

## EMBRYONIC AND LARVAL DEVELOPMENT OF THE BASAL GANGLIA CONNECTIONS IN THE BRAIN OF *XENOPUS LAEVIS*

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Recently, the afferent and efferent connections of the components of the amphibian basal ganglia, i.e., the striatum and the nucleus accumbens, have been studied in the brain of adult anurans and urodeles (Marín et al., 1995, 1996, 1997a,b). Significant differences in connections between the nucleus accumbens and the striatum were demonstrated, suggesting the presence of functional subdivisions. Moreover, those studies strongly supported the notion that a common pattern of connectivity exists among tetrapod vertebrates.

The aim of the present study was to establish the temporal sequence of appearance of striatal and accumbal connections in amphibians so that the comparison with other amniotes allows to gain more insight into the evolution of the basal ganglia of vertebrates. To reach this goal, the anuran *Xenopus laevis* has been selected because of the existence of an accurate timetable of development (Nieuwkoop and Faber, 1967) and the existence of recent histological data of basal ganglia connections in the adult brain of this species.

The methodology used in this study is based on the neuronal transport of low molecular weight (3kD) biotinylated dextranamines (BDA). Shortly, at appropriate times, embryos and larvae anesthetized in MS222 were cooled to a body temperature of 4°C and perfused transcardially with iced Ringer's solution. The brains were then removed and placed in fresh iced Ringer's solution. Applications of BDA were made unilaterally within the striatum (Fig.1) or the nucleus accumbens. After 15-24 h at 15°C in continuously oxygenated Ringer's solution the brains were fixed in 4% paraformaldehyde. Following crioprotection, the brains were cut on a freezing microtome and free-floating sections were reacted with avidin-biotin complex for BDA visualization. The detection of peroxidase activity was based on DAB-nickel as chromogen. After mounting the sections, the distribution of labeled cells and fibers in the brains of the tadpoles was studied.

At late embryonic stages, the basal forebrain of *Xenopus* receives inputs

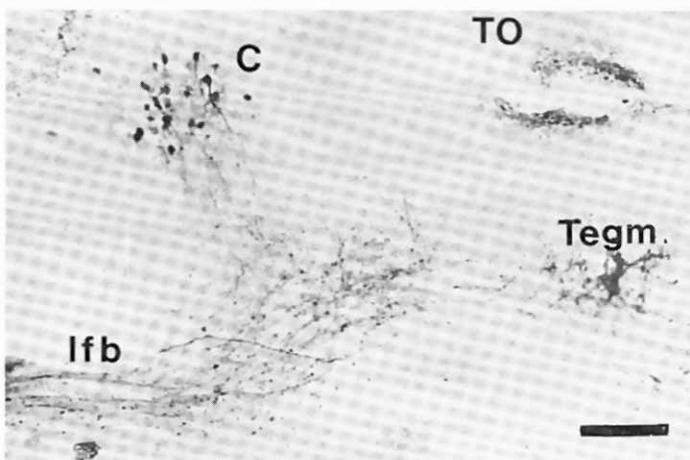


Figure 2. Sagittal section through the brain of a premetamorphic tadpole showing afferent cells in the central thalamic nucleus (C) and the terminal field in the mesencephalic tegmentum (tegm). (Ifb: lateral forebrain bundle; TO: optic tectum). Bar=100 µm.

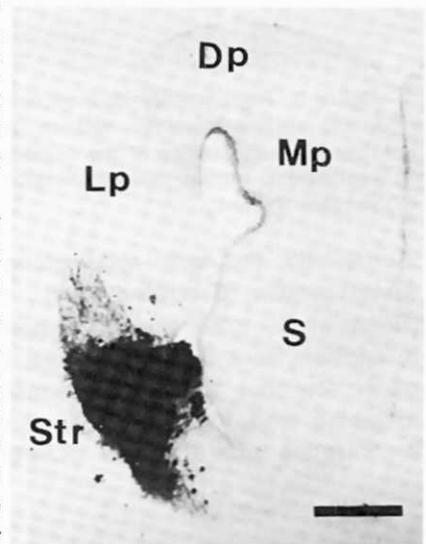


Figure 1. Photomicrograph illustrating a case of BDA application in the striatum at premetamorphic stages (Dp: dorsal pallium; Lp: lateral pallium; Mp: medial pallium; S: septum; Str: striatum). Bar=100 µm.

from cells located in the amygdala, the thalamus, the suprachiasmatic nucleus, the posterior tubercle, the raphe nucleus and the rhombencephalic reticular formation. During the *premetamorphosis*, the distinct connections of the striatum and the nucleus accumbens start to be patent.

Due to the bidirectional transport of BDA, both afferent cells and efferent fibers are distinguishable at these stages. Thus, afferent cells to the striatum are more numerous in the same areas observed in the embryos (mainly in the central dorsal thalamus, Fig. 2) but also new sources of inputs were detected in the mesencephalic and isthmic tegmentum, the parabrachial nucleus and the nucleus of the solitary tract. The efferent fibers leaving the telencephalon via the lateral forebrain bundle form a conspicuous terminal plexus in the mesencephalic tegmentum (Fig.

2). The afferent cells to the nucleus accumbens in premetamorphosis are located primarily in the olfactory bulb, medial amygdala, preoptic area, suprachiasmatic nucleus, anterior thalamic nucleus (Fig. 3), and in the same areas of the brainstem where striatal afferents are located.

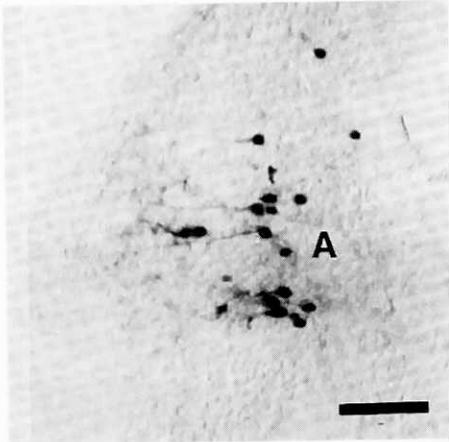


Figure 3. Retrogradely labeled cells in the anterior thalamic nucleus (A) after BDA application in the nucleus accumbens during premetamorphosis. Bar=100  $\mu$ m.

The *prometamorphosis* is characterized by a gradual increase in the number of cells that project to the basal telencephalon. These cells are located in the same areas found in previous larval stages and a strikingly high development of the afferents from the dorsal thalamus (the main source of basal ganglia afferences) is found during this period (Fig. 4). At the beginning of the *metamorphic climax*, the organization of the connections of the basal ganglia largely resembles the pattern observed in juveniles and adults.

The present study has shown that the early development of basal ganglia connections appears to be a common feature not only for amniotes, but probably for all tetrapod vertebrates.

Particularly, the early appearance of dopaminergic inputs to the developing basal ganglia (González et al., 1994) resembles the situation of amniotes (Voorn et al., 1988) and, as suggested previously, dopamine signaling could play a role in establishing connections. In addition, as a constant feature, the main efferent connections of the striatum and the nucleus accumbens develop much later than the afferent connections. All these developmental aspects are easily correlated to the situation in amniotes and, therefore, the notion that amphibians share an essentially similar pattern of basal ganglia organization with amniotes is further strengthened.

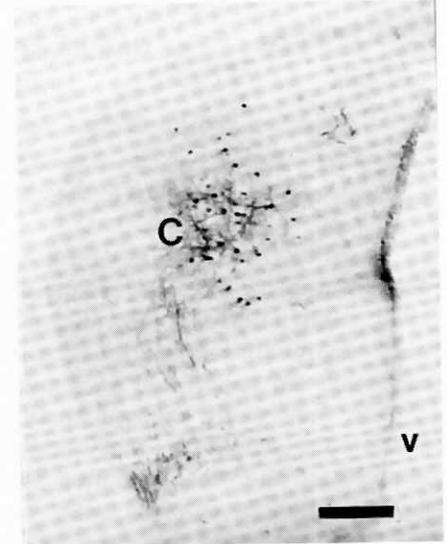


Figure 4. Retrogradely labeled cells in the central thalamic nucleus (A) after BDA application in the striatum during prometamorphosis. Bar=100  $\mu$ m.

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