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Calretinin and parvalbumin immunoreactive interneurons in the retrosplenial cortex of the rat brain: Qualitative and quantitative analyses

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ABSTRACT

The retrosplenial cortex (RSC) is a mesocortical region broadly involved with memory and navigation. It shares many characteristics with the perirhinal cortex (PRC), both of which appear to be significantly involved in the spreading of epileptic activity. We hypothesized that RSC possesses an interneuronal composition similar to that of PRC. To prove the hypothesis we studied the general pattern of calretinin (CR) and parvalbumin (PV) immunoreactivity in the RSC of the rat brain, its optical density as well as the morphological features and density of CR- and PV-immunoreactive (CR+ and PV+) interneurons. We also analyzed the overall neuronal density on Nissl-stained sections in RSC. Finally, we compared our results with our earlier analysis of PRC (Barinka et al., 2012).

Compared to PRC, RSC was observed to have a higher intensity of PV staining and lower intensity of CR staining of neuropil. Vertically-oriented bipolar neurons were the most common morphological type among CR+ neurons. The staining pattern did not allow for a similarly detailed analysis of somatodendritic morphology of PV+ neurons. RSC possessed lower absolute (i.e., neurons/mm^3) and relative (i.e., percentage of the overall neuronal population) densities of CR+ neurons and similar absolute and lower relative densities of PV+ neurons relative to PRC. CR: PV neuronal ratio in RSC (1:2 in area 29 and 1:2.2 in area 30) differed from PRC (1:1.2 in area 35 and 1:1.7 in area 36).

In conclusion, RSC, although similar in many aspects to PRC, differs strikingly in the interneuronal composition relative to PRC.

Abbreviations: CaBP, calcium binding protein(s); CB, calbindin; CR, calretinin; cROD, corrected relative optical density; PV, parvalbumin; PRC, perirhinal cortex; ROD, relative optical density; RSC, retrosplenial cortex; S.E.M., standard error of mean

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1. Introduction

In cerebral cortex, complementing the pyramidal cells forming excitatory glutamatergic connections, a large grouping of inhibitory GABAergic interneurons has been described. Various strategies to sort GABAergic interneurons into distinct subgroups have been adopted (for review see Barinka and Druga, 2010; DeFelipe et al. 2013; Markram et al., 2004). Based on the expression of cytosolic calcium-binding proteins (CaBP) calretinin (CR), parvalbumin (PV) and calbindin (CB), it is possible-with some limitations-to divide cortical GABAergic interneurons into three largely non-overlapping populations (Del Rio and DeFelipe, 1996; Gabbott and Bacon, 1996; Kawaguchi and Kubota, 1997; Kubota et al., 1994; Toledo-Rodriguez et al., 2004, 2005; Zaitsev et al., 2005, 2009). In particular, the PV-expressing (PV+) and CR-expressing (CR+) interneuronal subgroups exhibit virtually no overlap. CR+ interneurons differ from PV+ interneurons in many aspects (Barinka and Druga, 2010)-with regard to their morphology, their connectivity, their electrophysiological properties and at least in rodents, their site of origin. There are remarkable interareal and interspecies differences in the cellular composition of individual regions which must be taken into account when exploring and discussing the precise function of any cortical area (Barinka et al., 2010; Hof et al., 1993). Recently, we had shown that the transitional perirhinal cortex (PRC) in the rat possesses a specific complement of interneurons with a higher proportion of CR+ interneurons and less extensive dendritic and/or axonal arborization of PV+ interneurons when compared to temporal neocortex (Barinka et al., 2012) and prefrontal cortex (Gabbott et al., 1997). It remains unclear whether this is an evolutionary adaptation for a special function of PRC (a massive inhibitory mechanism with a gating function has been described by Curtis and Pare, 2004) or a general attribute of transitional cortical areas in the rat brain. To further clarify this matter, we herein investigated the interneuronal apparatus of retrosplenial cortex (RSC) in the rat. Retrosplenial cortex shares many structural and functional characteristics with PRC – [1] they both are transitional cortices located between neocortex and archi-/periarchicortex, [2] both are broadly involved in memory functions (Murray and Richmond, 2001; Suzuki, 1996; Vann and Aggleton, 2002; Van Groen and Wyss, 2003; Van Strien et al., 2009; Vann et al., 2009; Vogt et al., 2006;), and [3] both are part of networks involved in the generation and spreading of epileptic activity (Ampuero et al., 2007; Benini et al., 2011; Brevard et al., 2006; Cardoso et al., 2008; De Guzman et al., 2004; Duzel et al., 2006; Englot et al., 2008; Fukumoto et al., 2002; Holmes et al., 1992; Imamura et al., 1998; Kelly and McIntyre, 1996; Raisinghani and Faingold, 2005; Scholl et al., 2013; Sudbury and Avoli, 2007). Thus, we hypothesized that RSC possesses an interneuronal composition similar to that of PRC. In our present work we studied the general pattern of CR and PV immunoreactivity, the optical density of neuropil as well as the morphological features and density of CR- and PV-immunoreactive interneurons in rat RSC. The RSC is located on the medial surface of the hemisphere, while the PRC in the concavity of the rhinal sulcus. Hence, factors like a different packing density of cells could hamper direct comparison of interneuronal density between particular areas. To avoid this problem, in the present study, equivalently to previous study on PRC, the overall neuronal density was stereologically measured on Nissl-stained sections. The respective proportions of CR+ and PV+ interneurons were then expressed as a percentage of the overall (Nissl) neuronal population in cortical layers II–IV and V–VI, and collectively in layers II–VI. In the Discussion, we compare our results with our previous data obtained from PRC as well as with data obtained in various neocortical regions.

2. Results

2.1. *General features of calretinin and parvalbumin immunoreactivity in RSC*

Determination of the boundaries of cortical areas under study is illustrated in Figs. 1 and 3. General features of CR and PV staining as described below are shown in Figs. 2 and 3.

2.1.1. *Calretinin*

Unlike the perirhinal cortex, where the intensity of CR immunopositivity of neuropil was markedly higher than in the neighboring temporal neocortex (Barinka et al., 2012), we noted only a slight difference in the overall staining intensity between RSC and neighboring neocortex (Fig. 2B). However, differences in the pattern of immunoreactivity within individual cortical layers clearly differentiate retrosplenial areas 29 and 30 from the neighboring neocortex (Fig. 3A). In RSC the high layer I staining intensity, typical for all cortical regions, is less apparent than in the neighboring neocortex, and particularly so compared to PRC (Figs. 2B, 3A). Beneath layer I of RSC, a band of low CR immunoreactivity can be seen in layer II and the superficial portion of layer III (Fig. 3A). This band is very sharply delineated both superficially and profoundly in area 29, less sharply so in area 30. This difference is very prominent and enables a reliable distinction between these cortical areas. Further, in area 29, a thin and clearly demarcated band of higher CR immunoreactivity can be seen encompassing the thin layer IV as well as the most superficial portion of layer V (Fig. 3A). Again, the delineation of this highly CR-immunoreactive band is less sharp in area 30, where it also becomes markedly thicker relative to area 29. The deep portion of layer V and all of layer VI demonstrate a lower intensity of immunoreactivity (with the exception of the deepest parts of layer VI in area 29, as described below), which does not significantly change between areas 29 and 30 (Fig. 3A).

In the rostral portion of RSC, area 29 ventrally borders the corpus callosum (Fig. 1A–E). The corpus callosum proper is virtually devoid of CR label, while at the transition point between area 29 and corpus callosum a very thin band of higher CR+ neuropil spans the entire thickness of the cortex (Fig. 4A). Beneath this band, a very prominent CR+ field in the most medial portion of the cingulum, continuing in the deep portion of layer VI of area 29 (Fig. 4B) can be seen. It appears to be composed of transversely dissected CR+ fibers running along rostrocaudal axis under the RSC. Ultimately, they disappear in its caudal-most portion.

In the caudal portion of RSC, area 29 ventrally borders on dorsal subiculum and, finally, in its most caudal portion, on the postsubiculum (Fig. 1F–I). The border between area 29 and dorsal subiculum is clearly distinguishable: [a] the overall CR+ intensity in area 29 is higher than in subiculum, [b] a band of relatively