Expression of the Fos protein reveals functional subdivisions of the avian ventral lateral geniculate nucleus

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Abstract

The Fos protein was immunocytochemically detected in the chick ventral lateral geniculate nucleus after novel stationary and optokinetic stimulation. Fos-positive nuclei were mainly detected in the internal part of the ventral geniculate when the animals were submitted to stationary visual stimulation. On the other hand, Fos-positive nuclei were mainly seen in the external part of the nucleus when optokinetic stimuli were used. These data reveal functional subdivisions of the avian ventral geniculate, and support the hypothesis that this nucleus is involved in several aspects of the visual function.

Keywords: C-fos; Optokinetic nystagmus; Subcortical visual pathways; Ventral geniculate

Despite the massive retinal input of the ventral lateral geniculate nucleus (GLv), its visual function remains elusive [1]. There are suggestions of roles for the GLv in the pupillary reflex [13], color detection [11,16,20,25,26], circadian rhythms [4,22], and visuomotor integration [3,7,21]. The connections of the GLv appear to support the latter possibility, as the GLv is connected to the superior colliculus, pretectum, accessory optic nuclei, cerebellum, and vestibular complex [6,8,13,24]. In the present study, we analyzed immunocytochemically the expression of the Fos protein [18,19] in the avian GLv after visual stimulation.

Eighteen 10–15 day old chicks (Gallus gallus) were subjected to one of the following protocols. In the first group, plastic diffusers were applied to the feathers around one eye [28]. These diffusers attenuated light entering the eye by 0.6 log units. The chicks (n = 6) wore the diffuser for 5–7 days before the stimulus session, when it was removed and switched to the other, control eye. The period of 5–7 days of visual deprivation proved to be ideal to maximize Fos expression after visual stimulation. The animal was gently restrained and placed in a general illumination of about 1500 lx. The eye that had been deprived had the lower eyelid held open, and had a free view of the walls of the laboratory. As a control for novelty, two other birds were stimulated with the same procedures, but without having worn the diffuser. Chicks of the second group (n = 8) were kept for 5–7 days in a box covered with the same random-dot pattern used for optokinetic stimulation in the horizontal and vertical directions (1.0°/s). This procedure aimed at avoiding any Fos expression due to the novelty of the pattern. During the stimulation session, the birds were gently restrained, and the stimulus covered about 60° of the central visual field. One eye faced the pattern and the other faced a wall with the same (but stationary) pattern (control). The general illumination level was also around 1500 lx. Both stimulus situations lasted for 1–1.5 h, and were always conducted around 1000–1100 h. The animals were deeply anesthetized with ketamine and xylazine and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Two chicks not subjected to any specific stimulus were also anesthetized.
and perfused, in order to disclose any ‘basal’ Fos expression. The brains were postfixed, cryoprotected, and cut (30 μm) in the coronal plane. The sections were incubated with a rabbit antiserum (R. Bravo, Bristol-Myers Squibb, Princeton, NJ, USA), which has been shown to recognize the Fos protein and Fos-related antigens [15,23,28]. The antiserum was diluted 1:1000 in PB with 0.3% Triton X-100. Incubation times ranged from 12 to 16 h at 4°C. Details of the avidin-biotin protocol have been published [2]. Analysis of Fos-positive nuclei was subjective, and counterstaining with Giemsa [12] helped in the evaluation of the percentage of stained neurons. As the chick optic nerves are almost completely crossed [5], one side of the brain was used as a control.

Both novel stationary and optokinetic stimulation elicited specific Fos staining in visual nuclei of the chick brain. This staining was restricted to the side contralateral to the stimulated eye. Furthermore, no Fos-positive nuclei were detected in chicks not subjected to visual stimulation. The stimuli elicited Fos labeling in regions of the chick brain that are known to be related to diffuse light detection [6] and visual motion [6,23,27].

Many Fos-positive nuclei were observed in the internal part of the GLv (iGLv) (Fig. 1) after novel stationary stimulation. The percentage of cells in the iGLv averaged 52%. Very few nuclei were labeled in the iGLv of chicks that experienced the same stimulus, but without the preceding period of diffuser wear. Our experiments cannot distinguish between the Fos response being the result of the novelty of form vision after deprivation or the novelty of the brighter illumination which resulted from the removal of the diffuser. Only 2% of the iGLv neurons were labeled after optokinetic stimulation.

The external subdivision of the GLv (eGLv) exhibited a Fos response that was the opposite of the response of the iGLv. Following novel stationary stimulation, only 6% of the eGLv neurons were Fos-positive. The optokinetic stimulation, on the other hand, generated Fos responses in about 26% of the eGLv neurons (Fig. 2). The differential labeling of iGLv and eGLv in both conditions was very consistent among the animals of different groups. The percentage of labeled nuclei in response to horizontal stimulation was consistently higher (31%) than the percentage of Fos-positive nuclei in response to vertical stimulation (15%) (Fig. 3). The percentage of labeled nuclei was higher for temporal to nasal motion (43%) than for nasal to temporal motion (21%), whereas no obvious differences were noted for the different directions of vertical movement.

These data indicate that the chick GLv may contain at least two functional subdivisions, one that might be mainly...
involved in the detection of diffuse illumination (iGLv), and the other perhaps more related to a visuomotor role (eGLv). The iGLv may then be the subdivision of the GLv that could have a role in the pupillary reflex. This function was proposed for the GLv based on its connections with the pretectum [6,13]. The eGLv neurons have indeed been suggested to project to the chick pretectum [10]. The eGLv appeared to be more related to the optokinetic stimulus. This subnucleus could then be the responsible for the role of the GLv in the optomotor behavior [7,21]. With the protocol employed here, we could not decide, however, if the Fos labeling in the eGLv could have been generated by the actual motor responses and/or the visual input. Despite the fact that the avian GLv has been related to the horizontal and not to the vertical optokinetic nystagmus [7], Fos-positive nuclei were also detected in the GLv after vertical motion. Some GLv units were previously shown to respond to vertical movement [21].

Several cell types exist in the GLv [9,10,14]. It has been suggested that the medium-sized neurons of the chick GLv are motion-sensitive [10,21]. In our counterstained sections we could verify that several eGLv cells with Fos-positive nuclei were indeed medium-sized. The iGLv medium-sized neurons, however, were seldom seen to be labeled, but they might be sensitive to other types of dynamic stimuli. The rat GLv has been recently shown to present Fos-positive nuclei in response to a patterned stimulus displaced at 16°/s, although the labeling was similar after both stationary and moving stimuli [17].

In summary, despite the limitations of using the Fos expression as an index of activity [18,19], the present data reveal a functional parcellation of the chick GLv and support the hypothesis that the GLv might be involved in several aspects of the visual function.

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[12] Iniguez, C., Gayoso, M.J. and Carreres, J., A versatile and simple...


