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IMMUNOHISTOCHEMICAL DISTRIBUTION OF AMPA-TYPE LABEL IN THE PIGEON (*C. LIVIA*) HIPPOCAMPUS

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Abstract—The avian hippocampal formation (HF) is reported to have a role equivalent to that of the mammalian hippocampus, which may involve the glutamatergic system as well. In the present paper we offer evidence of the occurrence and distribution of the subunits composing AMPA-type glutamate receptors on neurons in the hippocampus region of the pigeon brain. The experiment analyzed the immunolabeling of glutamate receptor (GluR)$_1$, GluR$_4$, and GluR$_{2/3}$ receptor subunits in adult pigeons and found consistent evidence that neurons located in the five main areas of the avian HF have these AMPA-type subunits, but their incidence varies according to position and neuro-type. About 20%–35% of the irregular and 35%–70% of the triangular neurons on the lateral and medial "V" arms contain GluR$_1$ and GluR$_{2/3}$, while GluR$_4$ was found only at rounded neurons. The majority of the triangular neurons (over 90%) and about half of the irregular neurons in the medial area contain GluR$_1$ and GluR$_{2/3}$, whereas the rounded neurons contain primarily GluR$_4$ (95%). Labeling revealed GluR$_1$ (40%–60%) and GluR$_{2/3}$ (30%) in the dorsomedial and lateral areas but only in irregular neurons, while 60%–80% of the rounded neurons synthesize GluR$_4$. While triangular and irregular neurons appear to match the description of projecting neurons, rounded ones seem to participate in local circuits. A discussion of the functional significance of the avian HF concentrates on a postulation of the "V" arms as equivalent to the dentate gyrus and the dorsomedial area being similar to the Ammon's horn. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: glutamate receptors, avian brain, comparative neuroanatomy, neurochemical systems, dorsal pallium.

Many studies of the hippocampus of birds involve its anatomical, neurochemical and functional organization and were designed to compare aspects of this area with what is found in mammalian species. The investigations conducted with primates (Eichenbaum et al., 1992; Martin et al., 1993; Akhondzadeh, 1999), rodents (Sprick, 1995; Tischmeyer and Grimm, 1999; Guzowski et al., 2001), and birds as well (Reis et al., 1999; Colombo and Broadbent, 2000; Watanabe, 2001; Bingman and Sharp, 2006) have provided convincing evidence suggesting the equivalence of some of the essential roles of the hippocampus in these different vertebrate classes. However, in contrast to the consensus reigning in relation to the description of the structural and hodological characteristics of the mammalian hippocampal formation (HF), no such agreement is found for avian species [Krebs et al., 1991; Colombo and Broabent, 2000; Atoji and Wild, 2004; and specially Atoji and Wild (2006) for review].

The HF of the avian brain was first described in anatomical studies conducted in the 1930s (*apud* Campbell and Hodos, 1970). At that time, the dorsomedial (DM) telencephalon of birds was considered to be organized in two segments: the parahippocampal area (APH) and the hippocampus (HP); the latter is a laminated-like structure with two unique cellular columns in the shape of a "V" (Fig. 1A). This division has been used since then, as reported in the Brain Atlas of the Pigeon by Karten and Hodos (1967). Although the avian encephalon lacks the typical mammalian cortical structure (Casini et al., 1986; Colombo and Broabent, 2000), the "V"-shaped lamination provided a structure which can be compared to the CA (Ammon's horn) region of the mammalian APH (Erichsen et al., 1991; Montagnese et al., 1996; Kahn et al., 2003). Although the subdivisions of the avian HP have been widely discussed, there is no universal consensus; however the traditional correlation between the "V" arms and CA seems to provide an inaccurate analysis (Atoji and Wild, 2006; Bingman and Sharp, 2006; Suárez et al., 2006).

The equivalence of avian and mammalian brain areas was also investigated using correlations among neurochemical pathways, neurotransmitter systems and synaptic receptor classes. Immunodetection of various signaling agents within the avian hippocampal complex has been used to establish boundaries between HP and APH, as well as between subdivisions of these regions (Erichsen et al., 1991; Krebs et al., 1991; Esposito et al., 1994; Molnar et al., 1994; Kuenzel et al., 1997; Szèkely, 1999; Suárez et al., 2006). As consequence of the immunohistochemical approach, by the 1990s, a new organization of the pigeon's HP formation had been proposed. It included six different internal areas: the medial fiber tract, the "V"-shaped area, the medial area, the VIP-cell area, the DM, the lateral area and a dorsolateral (DL) boundary portion, called the substance P frontier (SPf) (Fig. 1B). A recent study reevaluated those morphochemical subdivisions of the APH and

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Abbreviations: APH, area parahippocampalis; CA, cornu ammonis; CDL, dorsolateral corticoid area; DG, dentate gyrus; DL, dorsolateral; DM, dorsomedial area; GluR, glutamate receptor; GluR1, AMPA-type glutamate receptor subunit 1; GluR4, AMPA-type glutamate receptor subunit 4; GluR2/3, AMPA-type glutamate receptor subunits 2 and 3; HF, hippocampal formation; HP, hippocampus; IEG, immediate-early-gene; IR, irregular cell; LAMP, limbic system-associated membrane protein; Ma, magnocellular region; NMDA, *N*-methyl-D-aspartate; Pa, parvocellular region; PB, phosphate buffer; Po, cell-poor region; R, rounded cell; SPf, substance P frontier; T, triangular cell; tr., triangular region.

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HF and highlighted the functional importance of the medial dorsal portion of the pallium of the avian brain. Suárez et al. (2006) introduced a radial subdivision for the APH area consisting of lateral, medial, and intermedial portions and Atoji and Wild's collaborative work (2004, 2006) suggested that the HF is organized with DL and DM divisions. In this latest arrangement, the DL area, which includes both a dorsal and a ventral portion, does not include the HP itself. This is considered to be the equivalent to the mammalian entorhinal cortex (Atoji and Wild, 2006). These authors also divide the DM into a lateral and a medial portion, corresponding, respectively, to the lateral and DM portions proposed by Erichsen (Erichsen et al., 1991; Krebs et al., 1991). Moreover, they renamed the former medial area triangular region (tr., instead of medial) and described the upper dorsal HP, a cell-poor area (lacking cellular bodies) in contrast to the magnocellular region (Ma) and the parvocellular region (Pa) which have rich neuronal populations (Atoji and Wild, 2006) (Fig. 1C, 1D).

All studies cited have provided data on neurochemical and circuitry organization which reinforce the correspondence between the avian HF and the mammalian HP. The amino acid glutamate, which underlies the functioning of HP in mammals, has been widely studied although little is known about its function in the bird brain. The only study even mentioning its presence in the avian HP is that of the Japanese quail (Cornil et al., 2000). This lack of knowledge about the glutamate system in the avian HP is in direct contrast with the enormous importance that has been attributed to glutamatergic synapses and receptors in neuronal circuits related to learning processes in the mammalian brain (Bramham et al., 1990; Petralia and Wenthold, 1992; Wenthold et al., 1996; Toyama and Koichi, 1998). The rapid glutamatergic actions in mammalian hippocampal areas are in part mediated by three classes of ionotropic glutamate receptors (GluRs) which are classified according to their specific agonists: AMPA, *N*-methyl-D-aspartate (NMDA), and kainate (kA). The first of these (AMPA-type receptors) play a significant role (Bramham et al., 1990; Jonas, 1993; Wenthold et al., 1996; Hall and Soderling, 1997; Venero and Sandi, 1997; Tsuzuki et al., 2000; Kakegawa et al., 2004). These AMPA-type receptors are heteromers composed of combinations of four subunits, $GluR_{1\text{-}4}$ that influences the channel kinetics (Hollmann et al., 1991; Seeburg, 1993; Hollmann and Heinemann, 1994).

In the present paper we intended to describe the presence, typology and distribution of the subunits of the AMPA-

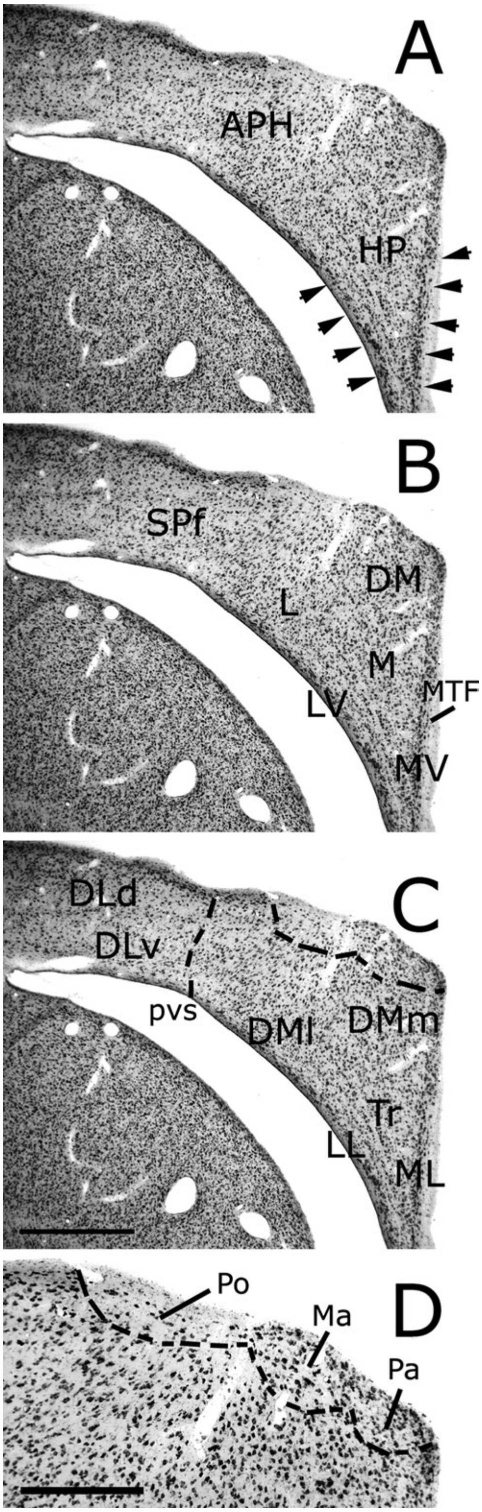

Fig. 1. Avian hippocampal areas. Low magnification images of the dorsal telencephalon of a pigeon brain, stained with Giemsa. (A) Classical view of the two original subdivisions adopted in the stereotaxic atlas of the pigeon brain by Karten and Hodos (1967), with arrowheads identifying the two cellular columns forming the "V" arms. (B) The image represents the proposition of Krebs et al. (1991) and Erichsen et al. (1991), as modified by Atoji and Wild (2006). (C) New subdivisions proposed for the avian dorsal pallium, including the dorsal cellular zone detailed in D. Note the SPf labeled area in (B) which marks the limits of the HF according to Krebs's and Erichsen's proposition, corresponds to the paraventricular sulcus (pvs), the boundary of DM and DL area of Atoji and Wild's proposition in C. Abbreviations: DLd: dorsal portion of the dorsolateral hippocampal formation, DLv: ventral portion of the dorsolateral hippocampal formation, DMl: lateral portion of the dorsomedial area, DMm: medial portion of the dorsomedial area, L, lateral portion of the hippocampus, LL: lateral layer of V-shaped structure, LV, lateral portion of the V arm, M, medial hippocampus, ML: lateral layer of V-shaped structure, MTF: medial tract fibers, MV: medial portion of the V arm, pvs: paraventricular sulcus. Scale bars=800 μm (A–C); 500 μm (D).