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RAS DOES NOT CONTRIBUTE TO THE FACILITATION OF HIPPOCAMPAL SYNAPTIC PLASTICITY ENABLED BY ENVIRONMENTAL ENRICHMENT

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Abstract—Environmental enrichment (EE), which mimics the wealth of sensory, motor and cognitive stimuli that arise through intense interactions with the ambient environment, results in enhanced hippocampal long-term potentiation (LTP) and spatial learning. A key molecular factor in the mediation of these changes is the brain-derived neurotrophic factor (BDNF). One of the downstream cascades that is activated by BDNF is the cascade linked to the small GTPase, Ras, that triggers mitogen-activated protein kinase (MAPK) activity and is part of the cAMP response element-binding protein (CREB) pathway that can lead to synaptic restructuring to support LTP. Here, we explored whether persistent activation of Ras in neurons further enhances LTP following EE of rodents. Immediately following weaning, transgenic mice that expressed constitutively activated neuronal Ras, or their wildtype (Wt) littermates, underwent 3 weeks of constant EE. In the absence of EE, theta burst stimulation (TBS) evoked LTP in the CA1 region of transgenic mice that was not significantly different from LTP in Wts. After 3 weeks of EE, hippocampal LTP was improved in Wt mice. Enriched transgenic mice showed an equivalent level of LTP to enriched Wts, but it was not significantly different from non-enriched synRas controls. Western blot analysis performed after a pull-down assay showed that non-enriched transgenic mice expressed higher Ras activity compared to non-enriched Wts. Following EE, Ras activity was reduced in transgenics to levels detected in Wts. These results show that constitutive activation of Ras does not mimic the effects of EE on LTP. In addition, EE results in an equivalent enhancement of LTP transgenics and Wts, coupled with a decrease in Ras activity to Wt levels. This suggests that permanent activation of Ras in neurons of synRas animals following EE results in an altered feedback regulation of endogenous Ras activity that is not a key factor in LTP enhancements. The maintenance of Ras within a physiological range may thus be required for the optimization of LTP in the hippocampus.

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Key words: Ras, GTPase, synaptic plasticity, hippocampus, BDNF, MAP kinase.

INTRODUCTION

Plasticity of synaptic connections enables the mammalian neural system to modify its structure and function in response to environmental stimuli. Sensory, motor and cognitive stimuli, resulting from interactions with the environment, play a key role in optimizing and modifying the neuronal circuitry required for normal brain development and function. In rodents, the effect of exposure to an environment that is replete with stimuli of this kind comprises environmental enrichment (EE). It comprises the exposure of laboratory animals to an improved habitat that includes, for example, toys, tunnels, running wheels and regularly-changing sensory-stimulating features, along with rearing in social groups. Subsequently, many effects of EE have been observed, including biochemical changes, dendritic arborization, synaptogenesis, neurogenesis, gliogenesis and increased neuronal survival (Volkmar and Greenough, 1972; Globus et al., 1973; Black et al., 1990; Van Praag et al., 1999; Gould et al., 1999; Rampon et al., 2000a; Birch et al., 2013). The hippocampus is particularly sensitive to EE (Teather et al., 2002). Enriched animals show enhanced long-term potentiation (LTP) (Duffy et al., 2001; Foster and Dumas, 2001; Artola et al., 2006), hippocampus-dependent learning and memory (Renner and Rosenzweig, 1987; Rampon et al., 2000b; Tang et al., 2001; Lee et al., 2003) and improved cortical reorganization and recovery after injury (Dahlqvist et al., 1999; Rampon et al., 2000b). Exposure to EE alters gene

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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element-binding protein; EE, environmental enrichment; Erk1 and Erk2, extracellular-regulated kinases 1 and 2; fEPSPs, field excitatory post-synaptic potentials; GAPs, GTP-ase activating proteins; GST, glutathione S-transferase; LTD, long-term depression; LTP, long-term potentiation; MEK, mitogen-induced extracellular kinase; Nf1, neurofibromin 1; RBD, Ras-binding domain; synGAP, synaptic Ras GTPase-activating protein; TBS, theta burst stimulation; Wt, wildtype.

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expression (Rampon et al., 2000a; McNair et al., 2007) with which protein products manage morphological and physiological synaptic alterations that in turn, mediate synaptic plasticity-related processes, as well as learning and memory (Migaud et al., 1998). Brain-derived neurotrophic factor (BDNF) contributes importantly to synaptic and neuronal restructuring following EE (Ickes et al., 2000; Rossi et al., 2006; Kuzumaki et al., 2011; Kondo et al., 2012). At the level of synaptic plasticity and learning, mice that are deficient in BDNF show impaired hippocampal LTP, long-term depression (LTD) and object recognition memory. BDNF is required for plasticity and learning enhancements elicited by EE (Novkovic et al., 2014) and it mediates the regulation of LTP predominantly by means of TrkB receptor activation (Lessmann et al., 2003). BDNF-TrkB signaling is believed to play an essential role in both the early and late phases of LTP (Figurov et al., 1996; Kang and Schuman, 1996; Korte et al., 1996). TrkB-BDNF signaling activates three major signaling pathways (PLC-γ, Ras/MAPK, PI3K/Akt) (Huang and Reichardt, 2003; Minichiello, 2009), whereby Ras/MAPK and PI3K/Akt mediate trafficking and translation of proteins, while PLC-γ controls intracellular Ca^{2+} levels and thereby, adenyl cyclase activity and cAMP response element-binding protein (CREB)-dependent transcription (Kelleher et al., 2004; Yoshii and Constantine-Paton, 2010). Thus, these cascades are believed to underlie synaptic restructuring that is triggered by, and sustains, synaptic plasticity (Kandel, 2001; Yin et al., 2002; Waterhouse and Xu, 2009).

We have previously shown that activated Ras in neurons mimics BDNF-induced survival and fiber outgrowth (Borasio et al., 1989). Furthermore, intracellular inhibition of endogenous Ras activity by Ras-function blocking $F_{a,b}$ fragments leads to an inhibition of BDNF-induced effects suggesting that Ras activity is essential for mediating neurotrophic actions (Borasio et al., 1993). Accordingly, small GTPase Ras becomes activated upon BDNF binding to the TrkB receptor (Jian et al., 1996; Iida et al., 2001) that then leads to activation of the Ras/Raf/MAPK pathway. The phosphorylation of the Ser/Thr-protein kinase, Raf-kinase at the threonine and serine residue activates Raf, which in turns phosphorylates and activates mitogen-induced extracellular kinase (MEK). Activated MEK then phosphorylates and activates MAPK. Through the activation of Ras/Raf/MAPK cascade, many MAP kinases are activated, four of which (Erk 1, 2, 4 and 5) are affected by neurotrophin/Trk signaling (Peng et al., 1996; Cavanaugh et al., 2001). MAPK regulates the transcription of certain genes through the activation of the transcription factor CREB (Adams and Sweatt, 2002) that comprises a key element in the enablement of structural changes that underlie persistent LTP and memory (Bourtchuladze et al., 1994; Lee and Silva, 2009). The activation of Ras leads not only to activation of the Ras/Raf/MAPK cascade, but also to the activation of the Ras/PI3K/AKT pathway, and the Ral-guanine nucleotide dissociation stimulator (RalGDS) signaling pathways (Castellano and Downward, 2011).

Given the importance of BDNF in the synaptic and functional changes that underlie EE, we explored whether Ras mediates these effects. We studied transgenic mice that express constitutively activated Ras in neurons (synRas) (Heumann et al., 2000). In these mice, the activity of the Ras/MAPK pathway is enhanced, whereas the PI3K/Akt pathway is not affected by constitutive Ras activation (Heumann et al., 2000). This gives us the means to focus on the role of the Ras/Raf/MAPK cascade in BDNF-mediated enhancements of learning and plasticity arising from EE.

We observed that LTP was equivalent in transgenic mice and their Wt littermates in the absence of EE. Three weeks of constant EE resulted in enhanced LTP in Wts. Transgenics exhibited enhanced LTP following EE, that was not significantly different to that seen in enriched Wt controls, but was also not different from LTP in non-enriched transgenics. Non-enriched transgenic mice expressed higher Ras activity compared to non-enriched Wts. Strikingly however, following EE, Ras activity was reduced in transgenics to the levels detected in Wts. This suggests that although the Ras cascade is not a key factor in LTP enhancements following EE, it restores total Ras activity to normal functional levels. The maintenance of Ras within a physiological range may thus be required to enable LTP to be expressed at an optimal functional level.

EXPERIMENTAL PROCEDURES

The present study was carried out in accordance with the European Communities Council Directive of September 22nd 2010 (2010/63/EEC) for care of laboratory animals and after approval of the local government ethics committee (Bezirksamt, Arnsberg). All efforts were made to minimize the number of animals used.

Animals

The transgenic (synRas) mice used in this study were heterozygotes that selectively express constitutively activated human RasG12V in postmitotic neurons under the direction of the neuronal rat synapsin 1 promoter (Heumann et al., 2000). The interaction between active Ras and GTP-ase activating proteins (GAPs) is prevented, leading to a lack of GAP-driven GTP-hydrolysis of Ras, which in turn causes constitutive activation of Ras (Heumann et al., 2000).

Transgenic synRas mice were bred from established lines (Heumann et al., 2000). SynRas mice express human constitutively activated Val12-Ha-Ras selectively in differentiated neurons. Permanently active Ha-Ras is the result of a point mutation leading to amino acid substitution (Gly to Val) at position 12. The created transgene construct that contains Valine12-Harvey-Ras (Val12-Ha-Ras) gene and LacZ reporter gene is expressed under control of the neuron specific synapsin 1 promoter while Ras expression of the endogenously regulated gene is still intact (Heumann et al., 2000). The transgene was detected by PCR using the following primers: 5′-TGACC ATCCAGCTGATCCAGAA-3′ for the mouse Ha-Ras gene and 5′-CTCCCCATCAATGACCACCTG-3′ for the human Ha-Ras gene (Biomers). New Wt C57BL/6 female mice