

## The role of $\gamma$ -aminobutyric acid and its receptors in the nucleus of basal optic root in pigeons\*

FU Yuxi (付煜西), GAO Hongfeng (高宏峰), Stephen A. George\*\*  
and WANG Shurong (王书荣)

(Laboratory for Visual Information Processing, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China)

Received April 6, 1996

**Abstract** The effects of  $\gamma$ -aminobutyric acid (GABA) and its antagonists bicuculline and 2-hydroxysaclofen on neuronal firings in the nucleus of basal optic root (nBOR) in pigeons were studied by using extracellular recording and microiontophoretic techniques. The results suggest that GABA may be an inhibitory neurotransmitter or modulator within nBOR, functioning by means of main mediation of GABA<sub>A</sub> receptors and of minor mediation of GABA<sub>B</sub> receptors. Furthermore, GABA and its GABA<sub>A</sub> receptors are involved in the modulation of directional selectivity in part of nBOR neurons.

**Keywords:** nucleus of basal optic root (nBOR), microiontophoresis,  $\gamma$ -aminobutyric acid (GABA), receptor, inhibition, pigeon.

Neuroanatomical, electrophysiological and behavioral studies have shown that the accessory optic system (AOS) in birds plays an essential role in optokinetic nystagmus (OKN). It mainly consists of two nuclei, i. e. the nucleus lentiformis mesencephali (nLM) involving the formation and modulation of horizontal OKN, and the nucleus of basal optic root (nBOR) playing its role in vertical OKN. 2-deoxyglucose labelling studies on homing pigeon<sup>[1]</sup> and chicken<sup>[2]</sup> have found that nBOR neurons are sensitive to whole-field stimuli moving slowly and particularly in vertical direction. Electrophysiological studies on nBOR neurons in these species<sup>[3-5]</sup> have led to similar conclusions. Nevertheless, Gioanni *et al.*<sup>[6]</sup> have claimed that the pigeon nBOR is essential in forming naso-temporal OKN. It is also indicated that the frog nBOR is at least partly mediated in horizontal OKN<sup>[7]</sup>. These reports have all indicated the existence of directional selectivity in nBOR neurons.

However, very little is so far known about the origin of directionality of nBOR neurons, their neurotransmitters and receptor subtypes. Immunocytochemical studies have shown that there are GAD (glutamic acid decarboxylase)-like immunoreactivity in nBOR of frogs<sup>[8]</sup> and pigeons<sup>[9]</sup>, and GABA ( $\gamma$ -aminobutyric acid)-like immunoreactivity in the chicken nBOR<sup>[10]</sup>, implying that GABA may be one of neurotransmitters or modulators within this nucleus. Furthermore, GABA probably plays some role in forming directional selectivity of neurons in the rat nucleus of optic tract (NOT)<sup>[11]</sup>. To further elucidate the functioning of GABA and its receptors within nBOR, we used extracellular recording and microiontophoretic techniques to examine the effects of GABA and its antagonists on spontaneous activity and visually evoked responses of

\* Project supported by the National Natural Science Foundation of China and Amherst College.

\*\* Department of Biology, Amherst College, MA 01002 - 5000, USA.

nBOR neurons in pigeons.

## 1 Materials and methods

Experimental animals were 15 adult homing pigeons (*Columba livia*) having body weight of 280–350 g. The pigeon was anesthetized with urethane (20%, 1 mL/100 g b. w.), and then positioned in a stereotaxic apparatus, and its body temperature was maintained at 41°C with a heating pad. The nictitating membrane of the right eye was cut to keep the eye open, and another eye was covered with an occluder. The caudal forebrain and rostral cerebellum contralateral to the eye were exposed, and dura matter removed by surgical operation. The stereotaxic coordinates of nBOR used in these experiments were AP 3.75–5.00, ML 1.20–2.10, DV 9.20–10.60, according to a pigeon brain atlas modified with cobalt sulfide stainings. A 5-barreled micropipette (3–5  $\mu\text{m}$  tip diameter) was stereotaxically advanced into nBOR. The recording channel was filled with a solution containing 2 mmol/L NaCl and 100 mmol/L  $\text{CoCl}_2$ , having impedance of 5–15 M $\Omega$ . The other channels contained the following compounds:  $\gamma$ -aminobutyric acid (GABA, Fluka, 0.5 mmol/L, pH 3.3), bicuculline (Sigma, 10 mmol/L, pH 3.3), and 2-hydroxysaclofen (RBI, 20 mmol/L, pH 3.0). These chemicals were microiontophoretically ejected with electrical current of appropriate polarity.

Visual stimuli were generated by workstation Silicon Graphics Indigo 2 and back-projected by a three-color projector (Electrohome ECP 4101) onto a screen, which was of size 90 cm width  $\times$  72 cm height, positioned at 45° to the longitudinal axis of the animal and 40 cm away from the stimulated eye. Two kinds of visual stimuli were used: one was whole-field gratings with black and white stripes being equal in width, oriented perpendicularly to their motion direction. Their spatial frequencies were 3–20 c/m, and motion velocities ranged from 0.02 to 2.0 m/s. Another was a light bar on dark background, being 1–23 cm wide, 90 cm long and swept across the screen at 0.02–2.0 m/s. Neuronal firings were amplified with a preamplifier and fed into a computer for on-line analysis.

By the end of the experiment, cobalt ions were ejected (3–5  $\mu\text{A}$ , 0.5 s duration, 1 Hz, 5–10 min) to histologically confirm location of the electrode tip. The pigeon was killed by over-dose of anesthetics, and decapitated. The brain was removed and then immersed in saline solution containing 10% ammonium sulfide for 30–45 min to produce black precipitate cobalt sulfide. After postfixation in 10% formalin for 48 h, frozen sections were cut at 60  $\mu\text{m}$  in thickness, histologically processed and counterstained with cresyl violet. The location of the recording electrode tips was observed and reconstructed under a microscope with a camera lucida tube.

## 2 Results

### 2.1 GABAergic inhibition is mainly mediated by GABA<sub>A</sub> receptors

A total of 86 nBOR neurons were extracellularly recorded, and 5 of them histologically located with cobalt sulfide. The marking spots were all positioned within nBOR. Generally speaking, these neurons were spontaneously active. Statistics of 47 out of 86 neurons showed an average spontaneous firing of 20 spikes per second. GABA exerted significant inhibition on 90% of the cells examined. Table 1 indicates the effects of GABA and its antagonists on 29 of these cells.

Here, excitation, inhibition and no-effect mean changes in either spontaneous firings or visual responses before and after application of chemicals. Both spontaneous activity and visual responses were parallelly changed in response to action of chemicals ejected. In 5–10 s after starting application of GABA with 50–200 nA current intensities, both spontaneous activity and visual responses began to reduce firings. On an average spontaneity was reduced by 80% and visually evoked discharge by 55% in 30–60 s. Neuronal firings could be completely recovered 30–60 s after stopping ejection. Following ejection of bicuculline, spontaneity and visual responses went up in 5–10 s, and this increase by bicuculline is parallel to the decrease by GABA in their time courses, implying that inhibition of GABA is mediated by GABA<sub>A</sub> receptors. On the other hand, the effects of 2-hydroxysaclofen were examined on 12 cells, 6 of which increased their spontaneous activity by 74% and visual responses by 66%, and 6 other cells were not sensitive to this GABA<sub>B</sub> receptor antagonist. Under the same conditions, these percentages of increase produced by bicuculline corresponded to 125% and 107%, respectively. Therefore, these results indicate that both GABA<sub>A</sub> receptors and GABA<sub>B</sub> receptors may coexist at least on part of nBOR neurons, and GABA inhibition is mainly mediated by GABA<sub>A</sub> receptors. Current intensities of 100–200 nA were needed for these two antagonists to start their actions in 5–10 s and the cells were recovered 1–2 min after stopping application of antagonists. Fig. 1 shows the effects of GABA and its antagonists on visual responses produced by nBOR neurons. It is indicated that visual responses of nBOR neurons could be inhibited by microiontophoretically applied GABA, and its antagonists could release this inhibition, with bicuculline being much stronger than 2-hydroxysaclofen in producing disinhibition. Therefore, it comes to a conclusion that GABA plays its role as inhibitory transmitter within the avian nBOR, and this inhibition is predominantly mediated by GABA<sub>A</sub> receptors, with minor involvement of GABA<sub>B</sub> receptors.

Table 1 The action of GABA and its antagonists on spontaneous firings and visual responses of pigeon's nBOR neurons

	GABA	Bicuculline	2-hydroxysaclofen
Excitation	0	26 (90%)	6 (50%)
Inhibition	26 (90%)	0	0
No effect	3 (10%)	3 (10%)	6 (50%)

The number of cells responding to the chemicals and their percentage (in parentheses).

## 2.2 GABA and its GABA<sub>A</sub> receptors are involved in modulating directional selectivity

Observations using gratings as visual stimuli indicate that all cells recorded from nBOR showed clear directional selectivity, i. e. a majority of cells preferred up- or downward movement, with only 10% of cells inclining toward to nasotemporal or temporonasal motion. Following application of bicuculline (50–300 nA, 5–10 s), differences in visual responses to various directions of motion were reduced, or directional selectivity of the cell decreased. However, this directionality could not be completely eliminated even though the application lasted for more than 10 min. While a light bar was used as visual stimulus, an inhibitory area of the receptive field was found in 44% of the recorded neurons. If the inhibitory area was located next to the excitatory region on the preferred direction within the receptive field, even long-lasting application of bicuculline (10 min) with stronger current (300–500 nA) still could not eliminate this inhibition. However, it was not the case with the inhibitory area appearing in the null direction (just opposite to the pre-

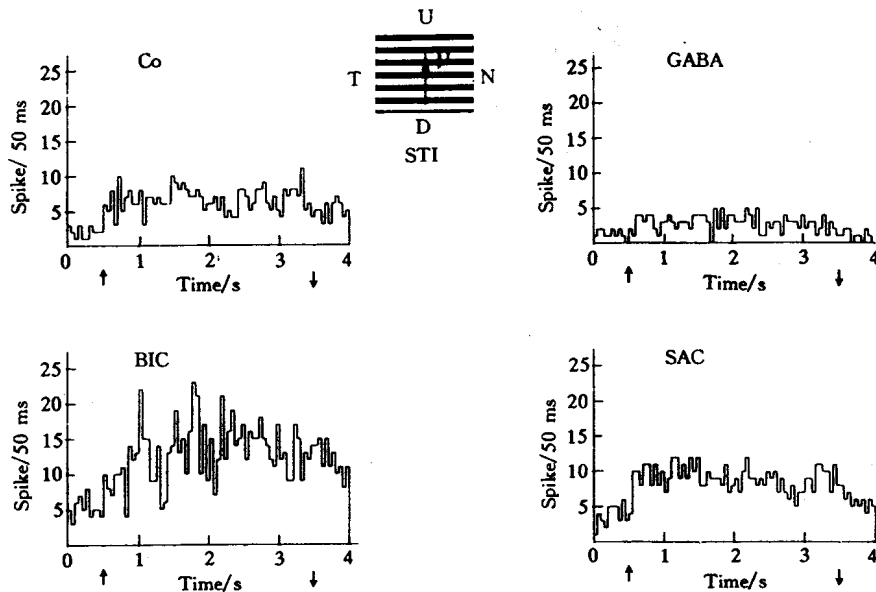


Fig. 1. The effects of GABA and its antagonists on visual responses of nBOR neurons. STI, visual stimuli (grating, spatial frequency 8.7 c/m); arrow  $v$ , motion direction (average velocity, 6.7°/s). U, D, T, N denote up, down, temporal and nasalward, respectively. GABA, BIC and SAC symbol visual responses under influence of GABA (100 nA, 15 s), bicuculline (100 nA, 60 s), or 2-hydroxysaclofen (100 nA, 60 s). Arrows beneath histograms point to onset and offset of visual stimulation. Co, control; Re, recovery. The cell was always recovered to its normal responsiveness following each tests.

ferred direction). Fig. 2 shows that a cell produced vigorous responses to a light bar making up-bottom motion across the screen, and its spontaneous activity or/and visual responses were increased by bicuculline while the bar was moved bottom-up, a clear inhibitory area appeared in the receptive field, which largely overlapped with the excitatory region produced by the up-bottom movement. This inhibitory area disappeared following bicuculline application (200 nA, 5 min). It suggests that GABA and its GABA<sub>A</sub> receptors may play an important role in the formation of directional selectivity and of inhibitory area of receptive fields in neurons of the avian nBOR.

### 3 Discussion

One of the main results of this study shows that GABA may be one of neurotransmitters within the avian nBOR, playing its inhibitory role predominantly through GABA<sub>A</sub> receptors. It is supported by immunocytochemical studies indicating the existence of GABA-like and GAD-like activities<sup>[8-10]</sup>. The facts that bicuculline and 2-hydroxysaclofen, antagonists of GABA, could increase visual responses of nBOR neurons, with the former being much stronger than the latter in evoking these responses, indicate that at least on part of nBOR neurons there coexist GABA<sub>A</sub> and GABA<sub>B</sub> receptors, and GABA<sub>A</sub> receptors are predominantly involved in GABA action. These are similar to those found by us in the pigeon nucleus rotundus<sup>[12]</sup>. We also pointed out in the previous paper<sup>[13]</sup> that the nucleus isthmi pars magnocellularis (Imc)-tectal pathway is partly glutamatergic and mediated by N-methyl-D-aspartate (NMDA), whereas the retino-tectal pathway is also glutamatergic, but mediated by AMPA receptors. Similarly, the coexistence of two subtypes

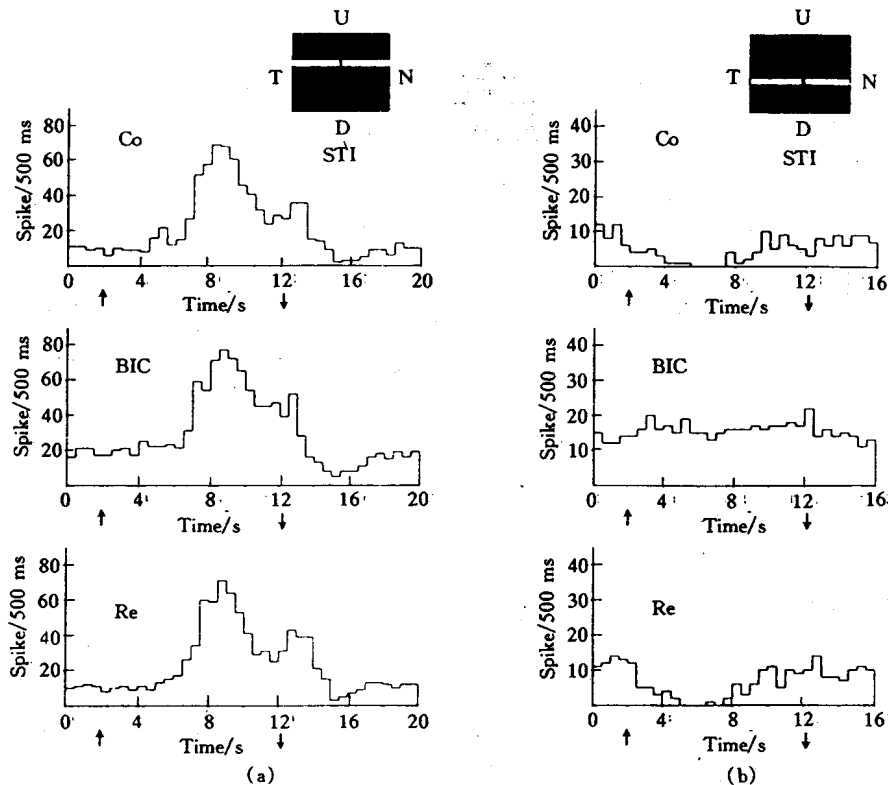


Fig. 2. Diagrams showing the influence of bicuculline on visual responses and inhibitory area of receptive fields in nBOR neurons. For abbreviations, see fig. 1. Iontophoresis parameters were 200 nA, 5 min. The light bar was 5.8 cm wide and its average velocity was  $8.1^{\circ}/s$ .

of receptors on nBOR neurons suggests that these cells receive at least two kinds of GABAergic inputs. However, the source(s) of these inputs are still unknown.

The other main result of this study is that GABAergic inputs play an important role in the formation of inhibitory area of receptive fields of nBOR neurons. Bicuculline can increase visual responses in the preferred direction, and completely eliminate visually inhibitory area in the null direction; it cannot essentially influence the directional selectivity of cells. It appears that the mechanism of eliminating the inhibitory area may be different from that of the reduction or elimination of orientation selectivity of cells in the cat lateral geniculate nucleus<sup>[14]</sup>, because the latter are explained as results of saturation discharge caused by bicuculline<sup>[15]</sup>. Therefore, it is suggested that there exists stronger GABAergic directional inhibition in part of cells in the pigeon nBOR. On the other hand, it appears that this inhibition is different from the above-mentioned neighbouring inhibition, which is not apparently influenced by bicuculline. The latter may be mediated by other subtypes of GABA receptors or other inhibitory transmitters. These suggestions are needed to be further studied.

## References

- 1 Frostm, B. J., Ramm, P., Morgan, B., Selective activation of pigeon nBOR with vertical whole field movement as revealed by

- <sup>14</sup>C-2DG autoradiography, *Neurosci. Abst.*, 1980, 6: 717.
- 2 McKenna, O. C., Wallman, J., Identification of avian brain regions responsive to retinal slip using 2-deoxyglucose, *Brain Res.*, 1981, 210: 455.
  - 3 Morgan, B., Frost, B. J., Visual response characteristics of neurons in nucleus of basal optic root of pigeons, *Exp. Brain Res.*, 1981, 42: 181.
  - 4 Gioanni, H., Rey, J., Villalobos, J. *et al.*, Single unit activity in the nucleus of the basal optic root (nBOR) during optokinetic, vestibular and visuo-vestibular stimulations in the alert pigeon (*Columba livia*), *Exp. Brain Res.*, 1984, 57: 49.
  - 5 Burns, S., Wallman, J., Relation of single unit properties to the oculomotor function of the nucleus of the basal optic root (accessory optic system) in chickens, *Exp. Brain Res.*, 1981, 42: 171.
  - 6 Gioanni, H., Villalobos, J., Rey, J. *et al.*, Optokinetic nystagmus in the pigeon (*Columba livia*), III. Role of the nucleus ectomamillaris (nEM): interactions in the accessory optic system (AOS), *Exp. Brain Res.*, 1983, 50: 248.
  - 7 Montgomery, N., Fite, K. V., Taylor, M, *et al.*, Neural correlates of optokinetic nystagmus in the mesencephalon of *Rana pipiens*: a functional analysis, *Brain Behav. Evol.*, 1982, 21: 137.
  - 8 Tyler, C. J., Fite, K. V., Devries, G. J., Distribution of GAD-like immunoreactivity in the retina and central visual system of *Rana pipiens*, *J. Comp. Neurol.*, 1995, 353: 439.
  - 9 Britto, L. R. G., Hamasaki, D. E., Keyser, K. T. *et al.*, Neurotransmitters, receptors, and neuropeptides in the accessory optic system: An immunohistochemical survey in the pigeon (*Columba livia*), *Visual Neuroscience*, 1989, 3: 463.
  - 10 Granda, R. H., Crossland, W. J., GABA-like immunoreactivity of neurons in the chicken diencephalon and mesencephalon, *J. Comp. Neurol.*, 1989, 287: 455.
  - 11 Schmidt, M., Lewald, J., Vandertogt, C. *et al.*, The contribution of GABA - mediated inhibition to response properties of neurons in the nucleus of the optic tract in the rat, *European J. Neurosci.*, 1994, 6(11): 1656.
  - 12 Gao, H. F., Wu, G. Y., Frost, B. J. *et al.*, Excitatory and inhibitory neurotransmitters in the nucleus rotundus of pigeons, *Visual Neuroscience*, 1995, 12: 819.
  - 13 Wang, S. R., Wu, G. Y., Felix, D., Imc-tectal projection is mediated by acetylcholine and glutamate, *Neuroreport*, 1995, 6: 757.
  - 14 Vidyasagar, T. R., Contribution of inhibitory mechanisms to the orientation sensitivity of cat dLGN neurones, *Exp. Brain Res.*, 1984, 55: 192.
  - 15 Soodak, R. E., Shapley, R. M., Kaplan, E., Linear mechanism of orientation tuning in the retina and lateral geniculate nucleus of the cat, *J. Neurophysiol.*, 1987, 58: 267.