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Research Report

Neurochemically defined cell types in the claustrum of the cat

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ABSTRACT

The claustrum is a subcortical structure reciprocally and topographically connected with all sensory and motor domains of the cerebral cortex. Previous anatomical and electrophysiological data suggested that most cells in the claustrum are large neurons that both receive cortical input and project back to cortex, forming excitatory connections with their cortical targets. These data have been interpreted to imply a relay function for the claustrum, with information from different functional cortical domains remaining segregated. The possibility that the claustrum might mediate a more "global" function has been recently been developed by Crick and Koch [Crick, F. C., Koch, C., 2005. What is the function of the claustrum? Philos. Trans. R. Soc. Lond., B Biol. Sci. 360, 1271–1279]. We have reexamined the anatomical substrate for information processing in the claustrum of the cat by analyzing the patterns of immunoreactivity to calcium-binding proteins, GAD, serotonin, nNOS and the glutamate transporter EAAC1. We found multiple neurochemically defined cell types, suggesting multiple classes of projection neurons and interneurons. Each class was found throughout the entire claustrum, in all functionally defined subdivisions. Many neurons in the claustrum were surrounded by parvalbumin, calretinin, GAD or nNOS immunoreactive terminals, suggesting that many neurons of the claustrum make extensive intraclaustral connections. The entire claustrum also receives a serotonergic input. The identification of multiple neurochemical cell classes, their distribution and the extent of their dendritic arborizations relative to functional compartments suggest a substrate for information processing in the claustrum that may allow integration of information across functional subdivisions.

1. Introduction

The claustrum is a subcortical nucleus present in most, if not all, mammalian brains, although its size and complexity vary among species (Ashwell et al., 2004; Butler et al., 2002; Kowiański et al., 1999; Sherk, 1986). Studies in several species have shown that the claustrum is reciprocally and topographically connected with many different functional areas of cerebral cortex (Baizer et al., 1993, 1997; Boussaoud et al., 1992; Clascá et al., 1992; Dinopoulos et al., 1992; Druga, 1989; Kowiański et al., 1998; Salin et al., 1989; Sherk, 1986 and additional older references therein; Sloniewski et al., 1986; Ungerleider et al., 1984; Updyke, 1993; Webster et al., 1993; Witter et al., 1988). Functional regions of the claustrum can thus be defined by anatomical connections. Electrophysiological data support the anatomical inferences, showing that

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cells in the part of the claustrum interconnected with visual cortex have visual responses, and those in the part of the claustrum interconnected with motor cortex are active in relation to movement (Boyapati and Henry, 1985; LeVay and Sherk, 1981b; Sherk and LeVay, 1981b; Shima et al., 1996).

Anatomical experiments in the cat using retrograde tracers have suggested that most of the cells of the claustrum are large projection or "principal" neurons. These cells receive glutamatergic input from cortex, primarily, (but not exclusively) from layer 6 and provide a glutamatergic projection back to cortex, predominantly (but not exclusively) to interneurons of layer 4 (Carey et al., 1980; Clascá et al., 1992; Grieve and Sillito, 1995; LeVay, 1986; LeVay and Sherk, 1981a; Macchi et al., 1981; McCourt et al., 1986; Pérez-Cerdá et al., 1996). Only about 10% of neurons in the cat visual claustrum were not retrogradely labeled (Sherk and LeVay, 1981b) and considered local interneurons. These data have suggested a "relay" role for the claustrum. By this view, the one that is implicit in many discussions of the claustrum, the claustrum serves a "local" function for each modality. Visual information, for example, would be sent to visual claustrum, processed in visual claustrum and the resultant signal relayed back to visual cortex, independent of activity in any other modality. In support of this view, early experiments suggested a role for visual claustrum in determining length sensitivity for receptive fields of cells in striate cortex (Sherk and LeVay, 1983), however later work did not support that observation (Boyapati and Henry, 1985). Another, altogether different, view is that the claustrum serves a more "global" or integrative function. This has long been suggested by various authors (e.g. Macchi et al., 1981). It was recently suggested again by data from a PET imaging study (Hadjikhani and Roland, 1998) implicating the claustrum in visual–tactile integration. This view has been elaborated by Crick and Koch (2005). They propose that processing for each modality is influenced by activity in some or all of the other modalities. This requires interconnections among different functional compartments in the claustrum so that "waves of information can travel within the claustrum…cementing the role of the claustrum in binding disparate events into a single percept…" (Crick and Koch, 2005). This idea seems to contradict the findings from electrophysiological studies showing only visual responses in visual claustrum and only motor responsive in motor claustrum, but the question of intermodality interactions was not explicitly addressed (LeVay and Sherk, 1981b; Sherk and LeVay, 1981a; Shima et al., 1996).

In an attempt to increase our understanding of the anatomical substrate for information processing in the claustrum, we have examined its neurochemical organization in the cat. One functional subdivision, the visual claustrum, has been very well-studied in this species, and its borders and visuotopic organization are well-established (LeVay and Sherk, 1981a,b; Sherk and LeVay, 1981a). We felt this information would facilitate the interpretation of a neurochemical analysis. We studied both frontal and parasagittal sections through the claustrum, to obtain a better picture of intraclaustral connectivity and to assess possible asymmetry in the disposition of somas and dendrites. The results show multiple neurochemically defined cell types, with more interneurons than previously suspected. Furthermore, the distribution of the different cell types and the patterns of dendritic branching support the possibility of interactions between neighboring functional domains. A brief report of some of these results has been presented (Rahman et al., 2003).

2. Results

2.1. *Overview of the cat claustrum*

In the cat, the claustrum is a long, thin sheet with its long axis in the anterior–posterior (A–P) direction. It extends for about 11.0 mm in the A–P direction, from about stereotaxic A19.5 to stereotaxic A8.5 (Snider and Niemer, 1961). Its shape and dimensions change with A–P level. Fig. 1 shows the claustrum in three frontal sections stained for cresyl violet. At the most anterior section (Fig. 1A), the claustrum is comma-shaped and curves over the anterior rhinal sulcus. The visual claustrum is not present at this level; there is a dorsal strip (Fig. 1A, s) interconnected with somatosensory cortex (Sherk, 1986). The approximate ventral border of this strip is indicated by the dashed line. Ventral to this line is a region termed the "ventral zone", (Fig. 1A, ve) by Sherk (1986). This zone contains the auditory claustrum; its other connections are not well-established. At a level about 3 mm more posterior (Fig. 1B) the dorsolateral portion of the claustrum is expanded and has a triangular shape, with the base of the triangle located dorsally and oriented at about 45° to the stereotaxic vertical. At this level, the visual zone occupies a dorsal strip less than 1 mm wide. The visual zone is bounded by the ventral zone (Fig. 1B; the dashed line marks the approximate border of the visual, v, and ventral, ve, zones). Still more posteriorly (Fig. 1C), the claustrum is much narrower in the medial–lateral dimension, with a slightly wider dorsal portion that extends ventrally to a narrow stem or tail. The orientation of the long axis of the claustrum at this level is close to vertical. The visual region is still present dorsally (Fig. 1C, v) and bordered by the ventral zone. Despite the differences in its shape and size at different A–P levels, the sizes, shapes and spacing of cells appear similar throughout. Furthermore, the borders of the different functional zones inferred from anatomical and electrophysiological experiments are anatomically invisible with this stain. In parasagittal sections, the shape of the claustrum is markedly different and changes with mediolateral level. Figs. 2(A, B) shows the appearance of the claustrum in two parasagittal sections about 2.5 mm apart. On the more medial section (Fig. 2A), 7 mm of the anterior–posterior extent of the claustrum is present. At the level of the more lateral section (Fig. 2B) the claustrum is boomerang-shaped and curves around the anterior rhinal sulcus. These sections again show the cytoarchitectonic uniformity of the claustrum in a Nissl stain.

2.2. *Calcium-binding proteins in the claustrum: frontal sections*

Fig. 3 shows the appearance of the claustrum stained for immunoreactivity to calci-binding proteins on three frontal sections at about stereotaxic A11.5 (3 mm anterior to its posterior pole). The black arrowheads in Fig. 3A indicate the