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Combination of fluoxetine and extinction treatments forms a unique synaptic protein profile that correlates with long-term fear reduction in adult mice

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Abstract
The antidepressant fluoxetine induces synaptic plasticity in the visual and fear networks and promotes the structural remodeling of neuronal circuits, which is critical for experience-dependent plasticity in response to an environmental stimulus. We recently demonstrated that chronic fluoxetine administration together with extinction training in adult mice reduced fear in a context-independent manner. Fear conditioning and extinction alter excitatory and inhibitory transmissions within the fear circuitry. In this study, we investigated whether fluoxetine, extinction or their combination produced distinct long-lasting changes in the synaptic protein profile in the amygdala, hippocampus and prefrontal cortex of conditioned mice. We determined that extinction induced synaptophysin expression and down-regulated the GluA1:GluA2 ratio throughout the fear network in water- and fluoxetine-treated mice, suggesting a common fluoxetine-independent mechanism for increased synaptic transmission and re-arrangement of AMPA-receptors by extinction training. In contrast to common changes, the presynaptic vesicular neurotransmitter transporters VGAT and Vglut1 were upregulated after extinction in water- and fluoxetine-treated mice, respectively. The cortical levels of the GABA transporter Gat1 were reduced in high-freezing water-drinking mice, suggesting a maladaptive increase of GABA spillover at cortical inhibitory synapses. Fear conditioning decreased, and extinction induced the expression of GABA-receptor alpha1 and alpha2 subunits in water- and fluoxetine-treated mice, respectively. Only a combination of fluoxetine with extinction enhanced GluN2A expression in the amygdala and hippocampus, emphasizing the

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role of this NMDA-receptor subunit in the successful erasure of fear memories. Our finding provides novel data that may become helpful in developing beneficial pharmacological fear-reducing treatment strategies.

1. Introduction

Fear conditioning and extinction paradigm is a widely accepted model for anxiety disorders and cognitive behavioral therapies. Although the model does not cover all symptoms of anxiety disorders, the model might help to examine the efficacy of extinction training in transgenic animals or in combination with pharmacological compounds. The extinction-enhancing treatments that have beneficial effects on the long-term reduction of the expression of pathological fear memory in different contexts are essential for anti-anxiety therapies. Several studies have aimed to understand the neurobiological processes that mediate the facilitation of extinction. Accumulating evidence implicates the amygdala, the hippocampus and the infralimbic and prelimbic areas of the medial prefrontal cortex in the expression of fear responses (Quirk et al., 2010). Because both fear acquisition and extinction alter synaptic transmission in these brain areas at excitatory and inhibitory synapses (Ehrlich et al., 2009; Lange et al., 2012), dissecting the synaptic mechanisms of fear reduction through the enhancement of extinction memories is necessary.

Pharmacological agents that potentiate or inhibit the function of proteins involved in synaptic transmission represent one of the most valuable tools for elucidating the molecular mechanisms of extinction facilitation. Over the past decade, D-cycloserine, a partial agonist of the N-methyl-D-aspartate receptor (NMDAR) has been consistently shown to enhance fear extinction learning and reduce several, although not all, anxiety symptoms (Walker et al., 2002; Mao et al., 2006; Guastella et al., 2007). Another important group of compounds are benzodiazepines, agonists of the gamma-aminobutyric acid A receptors (GABA(A)Rs) that have recently been shown to reduce fear potentiated startle responses (Smith et al., 2012). However, the beneficial effect of benzodiazepines seems to be context-dependent, thus reducing their clinical value (Harris and Westbrook, 2001; Otto et al., 2005). Acute treatment with diazepam before extinction training could prevent extinction retention in a shuttle avoidance task potentially due to the induction of anterograde amnesia (Pereira et al., 1989). Another limitation of benzodiazepines is the development of dependence in case of chronic treatment (Wolf and Griffiths, 1991). Finally, a critical function of the antidepressant group of pharmacological compounds, in particular serotonin-selective reuptake inhibitors (SSRIs) in synaptic plasticity and fear memory is currently under intensive investigation. Not all SSRIs were found to have the potential for a beneficial outcome on the combined treatment (Burghardt et al., 2013). The diverse effects of SSRIs are thought to be a result of their different affinities to the serotonin transporter (Owens and Nemeroff, 1998) and selectivity in potentiating various neurotransmitters (Goodnick and Goldstein, 1998). The SSRI fluoxetine has consistently been shown to facilitate extinction learning and retention and long-term context-independent fear reduction in rodents (Karpova et al., 2011; Camp et al., 2012) and has been shown to significantly reduce fear reinstatement even after discontinuation of fluoxetine exposure (Deschaux et al., 2011). However, the synaptic mechanisms of these effects are largely unstudied.

The trafficking of the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate receptors (AMPARs) is critically involved in the plasticity of excitatory synapses and the GluA1-dependent synaptic removal of GluA2-containing calcium-permeable AMPARs during extinction was shown to promote long-term depression in the lateral amygdala and fear memory erasure (Kim et al., 2007; Clem and Huganir, 2010). The GluA1-subunit of calcium-impermeable AMPARs has been recently found to be up-regulated in the basolateral amygdala shortly after the spontaneous fear recovery and context-dependent fear renewal tests (Xue et al., 2014). The key markers of the GABAergic system (different GABA(A)R subunits, GABA plasma membrane transporter-1 Gat1 and gephyrin) are dynamically regulated in the amygdala nuclei by the fear conditioning and/or extinction: the mRNA expression analysis revealed that the GABAergic markers were primarily down-regulated right after fear conditioning and up-regulated following fear extinction training (Chhatwal et al., 2005; Heldt and Ressler, 2007). Accordingly, the surface expression of the GABA(A)R β3 and γ2 subunits was decreased by the conditioning and increased by the extinction in the lateral amygdala (Lin et al., 2009). However, limited data exist on the fear conditioning/extinction-induced synaptic protein expression outside the amygdala and on the long-lasting effects of the accelerated extinction on synaptic protein changes associated with efficient fear erasure. Such data are of particular importance for developing effective pharmacological strategies in those clinical cases when the immediate behavioral or pharmacological therapies are not applicable.

We have recently shown that chronic fluoxetine applied before and during fear extinction training had a long-term effect on extinction acceleration and fear erasure in adult mice (Karpova et al., 2011). The aim of the present study was to use the same protocol for simultaneous analysis of the fear responses and of the synaptic protein profile permissive for long-term fear reduction in mice. We applied an immunohistochemical approach to provide spatial specificity of protein expression by outlining the brain areas critical for fear memory: the prelimbic and infralimbic cortices, the basolateral, lateral and central amygdala nuclei and the CA1 region of hippocampus. We evaluated how the combination of fluoxetine treatment and extinction training affected long-term expression of the markers of excitatory and inhibitory transmission: the presynaptic synaptophysin Syp, vesicular glutamate transporter Vglut1, vesicular GABA and glycine transporter VGAT, GABA transporter Gat1, and the postsynaptic major GABA(A)R subunits α1 and α2, postsynaptic density protein PSD95, as well as

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