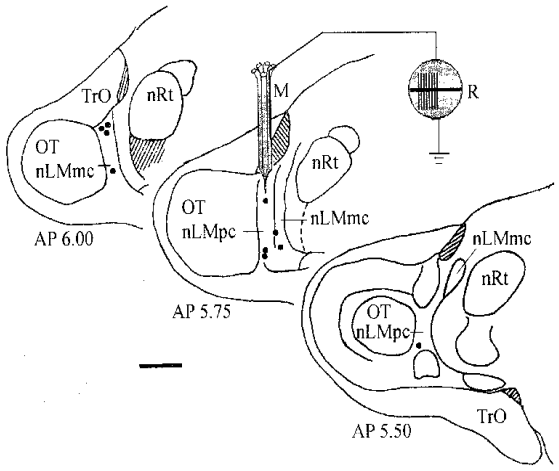

Fig. 1. Experimental arrangement and distribution of recording sites (solid dots) marked with cobalt-sulfide in the nucleus lentiformis mesencephali pars magnocellularis (nLMmc) and the nucleus lentiformis mesencephali pars parvocellularis (nLMpc). Cross-sections of the pigeon midbrain are arranged according to the anterior-posterior levels AP 6.00—5.50. M, Multibarrel micropipette; R, recording oscilloscope; nRt, nucleus rotundus; OT, optic tectum; TrO, tractus opticus. Scale bar: 1 mm.

Neurons in both divisions were characterized by their velocity- and direction-selectivities. About 90% of nLMmc cells and

nLMpc cells were slow cells preferring motion velocities of 1—16 deg/s, and 10% of pretectal cells were fast cells preferring motion velocities of larger than 32 deg/s. Most nLMmc cells preferred temporal-nasal motion (45%), or the opposite direction of motion (49%), and only a small population of cells (6%) responded maximally to vertical motion (fig.2). Three nLMpc unidirectional cells examined in the present study preferred nasalward or temporalward motion.

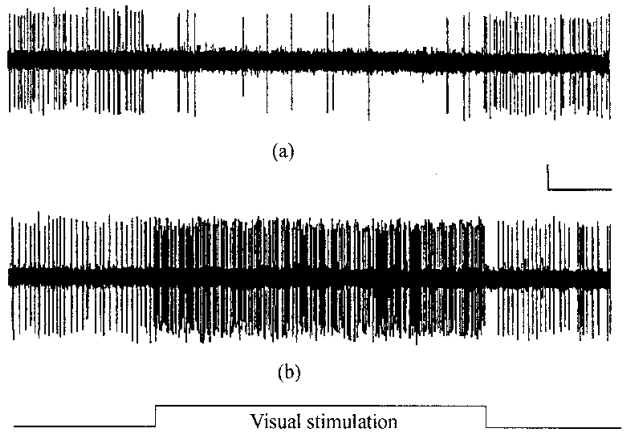

Fig. 2. Neuronal responses of a nLMmc cell to a computer-generated whole-field random-dot pattern with 250 white dots/m^2 (dot subtended 2 deg visual angles), which was moved at velocity of 8 deg/s in the nasal-temporal direction (a) and in the temporal-nasal direction (b). This cell was spontaneously active, with an average firing rate of 17 spike/s. Note that spontaneous activity was significantly inhibited by nasal-temporal motion, and visual excitation was produced by temporal-nasal motion. Upward deflection of bottom trace represents visual stimulation. Scales: 100 μV, 1 s.

The effects of GABAergic substances on visual responses evoked in the preferred direction were examined in 60 nLMmc cells and 7 nLMpc cells. The nLMmc neurons were spontaneously active, with an average firing rate of 18.1 ± 15.6 spike/s (mean ± SD, n = 60), and responded maximally to large-field stimuli moved at the optimal velocity in the preferred direction, with an average visual discharge of 50.1 ± 34.5 spike/s. Both spontaneous activity and visual responses were suppressed by GABA applied at currents of 20—200 nA (average = 60 nA). Their spontaneous and visual firing rates went down to 1.7 and 13.5 spike/s, respectively. Therefore, GABA could significantly reduce both spontaneous activity and visual responsiveness in nLMmc neurons (paired t-test, n =36, p<0.01). This reduction was recovered to normal in about 1.5 min after stopping GABA application. On the other hand, $GABA_A$ antagonist bicuculline (40—200 nA, average = 80 nA) significantly enhanced visual responses from 50.1 to 111.7 spike/s in 15—90 s following bicuculline application (t-test, n =60, p<0.01). Spontaneous activity was also enhanced by the antagonist. The enhancement of both visual responses and spontaneous activity was recovered to control levels about 5 min after stopping antagonist application, whereas $GABA_B$ antagonist 2-hydroxysaclofen applied even at higher current intensities (average =120 nA) for longer periods (average =120 s) did not change visual firing rates (n =36, p>0.05) (fig.3). Statistically, spontaneous activity of nLMpc cells was much weaker than that of nLMmc cells (9.2 vs. 18.1 spike/s). However, the effects of GABAergic substances on spontaneous activity and visual responsiveness of nLMpc cells were similar to those on nLMmc cells, in terms of that these activities were significantly inhibited by GABA (n=7, p<0.01) and enhanced

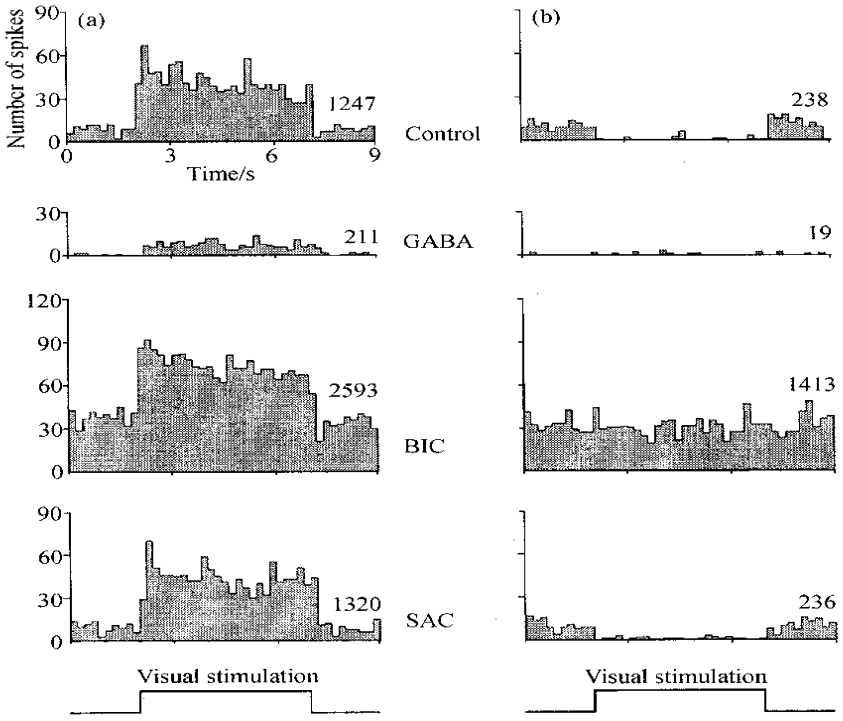

Fig. 3. Response histograms showing effects of gamma-aminobutyric acid (GABA, 20 nA) and its antagonists bicuculline (BIC, 200 nA) and 2-hydroxysaclofen (SAC, 200 nA) on visual responses of a nLMmc cell activated by motion in the preferred direction (a) and in the null direction (b). Numerals at the upper-right are the total number of spikes accounted for 3 sweeps. Upward deflection of bottom traces denotes visual stimulation using a random-dot pattern that was moved at 8 deg/s.

by bicuculline ($p<0.01$), but not affected by 2-hydroxysaclofen ($p>0.2$). It appeared that the two divisions of the nucleus, nLMmc and nLMpc, were not pharmacologically different at least with respect to the effects of GABAergic substances on their visual cells.

Seventy-five cells recorded from nLMmc included 2 omnidirectional cells that almost equally responded to all directions of motion, 1 bidirectional cell that produced stronger responses in two opposite directions, and 72 unidirectional cells that produced the maximal responses to a particular (preferred) direction. Among 7 nLMpc cells were 4 omnidirectional cells and 3 unidirectional cells. In most (80%) nLMmc unidirectional cells, an inhibition occurred when visual stimulus was moved in the null direction. However, no null-direction inhibition was observed in all 3 nLMpc unidirectional cells examined in the present study. Therefore, we only examined the effects of GABAergic substances on null-direction inhibition in 38 nLMmc unidirectional cells. Their average firing rate evoked by a visual stimulus moving in the null direction was 4.0 spike/s, and was significantly reduced by GABA (30 nA) to 1.2 spike/s. The inhibitory responses produced by motion in the null-direction were diminished and visual responses were enhanced by bicuculline application (60 nA) from 4.0 to 49.4 spike/s. Spontaneous activity was also significantly reduced (*n*

=38, $p<0.01$) by GABA and enhanced by bicuculline. On the other hand, 2-hydroxysaclofen did not affect spontaneous activity and visual responses ($n = 5$, $p>0.3$) of unidirectional cells at all. It was therefore likely that the null-direction inhibition of directional cells was mediated by GABA and $GABA_A$ receptors. However, after long-lasting (>2 min) application of bicuculline on unidirectional cells, the number of visual spikes activated by motion in the null direction was about 60% of that evoked by motion in the preferred direction. This remaining inhibition cannot be released by $GABA_B$ antagonist, either. It suggests that asymmetrical GABAergic input may be not the sole mechanism for producing directional selectivity of pretectal cells.

3 Discussion

The present study not only confirms the previous finding that the pigeon nLM cells are velocity- and direction-selective[5−7], but also shows that visual responses of pretectal cells to motion in the preferred direction are inhibited by GABA and enhanced by bicuculline, a $GABA_A$ antagonist, but not by 2-hydroxysaclofen, a $GABA_B$ antagonist, and that inhibition induced by motion in the null direction was reduced or eliminated by bicuculline, but not by 2-hydroxysaclofen. It appears that the roles of GABA and $GABA_A$ receptors are similar in both optokinetic nuclei, nLM and nBOR[9], indicating the involvement of GABA-mediated inhibition in forming directional asymmetry of optokinetic responses. These results imply that $GABA_A$ antagonists injected into nLM could increase OKN responses, which is in agreement with coil recordings of monocular OKN following microinjection of $GABA_A$ antagonist into the chicken nLM[11]. The effects of GABA antagonists on monocular asymmetry are supported by immunohistochemical finding that glutamic acid decarboxylase reactivity[12], as well as GABA and its receptors[13] exist in the pigeon nLM.

Within nLM and nBOR of pigeons, GABA can depress spontaneous activity and visual responses induced by motion in the preferred direction. However, there exist both $GABA_A$ and $GABA_B$ receptors in the pigeon nBOR[9] and only $GABA_A$ receptors in nLM, implying that there are more inhibitory ways within nBOR than in nLM. In both nuclei, GABA antagonists cannot completely block inhibition elicited by motion in the null direction[9]. Therefore, asymmetrical GABAergic input is unlikely to be the sole mechanism underlying directional selectivity. This directionality may also originate in part from the retinal ganglion cells that respond selectively to orientated edges moving in some particular directions[14]. A recent study shows that $GABA_A$ receptors are mediated in surround inhibition of receptive fields, whereas $GABA_B$ receptors involved in response habituation of visual neurons, indicating that two types of receptors play distinct roles in visual transmission and information processing[15].

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