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Comparative investigation of neuronal nitric oxide synthase immunoreactivity in rat and human claustrum

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ABSTRACT

We compared the distribution, density and morphological characteristics of nitric oxide synthase-immunoreactive (NOS-ir) neurons in the rat and human claustrum. These neurons were categorized by diameter into three main types: large, medium and small. In the human claustrum, large neurons ranged from 26 to 40 μm in diameter, medium neurons from 20 to 25 μm and small neurons from 13 to 19 μm. In the rat claustrum, large neurons ranged from 19 to 23 μm in diameter, medium neurons from 15 to 18 μm and small neurons from 10 to 14 μm. The cell bodies of large and medium neurons varied broadly in shape – multipolar, elliptical, bipolar and irregular, consistent with a projection neuron phenotype. The small neurons were most seen as being oval or elliptical in shape, resembling an interneuron phenotype. Based on a quantitative comparison of their dendritic characteristics, the NOS-ir neurons of humans and rats displayed a statistically significant difference.

1. Introduction

The claustrum is a telencephalic, subcortical structure present in nearly all mammalian brains. It was first depicted by the noted French physician and anatomist Felix Vicq d'Azyr, 1786 and described as a " . . . *separated cortical tract between the Sylvian fissure and the corpus striatum*" (Guirado et al., 2003; Real et al., 2003; Ashwell et al., 2004; Edelstein and Denaro, 2004). It was subsequently named the "claustrum" by the German physiologist Karl Burdach (Rae, 1954a,b).

The morphology and cytoarchitecture of the claustrum have been described in many mammals (Berke, 1960; Brockhaus, 1940; Druga, 1966a, 2014; Edelstein et al., 2010, 2011; Edelstein and Denaro, 2004; Filimonoff, 1966; Kowianski et al., 1998; Narkiewicz, 1964; Narkiewicz and Mamos, 1990; Rae, 1954a,b; Wójcik et al., 2004). Morphologically, the claustrum is a relatively simple nucleus. Traditionally, it is divided into two subdivisions: the dorsal (insular) claustrum and the ventral (piriform nucleus) claustrum or endopiriform nucleus. The dorsal claustrum is located deep to the insular cortex, whereas the ventral claustrum is located deep to the piriform cortex (Druga 1966a,b, 1968, 1971, 1975; Otellin and Makarov, 1972; Kunzle, 1975, 1978; Norita, 1977; Riche and Lanoir, 1978; Olson and Graybiel, 1980; Carey et al., 1980; Hinova-Palova et al., 1980a,b, 2012; Hinova-Palova and Paloff, 1982, 1984; Carey and Neal, 1985; Edelstein, 1986; Neal et al., 1986; Sloniewski et al., 1986a,b; Tanne-Gariepy et al., 2002; Guirado et al., 2003; Edelstein and Denaro, 2004; Ashwell et al., 2004; Sherk 1986; Witter et al., 1988; Dinopoulos et al., 1992; Baizer et al., 2014). Moryś et al. (1996) further divided the human claustrum into four parts: dorsal, orbital, temporal, and paraamygdalar. Namavar et al. (2005) distinguished three parts in the dorsoventral plane: cap, dorsal, and ventral parts.

In the human claustrum, five different types of neurons have been described: Type I are spiny neurons of various sizes and shapes; Type II are large aspiny neurons; Type III are large aspiny neurons devoid of pigment deposits; Type IV are small pigment-

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laden aspiny neurons, and Type V are small aspiny neurons devoid of lipofuscin granules (Braak and Braak, 1982).

The dorsal claustrum in mammals is broadly connected with the neocortex in rat (Edelstein and Denaro, 1980; Sloniewski et al., 1986a,b), cat (Narkiewicz, 1964, 1972; Druga, 1966a,b, 1968, 1975; Norita, 1977; Riche and Lanoir, 1978; Olson and Graybiel, 1980; Neal et al., 1986), dog (Narkiewicz, 1972), monkey (Kunzle, 1978; Riche and Lanoir, 1978; Edelstein and Denaro, 1979; 2004; Tanne-Gariepy et al., 2002), while the ventral claustrum is primarily interconnected with the prepiriform and entorhinal cortices (Druga, 1971; Witter et al., 1988; Dinopoulos et al., 1992; Tanne-Gariepy et al., 2002).

The claustrum is long known to be a site of heterosensory convergence in mammals and human (Olson and Graybiel, 1980; Ashwell et al., 2004; Hinova-Palova et al., 2007, 2008, 2012, 2014). The numerous neuronal populations as well as the topographical localization and physiologic attributes of its connections reveal the complexity of this irregularly-shaped and sheet-like telencephalic mass. The functional relationship between the claustrum and adjacent brain areas in different species of mammals continues to be the subject of debate (Edelstein and Denaro, 2004; Crick and Koch, 2005; Smythies et al., 2012, 2014a,b).

The dimensions of the claustrum vary greatly throughout the mammalian phylogenetic scale. In small lissencephalic animals (e.g., rat, mouse, bat, hedgehog) the claustrum is small, and due to a weakly developed extreme capsule, is sometimes difficult to distinguish from the adjacent cortex. In some other rodents (e.g., beaver, guinea pig), as well as in rabbits and primates, the volume of the claustrum increases and is easily differentiated from adjacent structures (De Vries, 1910; Landau, 1923; Berlucchi, 1927; Loo, 1931; Brockhaus, 1940; Macchi, 1948; Rae, 1954a,b; Stelmasiak, 1955; Berke, 1960; Pilleri, 1961, 1962; Filimonoff, 1966; Druga, 1974, 1975; Zilles et al., 1980; Paxinos and Watson, 1998; Kowiański et al., 2004). In the last few years there are also some studies describing mediators in different populations of claustral cells (Cozzi et al., 2014; Hinova-Palova et al., 2014; Pirone et al., 2014; Rockland, 2014; Watakabe et al., 2014).

Nitric oxide (NO) has been recognized as a unique gaseous neurotransmitter (Garthwaite, 1991; Holstein et al., 2001; Martinelli et al., 2002), and neurons expressing the NO-synthesizing enzyme, nitric oxide synthase (NOS), exist in certain cell populations of the brain (Snyder, 1992; Olmos et al., 2005). Nitric oxide takes part in many important physiologic processes such as synaptic plasticity in the hippocampus (Krushkov et al., 1996), as well as eye movements (Moreno-López et al., 1996, 1998, 2001), blood pressure control (Maeda et al., 1999), and the functional integrity of the claustrum (Hinova-Palova et al., 1997), thalamus (Krushkov et al., 1996), inferior colliculus (Paloff and Hinova-Palova, 1998), and vestibular complex (Papantchev et al., 2005, 2006). Histochemical mapping studies of the claustrum reveal NOS and NADPH-d activity (Vincent and Kimura, 1992; Paloff et al., 1994; Rodrigo et al., 1994; Hinova-Palova et al., 1997, 2013; Paloff and Hinova-Palova, 1998; Paxinos and Watson, 1998; Vincent, 2000; Edelstein et al., 2012a,b). The neurochemical organization of the claustrum has been extensively studied in many species (Edelstein and Denaro, 2004), however, data comparing this structure in rat and human is very scant.

Therefore, the aim of this study was a comparative investigation of NOS-ir in the rat and human claustrum, which included: (1) the demonstration of NOS-ir in neurons and fibers, (2) a description and analysis of their morphology and distribution via light microscopy, and (3) verifying whether the NOS-ir neurons comprise a specific subpopulation and if they have a specific pattern of organization.

2. Material and methods

The brains of 3 males and 3 females (between 30 and 54 years of age) with no evidence of neurogical disorders were obtained at autopsy. The portion of each cerebral hemisphere containing the claustrum was obtained and sectioned into blocks (1–2 cm in frontal plane), then fixed for two days in 4% paraformaldehyde. Serial coronal sections of 40 μm were cut on a freezing microtome and collected in the same phosphate buffer.

A total of six adult male and female Wistar rats (average weight of 250–300 g) were used for this study. The animals were irreversibly anesthetized with thiopental (40 mg/kg b.w.). Transcardial perfusion was performed using 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed and postfixed overnight in the same fixative solution at 4 °C.

Serial coronal sections from humans and rats were cut on a freezing microtome (Reichert-Jung) at a thickness of 40 μm. For the rat claustrum, sections were collected at levels of +2.20 mm to −1.30 mm from bregma (Paxinos and Watson, 1986). For the human claustrum, all planes from rostral to caudal poles were examined. Every fifth section was processed for NOS immunohistochemistry. Free-floating sections were preincubated for 1 h in 5% normal goat serum in PBS. After that, incubation in primary antibody was done for 48 h at room temperature. We used monoclonal anti-NOS1 antibody (Santa Cruz Biotechnology Cat# sc-5302, RRID:AB_626757) in a dilution of 1:1000.After rinsing in PBS, sections were incubated for 2 h in biotinylated goat anti-mouse IgG antibody (Vector Laboratories Cat# BA-9200, RRID: AB_2336171) in a dilution of 1:500. The sections were rinsed in PBS and incubated for 1 h in avidin-biotin-peroxidase complex (Vector Laboratories Cat# PK-6100, RRID:AB_2336819). This was followed by a rinse in PBS and then 0.05 M Tris-HCl buff ; ;er, pH 7.6, which preceded incubation in 0.05% 3,3′-diaminobenzidine (Sigma) containing 1% H_2O_2 (1:100) for reaction product visualization. Sections were then collected in Tris-HCl buff ; ;er 0.05 M, pH 7.6, air-dried for 24 h, rinsed three times in distilled water (5 min per rinse), and air-dried again. They were mounted on gelatin-coated glass slides, dried for 24 h and coverslipped with Entellan. Coronal sections were used to calculate neuronal packing density. Twelve sections were used as controls. All were incubated as previously described, but omitting the primary or secondary antibody. All controls were negative.

The routine Nissl staining (Cresyl violet) technique was used to examine the overall morphology and localization of cell bodies in claustrum.

2.1. Morphometric analysis

A light microscope (Olympus) was used for the examination of sections. A total of 480 NOS-ir neurons (240 from human claustrum and 240 from rat claustrum) were analyzed in order to determine morphology, diameter, dendrites, dendritic arborizations and classification. Randomly-selected slides – three from rostral (in rat: between bregma +3.20 and +1.70; in human: olfactory tract), three from middle (in rat: between bregma +1.60 and −0.30; in human: amygdaloid body) and three from caudal (in rat: between bregma −0.40 and −1.80; in human: hippocampal digitations) parts of each of the twelve brains were studied. For each stereotaxic plane the number of NOS-ir cells were calculated as an average from the total number of neurons counted from all sections per plane. Standard planar morphometry and including linear analysis (i.e., line length and width) was performed. A mean of the maximum and minimum diameter of all neurons and number of its branches was then calculated. For all measurements, only neurons with clearly visible nucleus were included. Morphometric analysis was performed using a microanalysis system