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Research Report

Calretinin, parvalbumin and calbindin immunoreactive interneurons in perirhinal cortex and temporal area Te3V of the rat brain: Qualitative and quantitative analyses

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ABSTRACT

The perirhinal cortex (PRC) composed of areas 35 and 36 forms an important route for activity transfer between the hippocampus–entorhinal cortex and neocortex. Its function in memory formation and consolidation as well as in the initiation and spreading of epileptic activity was already partially elucidated. We studied the general pattern of calretinin (CR), parvalbumin (PV) and calbindin (CB) immunoreactivity and its corrected relative optical density (cROD) as well as morphological features and density of CR and PV immunoreactive (CR+, PV+) interneurons in the rat PRC. Neighboring neocortical association area Te3V was analyzed as well.

The PRC differed from the Te3V in higher CR and lower PV overall immunoreactivity level. On CR immunostained sections, the difference between high cROD value in area 35 and low cROD value in area Te3V reached statistical significance ($p<0.05$). The pattern of CB immunoreactivity was similar to that of the neocortex. Vertically oriented bipolar neurons were the most common morphological type of CR+ neurons, multipolar neuronal morphology was typical among PV+ neurons and vertically oriented bipolar neurons and multipolar neurons were approximately equally frequent among CB+ neurons. The density of CR+ and PV+ neurons was stereologically measured. While the density of PV+ neurons was not significantly different in PRC when compared to Te3V, density of CR+ neurons in area 35 was significantly higher by comparison with Te3V ($p<0.05$). Further, the overall neuronal density was measured on Nissl stained sections and the proportion of CR+ and PV+ interneurons was expressed as a percentage of the total neurons counts.

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Abbreviations: Asf, area sampling fraction; CaBP, calcium-binding proteins; CB, calbindin; CB+, calbindin immunoreactive (neurons); CE, coefficient of error; CR, calretinin; CR+, calretinin immunoreactive (neurons); EC, entorhinal cortex; POR, postrhinal cortex; PRC, perirhinal cortex; PV, parvalbumin; PV+, parvalbumin immunoreactive (neurons); ROD, relative optical density; cROD, corrected relative optical density; ssf, section sampling fraction; tsf, tissue sampling fraction

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The percentage of CR+ interneurons was higher in area 35 by comparison with area Te3 ($p<0.05$), while the percentage of PV+ interneurons did not significantly differ among the examined areas. In conclusion, the PRC possesses specific interneuronal equipment with unusually high proportion of CR+ interneurons, what might be of importance for the presumed gating function of PRC in normal and diseased states.

1. Introduction

The perirhinal cortex (PRC) is a periallocortical region composed of areas 35 and 36. In the rat brain, PRC is located in the posterior rhinal sulcus. Rostrally it borders on the insular cortex, dorsally on the temporal association cortex, ventrally the PRC abuts on the entorhinal cortex (EC) and finally, caudally it is bordered by the postrhinal (POR) cortex (Burwell, 2001; Burwell et al., 1995). The role of PRC in various physiological and pathophysiological processes has been partially elucidated. For instance, various forms of memory were found to be critically dependent on the parahippocampal region, with PRC being the requisite component in both memory formation and consolidation (reviewed in Murray and Richmond, 2001; Suzuki, 1996; van Strien et al., 2009). Importantly, clinical and experimental evidence indicates that the perirhinal cortex contributes significantly to limbic seizure initiation, spread to other limbic structures as well as secondary generalization (Benini et al., 2011 ; Bernasconi et al., 2003; de Guzman et al., 2004; Fukumoto et al., 2002; Holmes et al., 1992; Imamura et al., 1998; Jutila et al., 2001; Kelly and McIntyre, 1996; McIntyre and Kelly, 2000; McIntyre et al., 1993; McIntyre et al., 1999; Mirnajafi-Zadeh et al., 1999; Morimoto et al., 2004; Nairismagi et al., 2006; Raisinghani and Faingold, 2005; Sato et al., 1998; Sudbury and Avoli, 2007; Tortorella et al., 1997). In general, the PRC forms a main route for activity transfer between the hippocampus–entorhinal cortex on one side and the neocortex on the other. For a long time, PRC was thought to be a "simple" relay station, which reliably transmits information between hippocampal formation and neocortex. However, in the last decade, a powerful inhibitory mechanism which strongly restricts propagation of activity across the rhinal sulcus was described in PRC (Biella et al., 2002; Biella et al., 2003; de Curtis and Pare, 2004; de Curtis et al., 1999; Naber et al., 2000; Pelletier et al., 2004). It is proposed that PRC (together with EC) actively gates the information flow between hippocampus and neocortex, permitting only the relevant inputs to pass through the rhinal cortices (de Curtis and Pare, 2004). While the amygdala (Paz et al., 2006) and medial prefrontal cortex (Paz et al., 2007) with their massive excitatory projections to EC-PRC, as well as subcortical cholinergic inputs (Apergis-Schoute et al., 2007) are anticipated to act as gate-opening structures, the structural substrate of the inhibitory/gate-closing mechanism is less well defined. However, it can be presumed that the intrinsic GABAergic interneurons of EC-PRC play an important role in restricting the impulse traffic through this region. For further exploration of inhibitory function of the EC-PRC region in normal and diseased states, a precise description of distribution and morphology of inhibitory interneurons might be of great value. While this was done extensively in EC of the rat (Miettinen et al., 1997; Wouterlood et al., 1995; Wouterlood et al., 2000), only few authors have studied the inhibitory interneurons in the perirhinal cortex (Moyer et al., 2011). Therefore, in the present study we wanted to characterize the interneuronal populations of the perirhinal cortical areas 35 and 36 in the rat in terms of quality and quantity. Because of its prominent and functionally significant interconnectivity with PRC (Agster and Burwell, 2009; Burwell and Amaral, 1998a; Furtak et al., 2007b), the neighboring temporal association area Te3V was analyzed as well. We used calretinin (CR), parvalbumin (PV) and calbindin (CB) immunohistochemistry, as these calcium-binding proteins (CaBP) are well known as markers of different and largely non-overlapping interneuronal populations (see DeFelipe, 1997 and Druga, 2009 for review). PV, CR and CB immunoreactivity was qualitatively and quantitatively analyzed, morphological features of PV+, CB+ and CR+ neurons were qualitatively evaluated and the density of CR+ and PV+ neurons was stereologically quantified. The PRC is located in the rhinal sulcus, while the area Te3V on the convexity of the hemisphere. Hence, factors like a different packing density of cells in and outside the sulcus could hamper direct comparison of interneuronal density between particular areas. To avoid this problem, the overall neuronal density in areas 35, 36 and Te3V was stereologically measured on Nissl-stained sections to acquire an approximate ratio between the overall neuronal and the interneuronal counts in each area under study.

2. Results

2.1. *General features of calretinin, parvalbumin and calbindin immunoreactivity*

See Fig. 1 for determination of boundaries of cortical areas under study. For general features of CR, PV and CB staining as described below see Figs. 2 and 3.

2.1.1. *Calretinin*

The overall level of CR immunopositivity in PRC is markedly higher than in the neighboring entorhinal cortex. A prominent and abrupt decrease of CR immunopositivity at the perirhinal/entorhinal border reliably demarcates the ventral border of PRC. The high layer I staining intensity, typical for all cortical regions, is in PRC and especially in area 35 even more obvious than in the adjacent temporal neocortex. In area 35, staining intensity in layers II, III, V, and VI (area 35 does not posses layer IV) is relatively homogenous with only slightly higher levels in deep layer III and deep layer VI. In area 36, the pattern of immunopositivity shows trilaminar appearance with a notably higher level of immunopositivity in layers I, deep III+IV, and deep VI, and lower staining intensity in layers II and superficial III and in layer V. In temporal