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Distribution of NADPH-diaphorase reactivity in the central nervous system of the common toad (*Bufo bufo*)

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ABSTRACT

We examined the distribution of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-reactive elements in the central nervous system (CNS) of the common toad, *Bufo bufo*. The investigation involved adult male and female toads collected during the breeding season. Labeled neurons of different morphological appearances (weakly or darkly stained, unipolar, bipolar, and multipolar) and fibers were observed across all subdivisions of the amphibian brain. Overall, a similar distribution of NADPH-D-labeled neurons was observed in the brain of male and female toads. In the secondary prosencephalon NADPH-D-labeled neurons were observed in the olfactory bulbs, pallial regions, nucleus accumbens, diagonal band of Broca, septum, striatum, amygdala, suprachiasmatic and magnocellular preoptic nuclei, dorsal and ventral hypothalamus. In the diencephalon, NADPH-D-positive neurons were seen in the anterior thalamic nuclei, ventromedial and ventrolateral nuclei, central and lateral thalamic nuclei, posterior tubercle, posterodorsal division of the lateral thalamic nucleus, and in the pretectal and pretoral gray. In the mesencephalon, heavily stained neurons were present in the anterodorsal and anteroventral tegmental nuclei, magnocellular, principal and laminar nuclei of the torus semicircularis, and nucleus profundus mesencephali. In the isthmus, stained cells were observed medially and ventrally in the posterodorsal and posteroventral tegmental nuclei. In the rhombencephalon, numerous NADPH-D-stained neurons were distributed in the cerebellar nucleus, sensory and descending trigeminal nuclei, motor nuclei of the glossopharyngeal and vagus nerves, the nucleus of the solitary tract, nuclei of the hypoglossal and octaval nerves, dorsal column nucleus, central gray region, and in reticular formation. However, the complete absence of NADPH-D-stained neurons in the cerebellar cortex was an unusual feature observed in this study. The widespread distribution of NADPH-d staining in diverse cell types, belonging to a variety of neuronal systems suggests a widespread role for NADPH-d in modulating diverse functions, including sensory coding in the amphibian nervous system.

1. Introduction

Nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-positive cells are involved in the production of nitric oxide (NO), a widely recognized and unique cell signaling molecule that plays multiple roles in the brain and peripheral tissues of vertebrates and

Abbreviations: A, anterior thalamic nucleus; AC, anterior commissure; AD, anterodorsal tegmental nucleus; AMYl, lateral amygdala; AMYm, medial amygdala; AOB, accessory olfactory bulb; OTr, olfactory tract; AQ, aqueduct of Sylvius; AV, anteroventral tegmental nucleus; C, central thalamic nucleus; CB, cerebellar nucleus; CBL, cerebellum; CG, central gray; CGT, thalamic geniculate body; DBB, nucleus of the diagonal band of Broca; DCN, dorsal column nucleus; DHyp, dorsal hypothalamus; DP, dorsal pallium; gl, glomerular layer; HB, habenula; HR, hindbrain reticular formation; ig, internal granular layer; III, third ventricle; IR, infundibular recess; IV, fourth ventricle; IXm, motor nucleus of glossopharyngeal nerve; La, lateral thalamic nucleus; LAM, laminar nucleus of the torus semicircularis; LP, lateral pallium; Lpd, lateral thalamic nucleus posterodorsal division; LV, lateral ventricle; MOB, main olfactory bulb; MP, medial pallium; MT, magnocellular nucleus of the torus semicircularis; NAC, nucleus accumbens; NI, nucleus isthmi; OB, olfactory bulbs; OC, optic chiasma; ON, optic nerve; OT, optic tectum; OTr, olfactory tracts; OV, optic ventricle; P, posterior thalamic nucleus; PalC, pallial commissure; PC, posterior commissure; PD, posterodorsal tegmental nucleus; POA, preoptic area; POAp, posterior preoptic area; PON, preoptic nucleus; PONa, anterior preoptic nucleus; POR, preoptic recess; PR, principal nucleus of the torus semicircularis; PRM, nucleus profundus mesencephali; PTG, pretectal gray; PTRG, pretoral gray; PV, posteroventral tegmental nucleus; SCN, suprachiasmatic nucleus; SOC, supraoptic commissure; Sl, lareral septum; Sm, medial septum; Sol, nucleus of the solitary tract; STR, striatum; TC, tectal commissure; TEL, telencephalon; TP, posterior tubercle; Vds, descending trigeminal nucleus; VIII, octaval nucleus; VL, ventrolateral thalamic nucleus; VM, ventromedial thalamic nucleus; Vpr, principal sensory trigeminal nucleu; Xm, motor nucleus of the vagus; XII, nucleus of hypoglossal nerve

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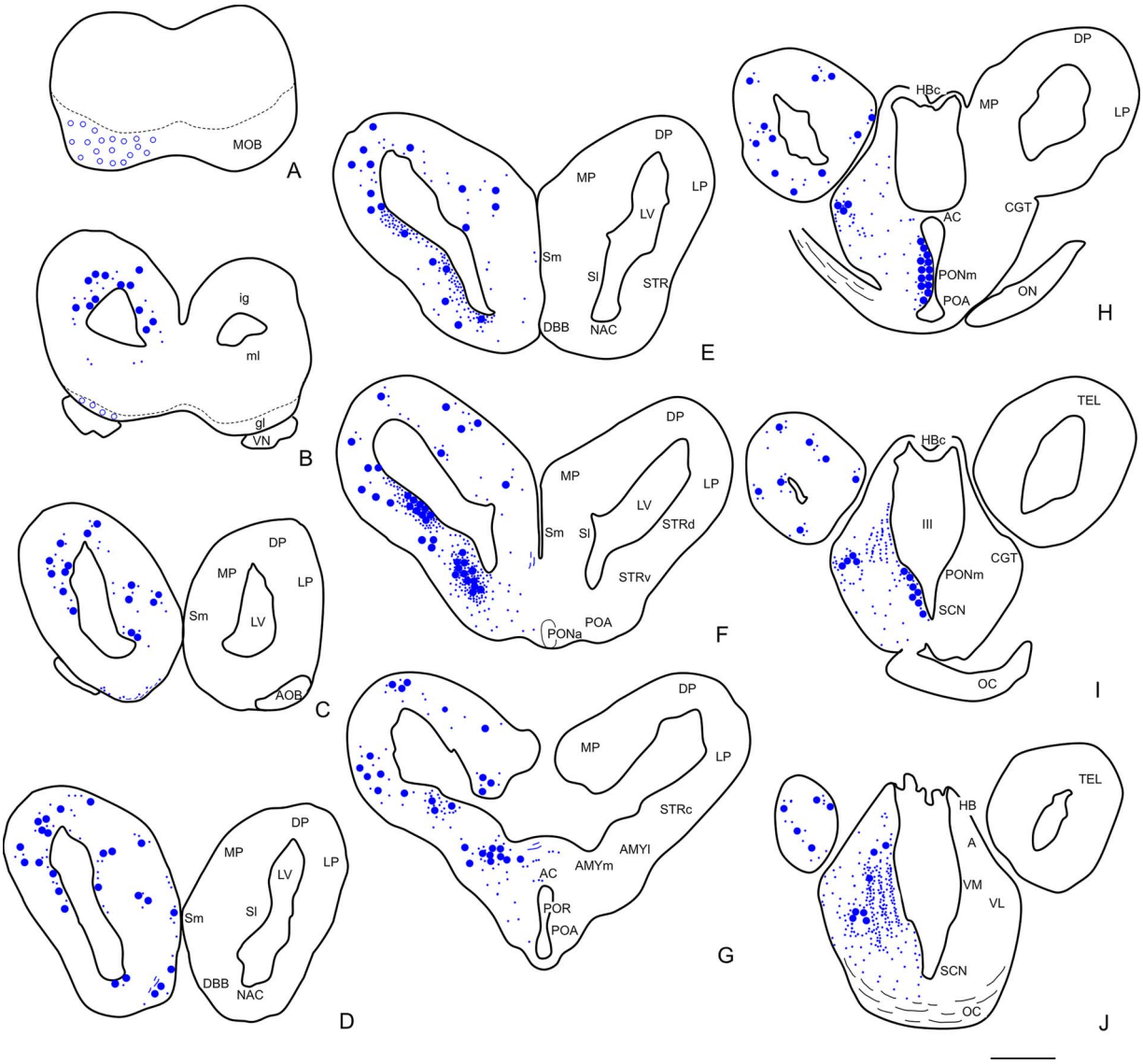

Fig. 1. (Parts I and II). Distribution of NADPH-d-positive cell bodies and fibers in the toad brain. Camera lucida drawings of rostro-caudal progressive transverse sections of the toad brain at the levels indicated by the letters in the schematic lateral view of the brain at right side lower panel. NADPH-d-positive cell bodies are indicated as large dots and the fibers are indicated as dashes and fine dots. The number of dots corresponds to the densities of the NADPH-d-positive elements, as described in the Results section. Empty dots in the olfactory bulbs indicate the glomeruli. Scale bar: 500 μm.

invertebrates (Toda and Ayajiki, 2006; Knott and Bossy-Wetzel, 2009).

NO has been considered a neurotransmitter and may also modulate synaptic functions in the central nervous system (CNS) with implications in olfaction, food and liquid intake, modulation of nociception, release of other neurotransmitters, neuroendocrinology of reproduction, reproductive behavior, learning and memory processes (Nelson et al., 1997; Park et al., 1998a,b; Ernst et al., 1999; McCann et al., 1999, 2003; Rentería and Constantine-Paton, 1999; Steinbusch et al., 2000; Nelson and Chiavegatto, 2001; Prast and Philippu, 2001; Villani et al., 2001; Esplugues, 2002; Moreno et al., 2002a,b; Paul and Ekambaram, 2011)

NO originates from L-arginine through a reaction, requiring NADPH and oxygen (O_2), and catalyzed by a constitutive neuronal isoform of nitric oxide synthase (NOS) called neuronal nitric oxide synthase (nNOS). During the histochemical reactions, NADPH-d reduces chromogens such as tetrazolium salts to form insoluble dark formazan products, thereby allowing the use of NADPH-d histochemistry for the localization of nNOS in the nervous system (Thomas and Pears, 1961; Dawson et al., 1991; Hope et al., 1991). The direct relationship between NADPH-d staining and NOS expression has been well documented (Bredt et al., 1991; Dawson et al., 1991) although the presence of NOS does not seem to be always reflected by NADPH-d histochemistry (Hope et al., 1991; Holmqvist et al., 1994; Brüning et al., 1994a,b; Brüning and Mayer, 1996; López and González, 2002; Moreno et al., 2002b).

The neurons producing NO/nNOS have been localized to several areas of the vertebrate CNS using both histochemical and immunohistochemical methods. Some studies have demonstrated colocalization in a few areas of the vertebrate brain with choline acetyltransferase, tyrosine hydroxylase (TH), serotonin (5-HT), and numerous neuropeptides (Panzica et al., 1998; Prast and Philippu, 2001; López et al., 2005).

Interestingly, the scientific reports focusing on the pattern of NADPH-d distribution in the amphibian brain reveal a large variability in the brain NADPH-d neuronal system (Artero et al., 1995; Brüning and Mayer, 1996, 2001; González et al., 1996, 2002; Muñoz et al., 1996, 2000; Porteros et al., 1996; Pitzer and Wirtshafter, 1997; Prasada Rao et al., 1997; Lázár and Losonczy, 1999; Mühlenbrock-Lenter et al., 2005; Huynh and Boyd, 2007; Pinelli et al., 2014; Jadhao et al., 2017) with differences recorded also in closely related species (Pinelli et al., 2014). Therefore, an attempt to define a wider neuroanatomical pattern