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Research Report

Long-term effects of early life deprivation on brain glia in Fischer rats

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ABSTRACT

Both clinical and experimental studies have indicated that depression and depression-like animal conditions are associated with disruption of the intrinsic plasticity of the brain, resulting in neuronal atrophy. However, little is known about the brain glia in these conditions. Early life stress in the form of infant abuse or neglect constitutes a risk factor in the aetiology of major depressive disorder in later life. It is possible to model this relation between early life stress and depression in the rat through maternal deprivation; in adulthood, this postnatal manipulation is known to lead to depression-like behaviour. In the stress-hyperresponsive Fischer strain, P1–14 pups were isolated for 4 h/day (early deprivation, ED, $n=6$) or were nonhandled (NH, $n=6$); they were left undisturbed until adulthood. *Postmortem* quantitative analysis of regional astroglial distribution and morphology based on glial fibrillary acidic protein (GFAP) immunohistochemistry indicated a significant effect of ED on the density of GFAP-reactive astrocytes in brain areas implicated in stress-related behaviour. A moderate (10–22%) but consistent reduction in GFAP-reactive astrocyte density was seen in dorsal dentate gyrus, prefrontal cortex, ventral hippocampal CA1, cingulate cortex, dorsal hippocampal CA1 and basolateral amygdala. The ED-related reduction in GFAP-immunoreactive astrocyte density was more marked than the reduction in total cell density, which suggests that GFAP immunoreactivity, rather than the number of astrocytes, was reduced. This study provides evidence that early life stress leads to long-term changes in the density of astroglia in the brain regions involved in stress responses in the rat.

1. Introduction

Neurobiological effects of stress, both immediate and delayed, have been studied with a focus on brain neurones, with little attention to glia. There are several lines of evidence which indicate that both long-term stress and depression may affect neurones, mostly in the limbic system and prefrontal cortex (reviewed by McEwen, 2001, 2005). Dendritic atrophy observed in the hippocampi of stress-exposed rodents and nonhuman primates as well as human patients with recurrent depression

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Abbreviations: DAB, 3,3′-diaminobenzidine; ED, early deprivation; GFAP, glial fibrillary acidic protein; MDD, major depressive disorder; NH, nonhandled; PBS, phosphate-buffered saline; ROI, region of interest

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or posttraumatic stress disorder is consistent with losses in neuroplasticity; they are so pronounced that depression is considered a disorder of brain plasticity and resultant brain structural remodelling (McEwen, 2001, 2005; Duman, 2002a,b, 2004; Fuchs et al., 2004).

Although less is known about the role of glia in depression-related brain remodelling, there is growing evidence that indeed both neuronal and glial changes constitute the neuropathology of primary mood disorders, including depression (Harrison, 2002). Rajkowska (2000) and Harrison (2002) have reviewed the evidence for glial pathology in major depression, indicating that glial cell number and packing density are reduced in the prefrontal and anterior cingulate cortex, while Cotter et al. (2002) have reported reduction in glial cell density in the prefrontal cortex in major depressive disorder (MDD) and bipolar disorder. The methods employed in these studies did not allow for identification of the class of glia implicated (astrocytes, oligodendrocytes or microglia), and to date there are few published reports that target specific subtypes of brain glia in the context of depression. Miguel-Hidalgo et al. (2000), using glial fibrillary acidic protein (GFAP) as a selective immunohistochemical marker of astroglia, found a reduction in GFAP-stained cell count in the prefrontal cortex of a younger subgroup (below 46 years) of MDD patients. Müller et al. (2001) reported a modest decrease in GFAP-immunoreactivity in the hippocampal areas CA1 and CA2 in MDD with no parallel changes in neuronal density or distribution in those areas. A study by Hamidi et al. (2004) has shown oligodendrocyte deterioration in the amygdala of MDD patients. Astroglial changes in depression and experimental animal models are of particular interest since astrocytes play vital roles in maintaining neuroplasticity, and do so via multiple mechanisms including support of synaptogenesis, synapse maintenance and secretion of neurotrophins (for reviews, see Newman, 2003; Slezak and Pfrieger, 2003).

Stressful experiences in early life are important risk factors in the aetiology of depression (e.g. Heim and Nemeroff, 1999, 2001; McEwen, 2000; Shea et al., 2004). In Wistar and Fischer rats, we have demonstrated that early deprivation of maternal care leads to development of adult offspring with depression-like behavioural phenotypes, including reduced motivation for reward and reduced coping (Pryce et al., 2001, 2002a,b; Mintz et al., 2005; Rüedi-Bettschen et al., 2004, 2006; reviewed in Pryce et al., 2005). In the present study conducted with Fischer rats, we test the hypothesis that the *in vivo* depression-like phenotypes are associated with changes in astroglial density in brain regions that regulate emotionality and that are implicated in the neurobiology/neuropathology of depression. The regions of interest considered here are the cingulate and prefrontal cortices, basolateral amygdala, hypothalamic paraventricular nucleus and hippocampus. Of the hippocampal subregions, we have focused on dentate gyrus and CA1, because of their known role in neuroplasticity and implication in stress-related conditions (McEwen, 2000; Sousa et al., 2000; Fuchs et al., 2004). Both ventral and dorsal hippocampal regions were considered because of the functional dissociation of these two, with the ventral hippocampus primarily involved in emotional processing and the dorsal hippocampus primarily implicated in learning and memory (e.g. Bannerman et al., 2004).

2. Results

2.1. *Immunohistochemistry*

No false-positive stained astrocytes were found in the negative controls (blank sections) processed through the immunohistochemistry procedure with exclusion of the primary anti-GFAP antibody. Profiles of GFAP-reactive cells displayed typical astrocytic morphology with GFAP-positive cell bodies and processes. This is illustrated in Fig. 1, which shows a representative GFAP stain in the frontal cortex of an NH rat.

For the density of GFAP-reactive astrocytes (Fig. 2), there was a significant main effect of treatment ($F(1, 10)=109.96$, $p<0.0001$) and a significant main effect of ROI ($F(9, 90)=54.94$, $p<0.0001$), in the absence of a significant treatment×ROI interaction ($p>0.38$). The treatment main effect reflected the moderate (10–22%) but consistent reduction in GFAP-reactive astrocyte density in ED rats. In terms of the relative extent of the ED effect on individual ROIs, the average ED effect was DHippoDG>PfCx>VHippoCA1>CgCx>DHippoCA1>Amgd>Other. The significant main effect of ROI was attributable to the higher GFAP-reactive astrocyte density in the PVH, FrCx and Amgd relative to other ROIs (Bonferroni post hoc test: $p<0.0001$ in all cases) (Fig. 2).

For total cell density, assessed by counting of hematoxylin-stained cell nuclei (Fig. 3a), there was a significant main effect of treatment ($F(1, 10)=8.84$, $p<0.02$) and a significant main effect of ROI ($F(8, 83)=75.39$, $p<0.0001$), in the absence of a significant treatment×ROI interaction ($p>0.08$). The treatment main effect reflected the small (up to 6%) but consistent reduction in the total cell density in ED rats. In terms of the relative extent of the ED effect in individual ROIs, the average ED effect was DHippoDG>Amgd>DHippoCA1>CgCx>Other.

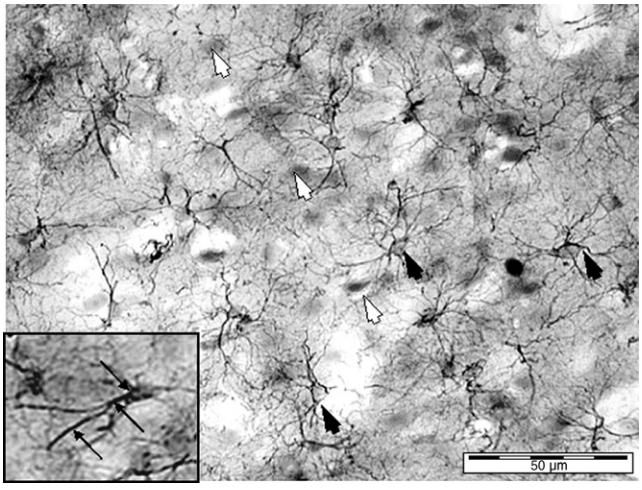

Fig. 1 – Immunohistochemistry of astroglia—a typical micrograph representing frontal cortex of a nonhandled (NH) rat. Astrocytes stained with antibody to glial fibrillary acidic protein (black arrows); cell nuclei stained with hematoxylin (white arrows). Scale bar: 50 µm. Inset refers to the morphometric analysis of astroglia: arrows indicate primary astrocytic processes.