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TrkB but not trkC receptors are necessary for postnatal maintenance of hippocampal spines

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Abstract

Dendritic spines are major sites of excitatory synaptic transmission and changes in their densities have been linked to alterations in learning and memory. The neurotrophins brain-derived neurotrophic factor and neurotrophin-3 and their receptors, trkB and trkC, are thought to be involved in learning, memory and long-term potentiation (LTP). LTP is known to induce trkB and trkC gene expression as well as spinogenesis in the hippocampus. In the aging hippocampus, declines in trkB and trkC mRNA levels may underlie, at least in part, impairments in spatial memory and reductions in spine densities. To determine the significance of trkB and trkC for the maintenance of dendritic spines, we have analyzed Golgi-impregnated hippocampi of adult and aged mice heterozygous for trkB, trkC, or both along with respective wildtype littermates. Deletion of one allele of trkB, but not trkC, significantly reduces spine densities of CA1 pyramidal neurons in both adult and aged mice, as compared to age-matched controls. This indicates that trkB, but not trkC, receptors are necessary for the maintenance of hippocampal spines during postnatal life.

Keywords: Spines; Dendritic spines; Neurotrophin; Trk; Hippocampus; CA1; BDNF; NT-3

1. Introduction

The neurotrophins brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), along with their cognate receptors trkB and trkC, respectively, are prominently expressed in the hippocampus (Linden et al., 2000; Mudo et al., 1996). Both neurotrophins have been implicated in hippocampal long-term potentiation (LTP) and hippocampus-dependent learning (Chen et al., 1999; Hall et al., 2000; Korte et al., 1995; Linnarsson et al., 1997; Lu and Chow, 1999; Mizuno et al., 2003). Moreover, it has been shown that induction of LTP in the hippocampus induces an elevation in trkB and trkC gene expression (Bramham et al., 1996).

Dendritic spines are small protrusions extending from the dendrites of neurons; these protrusions bear the majority of synapses. Excitatory synapses on hippocampal pyramidal neurons are exclusively located to dendritic spines, usually in a 1:1 proportion (Andersen, 1990). LTP seems to be associated with increased spine densities (Muller et al., 2000) and has been shown to induce the formation of new, mature and probably functional synapses (Toni et al., 1999). In addition, some forms of learning have been shown to increase the number of dendritic spines (Geinisman, 2000; Leuner et al., 2003; Nimchinsky et al., 2002; Yuste and Bonhoeffer, 2001). There is also evidence to suggest that variations in spine length may contribute to behavioral alterations (Matsuzaki et al., 2001; Vanderklish and Edelman, 2002). For example, spine length on neurons in the piriform cortex has been shown to be reduced after learning (Knafo et al., 2005).

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Several lines of circumstantial evidence suggest that signalling through trkB and trkC may not only be important for spine development and plasticity, but also for their long-term maintenance during aging. Thus, it has been shown that trkB mRNA (Silhol et al., 2005; Webster et al., 2006) in the hippocampus and trkC mRNA (Beltaifa et al., 2005), at least in the cortex, decline during aging in the hippocampal formation. These losses are accompanied by reductions in hippocampus-related spatial memory and spine densities in the hippocampal area CA1 (von Bohlen und Halbach et al., 2006b). It has been speculated that such changes may contribute to the reduced plasticity of specific brain areas in aged animals (Silhol et al., 2005). We therefore wanted to determine whether reduced availability of trkB and trkC receptors affects hippocampal spine densities during aging. We used mice heterozygous for trkB$^{(+/-)}$, trkC$^{(+/-)}$ and trkB/C$^{(+/-)/(+/-)}$, which – in contrast to the homozygous mutants – survive into adulthood (Minichiello and Klein, 1996).

2. Materials and methods

2.1. Generation and genotyping of mice

Male heterozygous adult (6–7 months old) and aged (21–22 months old) trkB$^{(+/-)}$, trkC$^{(+/-)}$, trkB/C$^{(+/-)/(+/-)}$ mice, and age-matched littermates (trkB/C$^{(+/+)/(+/+)}$, named: "wild types") were used and maintained in accordance with the institutional guidelines for animal welfare.

The generation and genotypic analysis of the single mutant mice (trkB and trkC) has previously been described (Klein et al., 1993, 1994). By crossbreeding first heterozygous trkB and heterozygous trkC mice (Minichiello and Klein, 1996) heterozygous trkB/C$^{(+/-)/(+/-)}$ mice, trkB$^{(+/-)}$, trkC$^{(+/-)}$, trkB/C$^{(+/-)/(+/-)}$ mutant mice, and control littermates (trkB/C$^{(+/+)/(+/+)}$) were obtained (von Bohlen und Halbach et al., 2005). Genotypes were determined by PCR from tail biopsies of the offsprings as previously described (Schimmang et al., 1995). In detail, the *trkB* genotype was determined by PCR amplification using a common 5′ primer (5′-TCG CGT AAA GAC GGA ACA TGA TCC-3′) and either a 3′ primer for the wildtype allele (5′-AGA CCA TGA TGA GTG GGT CGC C-3′) or a 3′ primer from the pgk-1 promotor of the neo cassette (5′-GAT GTG GAA TGT GTG CGA GGC C-3′). The *trkC* genotype was determined using a common 5′ primer (5′-CTG AAG TCA CTG GCT AGA GTC TGG G-3′) and either a 3′ primer for the wildtype allele (5′-GTC CCA TCT TGC TTA CCC TGA GG-3′) or a 3′ primer from the pkg-1 promotor of the neo cassette (5′-CCA GCC TCT GAG CCC AGA AAG C-3′). The PCR amplified DNA was analyzed on a 1.5% agarose gel.

2.2. Golgi method

Mice were deeply anesthetized with ether and transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffer. Brains were left in situ for 30 min prior to dissection, and postfixed in 4% PFA. Golgi-staining was performed according to the Golgi-Kopsch method, which results in well-impregnated neurons (Rosoklija et al., 2003). Blocks of about 2.5 mm thickness were prepared from each hemisphere, and brain pieces were soaked in a solution containing 2.5% potassium dichromate for 1 day at room temperature in the dark. This solution was then replaced by fresh solution of 2.5% potassium dichromate, and tissues were incubated for another 6 days. Tissues were then washed and transferred to a solution containing 0.75% $AgNO_3$. After 6 days, brain pieces were removed, washed in 40 and 20% ethanol, and cut into 60 μm coronal sections using a vibratome (Leica, Germany). Sections were mounted on gelatine coated slides and coverslipped using Merkoglas (Merk, Germany).

2.3. Tissue shrinkage

Since differential tissue shrinkage may compromise measurements, it is theoretically possible that, e.g., tissues of the different knockout mice might shrink more or less, respectively, than tissues of control mice. To avoid such a potential error, we measured the thickness of the dendritic fields of interest using a computer-controlled reconstruction and analysis system (NeuroLucida; MicroBrightField Inc., USA). For each investigated animal, the thickness of the basal (distance from the pyramidal cell layer to the alveus) and apical (distance from the pyramidal cell layer to the hippocampal fissure) dendritic fields and the thickness of the molecular layer of the DG (distance from the granule cell layer to the hippocampal fissure) were measured in six different sections using a ×20 objective.

In addition, tissue shrinkage in the *z*-axis was determined for each analyzed group by measuring the thickness in the *z*-axis in the area of the molecular layer of the DG in three different sections of each animal. A Zeiss Axioscop Imaging microscope (Zeiss, Germany) equipped with a ×100 oil objective (NA: 1.25) and a *z*-axis computer-controlled stage from Merzhäuser (Model No.: EK32; Germany) were used for this analysis (Baryshnikova et al., 2006). The custom-made software StereoInvestigator 5.04.1 (MicroBrightField Inc., USA) was used for measuring section thickness.

The obtained values were statistically evaluated by using two-way ANOVA (grouped for age and genotype), followed by a Bonferroni post-hoc test.

2.4. Spine analysis

Spine analysis was conducted blind to the experimental conditions. We examined spines on apical dendrites of CA1 pyramidal neurons and on basal dendrites of CA1 pyramidal neurons, as well as on dendrites of the dorsal leaf of the dentate gyrus (Fig. 1A). Spines were analyzed in the dorsal, but not ventral leaf of the dentate gyrus, since spine densities are different in both locations (Desmond and Levy, 1985). Analyses were conducted on Golgi-impregnated sections that