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Astrocytes support hippocampal-dependent memory and long-term potentiation via interleukin-1 signaling

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ABSTRACT

Recent studies indicate that astrocytes play an integral role in neural and synaptic functioning. To examine the implications of these findings for neurobehavioral plasticity we investigated the involvement of astrocytes in memory and long-term potentiation (LTP), using a mouse model of impaired learning and synaptic plasticity caused by genetic deletion of the interleukin-1 receptor type I (IL-1RI). Neural precursor cells (NPCs), derived from either wild type (WT) or IL-1 receptor knockout (IL-1rKO) neonatal mice, were labeled with bromodeoxyuridine (BrdU) and transplanted into the hippocampus of either IL-1rKO or WT adult host mice. Transplanted NPCs survived and differentiated into astrocytes (expressing GFAP and S100β), but not to neurons or oligodendrocytes. The NPCs-derived astrocytes from WT but not IL-1rKO mice displayed co-localization of GFAP with the IL-1RI. Four to twelve weeks post-transplantation, memory functioning was examined in the fear-conditioning and the water maze paradigms and LTP of perforant path-dentate gyrus synapses was assessed in anesthetized mice. As expected, IL-1rKO mice transplanted with IL-1rKO cells or sham operated displayed severe memory disturbances in both paradigms as well as a marked impairment in LTP. In contrast, IL-1rKO mice transplanted with WT NPCs displayed a complete rescue of the impaired memory functioning as well as partial restoration of LTP. These findings indicate that astrocytes play a critical role in memory functioning and LTP, and specifically implicate astrocytic IL-1 signaling in these processes. The results suggest novel conceptualization and therapeutic targets for neuropsychiatric disorders characterized by impaired astrocytic functioning concomitantly with disturbed memory and synaptic plasticity.

1. Introduction

Recent studies indicate that astrocytes are not merely the supportive cells of the brain, but that they also play an important integral role in neural and synaptic functioning (Haydon and Carmignoto, 2006; Henneberger et al., 2010; Newman, 2003; Volterra and Meldolesi, 2005). Specifically, astrocytic processes ensheath most synapses in the brain, and express receptors for several transmitters. Signaling via these receptors evokes elevations in astrocytic Ca^{2+} concentration, resulting in the regulated secretion of various gliotransmitters, which modulate neuronal excitability and synaptic strength (Haydon and Carmignoto, 2006; Jourdain et al., 2007; Perea and Araque, 2007). Astrocytes-to-neurons communication also plays a critical role in synaptic plasticity processes, including long-term potentiation (Nishiyama et al., 2002; Yang et al., 2003) and synaptic scaling following prolonged inhibition of neuronal activity (Stellwagen and Malenka, 2006). Corroborating these findings, in neuropsychiatric disorders that are characterized by impaired astrocytic functioning, e.g., major depression and neurodegenerative diseases (Bowley et al., 2002; Cotter et al., 2001a; Mrak and Griffin, 2005; Ongur et al., 1998; Seifert et al., 2006), behavioral and neural plasticity are also severely disturbed (Duman et al., 2000; Selkoe, 2002).

To directly assess the possible role of astrocytes in these processes we used a mouse model of impaired learning, memory and long-term potentiation (LTP), caused by a genetic deletion of the receptor for the cytokine interleukin-1 (IL-1). In the brain, this receptor is extensively (but not exclusively) expressed by astrocytes (Ban et al., 1993; Hammond et al., 1999; Pinteaux et al., 2002; Wong and Licinio, 1994) and plays an important role in the functioning of

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these cells (Beskina et al., 2007; Holliday and Gruol, 1993). Previous research indicated that mice with a genetic deletion of the IL-1 receptor type I display markedly impaired hippocampal-dependent memory functioning and LTP, although memories that do not depend on the integrity of the hippocampus seem to be spared (Avital et al., 2003; Goshen et al., 2007; Schmid et al., 2009). These findings were corroborated by other lines of evidence for the involvement of IL-1 signaling in normal memory functioning and LTP, including the induction of IL-1 related genes during hippocampal-dependent memory tasks (Depino et al., 2004; Goshen et al., 2007; Labrousse et al., 2009) and LTP (Balschun et al., 2003; Schneider et al., 1998), the facilitation of memory consolidation by low doses of IL-1 (Brennan et al., 2003; Goshen et al., 2007; Song et al., 2003; Yirmiya et al., 2002), as well as the interference with hippocampal-dependent memory consolidation and LTP by IL-1 receptor antagonist (IL-1ra) (Goshen et al., 2009, 2007, 2003; Goshen and Yirmiya, 2009; Ross et al., 2003; Schmid et al., 2009; Spulber et al., 2009).

To test the hypothesis that astrocytes in general, and astrocytic IL-1 signaling in particular, are critical for memory functioning and LTP, mice with deletion of the IL-1 receptor type I (the only known signaling receptor for IL-1) (IL-1rKO mice) and wild type (WT) mice were transplanted intrahippocampally with neural precursor cells (NPCs) derived from either WT or IL-1rKO mice, which differentiate exclusively into astrocytes (Ben Menachem-Zidon et al., 2008; Raedt et al., 2009). Memory functioning in two hippocampal-dependent tasks and *in vivo* LTP were tested several weeks later. We report that astrocyes derived from WT, but not IL-1rKO mice completely rescued the impaired memory functioning and partially restored LTP in IL-1rKO mice, providing direct evidence for the essential role of astrocytic IL-1 signaling in hippocampal-dependent memory functioning and synaptic plasticity.

2. Materials and methods

2.1. Subjects

Subjects were male IL-1rKO mice and their 129/Sv X C57BL/6 WT controls (Jackson Laboratories, Bar Harbor). Mice were 9–10 weeks old, housed in an air-conditioned room (23 ± 1 °C), with food and water ad libitum. The behavioral experiments were conducted during the first half of the dark phase of the12-h light/dark cycle. Newborn IL-1rKO mice and their 129/Sv X C57BL/6 WT controls were used as a source for neural precursor cells. The experiments were approved by the Hebrew University Committee on Animal Care and Use.

2.2. Preparation of neural precursor cells and growth of neurospheres

Neural precursor cell spheres were prepared from newborn WT or IL-1rKO mice as previously described (Ben-Hur et al., 2003; Ben Menachem-Zidon et al., 2008). Briefly, the hemispheres were dissected, followed by removal of the meninges. The tissue was minced, digested by trypsin for 20 min, and dissociated. Following suspension in N2 medium, 10×10^6 cells were plated in a T-75 uncoated flask and supplemented with 10 ng/ml FGF2 and 20 ng/ml EGF, added daily. Un-differentiated neurospheres were collected for characterization *in vitro* or for transplantation after 5 days of growth.

2.3. Characterization of neural precursor cells in vitro

For the assessment of differentiation, floating spheres obtained from WT and IL-1rKO newborn mice were adhered to polylysine-coated dishes. Spheres were allowed to differentiate for 5 days, then fixed and stained for lineage-specific markers. Experiments were performed in triplicates and repeated three times, with at least 30 spheres per plate. At least 1000 cells were counted for each experimental condition to determine cell fate.

2.4. Sphere transplantation

Prior to transplantation, spheres (from either WT or IL-1rKO newborn mice) were incubated with 25 μg/ml BrdU for 72 h. The spheres (4000-spheres/4 μl N2 medium) were bilaterally transplanted into the hippocampus using a stereotaxic instrument. Mice from the Sham group were injected with 4 μl of the N2 medium. Coordinates of the injection site (in mm) relative to Bregma were as follows: AP −2.6, L ± 1.4, DV −1.6, for all groups. Injections were conducted using a 25-μl Hamilton syringe. After each injection, the needle was left in situ for 5 min before being retracted, to allow complete diffusion of the spheres. Four weeks after transplantation, animals were perfused with PBS followed by 4% paraformaldehyde. Tissues were deep frozen in liquid nitrogen. Serial 8 μm brain coronal frozen sections were cut for histological analyses.

2.5. Immunofluorescent staining and quantification of the transplanted cells in vivo

The transplanted cells were detected by immunostaining for BrdU. Sections were incubated with 2 N HCl for 30 min at 37°, washed, and incubated with 3% normal goat serum for 1 h in room temperature (RT), followed by incubation with rat anti-BrdU (clone BU1/75 (ICR1), serotec, 1:200 dilution) overnight at 4 °C. A goat anti rat IgG secondary antibody, conjugated to Alexa 555, was added for 50 min at RT, and counterstaining was done with DAPI.

To determine the fate of the transplanted cells, sections were double stained for BrdU and the astrocytic markers GFAP (rabbit anti-GFAP:Dako, 1:100) and S100β (mouse anti S100β:Abcam, 1:100), the neuronal marker NeuN (mouse anti-neuronal nuclei: Chemicon, 1:50), or the oligodendrocyte marker galactocerebroside (rabbit anti-GalC:Millipore Bioscience Research Reagents, 1:100), followed by incubation with goat anti rabbit IgG or goat anti-mouse IgG secondary antibodies, respectively, conjugated to Alexa-488 (Molecular Probes) for 50 min at RT. To examine the phenotype of the cells that express IL-1RI, sections were double stained using goat anti-IL-1RI (1:100) together with rabbit anti-GFAP (1:100), mouse anti-NeuN (1:50) or rabbit anti-GalC (1:100) antibodies, followed by incubation with donkey anti goat IgG, goat anti rabbit IgG or goat anti-mouse IgG secondary antibodies, respectively, (Molecular Probes) for 50 min at RT. Images were taken using a confocal microscope (Leica) or a Nikkon E-600 fluorescent microscope.

BrdU-labeled cells were counted on every sixth 8 μm coronal section, covering the dentate gyrus in its rostro-caudal extension. Quantification of cell numbers was based on counting immunopositive nuclei that were completely filled with the fluorescent stain; given that cells were counted in sections 48 μm apart and that the nuclei sizes in any one dimension were smaller than 30 μm, this procedure ensured that no cells were counted more than once. The total number of transplanted cells was extrapolated for the entire volume of the dentate gyrus (Kempermann et al., 1997). Differentiation of transplanted cells into astrocytes, neurons and oligodendrocytes was determined by immunofluorescent staining for GFAP, S100β, NeuN and Gal C in brains transplanted with BrdU labeled neurospheres. As no BrdU-labeled cells were also stained for NeuN or Gal C, quantitative analysis focused on astrocytes. The percentage of graft-derived cells that differentiated into astrocytes was determined by dividing the number of cells manifesting double labeling for BrdU and GFAP by the total number of BrdU positive cells. The percentage of IL-1RI positive cells that also expressed GFAP was calculated by dividing the number of cells with IL-1RI + GFAP double labeling by the total number of IL-1RI positive cells. All analyses were conducted by an experimenter