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Vortioxetine promotes early changes in dendritic morphology compared to fluoxetine in rat hippocampus

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Abstract
Preclinical studies reveal that the multimodal antidepressant vortioxetine enhances long-term potentiation and dendritic branching compared to a selective serotonin reuptake inhibitor (SSRI). In the present study, we investigated vortioxetine's effects on spines and dendritic morphology in rat hippocampus at two time points compared to the SSRI, fluoxetine.
Rats were dosed for 1 and 4 weeks with vortioxetine and fluoxetine at doses relevant for antidepressant activity. Dendritic morphology of pyramidal neurons (i.e., dendritic length, dendritic branch, spine number and density, and Sholl analysis) was examined in Golgi-stained sections from hippocampal CA1.
After 1 week of treatment, vortioxetine significantly increased spine number (apical and basal dendrites), spine density (only basal), dendritic length (only apical), and dendritic branch number (apical and basal), whereas fluoxetine had no effect. After 4 weeks of treatment, vortioxetine significantly increased all measures of dendritic spine morphology as did fluoxetine except for spine density of basal dendrites. The number of intersections in the apical and basal dendrites was also significantly increased for both treatments after 4 weeks compared to control. In addition, 4 weeks of vortioxetine treatment, but not fluoxetine, promoted a decrease in spine neck length.
In conclusion, 1-week vortioxetine treatment induced changes in spine number and density and dendritic morphology, whereas an equivalent dose of fluoxetine had no effects. Decreased spine

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neck length following 4-week vortioxetine treatment suggests a transition to mature spine morphology. This implies that vortioxetine's effects on spine and dendritic morphology are mediated by mechanisms that go beyond serotonin reuptake inhibition.

1. Introduction

Around 30–40% of major depressive disorder (MDD) patients fail to respond to antidepressant treatment, and 5–10% remain depressed despite repeated medical treatment (Mathers et al., 2006). Whereas the monoamine hypothesis of depression has prevailed for the last 5 decades, a more recent neuroplasticity hypothesis is gaining momentum. This suggests that neuronal plasticity in cortico-limbic brain structures plays an important role in the pathophysiology of MDD (Duman 2004; Licznerski and Duman, 2013). In support of this hypothesis, reduced hippocampal neurogenesis, loss of dendritic spines, dendritic atrophy, and loss of synapses have been observed in the mammalian brain in response to stress and depression (Kang et al., 2012; Pittenger and Duman, 2008; Wainwright and Galea, 2013). Numerous *in vivo* imaging studies in humans indicate that the volume of the hippocampus may be reduced in depression (Campbell and Macqueen, 2004; Sheline et al., 2003). Moderate apoptosis and atrophy of hippocampal neurons in depressed patients (Campbell and Macqueen, 2004) and reduced spine number and dendrite complexity could contribute to the decreased hippocampal volume (Licznerski and Duman, 2013).

Antidepressant treatment may trigger molecular and cellular mechanisms linked to neuronal plasticity and counteract the structural impairments of MDD by neurogenesis, gliogenesis, dendritic arborization, and new synapse formation (Duman, 2004; Pittenger and Duman, 2008). Animal studies have demonstrated changes in number and type of synapses in the hippocampus after antidepressant treatment (Chen et al., 2008, 2009, 2010; Hajszan et al., 2009, 2010). Antidepressant drugs induce rapid hippocampal synaptogenesis in the CA1 (Hajszan et al., 2009), whereas onset of dentate gyrus (DG) neurogenesis often occurs 3–4 weeks after treatment (Malberg 2004; Marcussen et al., 2008). Recent evidence showed that ketamine, rapidly increases spine density and function in limbic brain regions and that the behavioral effects of ketamine required synaptic protein synthesis and spine formation in rats (Li et al., 2010, 2011; Licznerski and Duman 2013). These findings suggest that recovery of synaptic connections is critical for a rapid antidepressant response and is not mediated entirely via neurogenesis (Kang et al., 2012; Li et al., 2010, 2011).

Animal studies of 5-hydroxytryptamine (5-HT) receptor subtype selective ligands indicate that they have opposing effects on the activity of selective serotonin reuptake inhibitors (SSRIs) in the brain networks underlying their therapeutic effects (Lucas et al., 2007; Scorza et al., 2012). Therefore, one antidepressant strategy has been to develop drugs that combine serotonin transporter (SERT) blockade and favorable 5-HT receptor modulation (Artigas, 2013). Vortioxetine has recently gained approval for the treatment of MDD and has a multimodal mechanism of action that combines antagonism at 5-HT_3, 5-HT_7 and 5-HT_{1D} receptors, agonism at 5-HT_{1A} and partial agonism at 5-HT_{1B} receptors, with SERT inhibition (Sanchez et al., 2015). Preclinical studies of vortioxetine revealed enhanced neuroplasticity, i.e. long-term potentiation (LTP) formation *in vitro* and dendritic branching *in vivo* compared to an SSRI (Dale et al., 2014; Guilloux et al., 2013). Gene expression studies have shown that a single dose of vortioxetine activates genes encoding dendritic spine plasticity proteins (du Jardin et al., 2013). Based on these results, we hypothesized that vortioxetine would show an enhanced effect on spinogenesis and dendritic morphology *versus* an SSRI. In the present study, we investigated vortioxetine's effects on spine number and density, dendritic length, and the number of branches in the rat hippocampus compared with the SSRI fluoxetine.

2. Experimental procedures

2.1. Animals

Adult male Sprague-Dawley rats (180–200 g) (n=96) were kept on a normal light: dark cycle and given free access to food and water. There were 12 rats in each group for each time point and dose. All rats were handled for 7 days before any procedure was initiated.

2.2. Antidepressant treatments

Vortioxetine was given in food chow (1.6 g/kg), which was provided by H. Lundbeck A/S (Copenhagen, Denmark) for 7 and 28 days. The selection of this dose was based on previously obtained data that this dosage produces >80% SERT occupancy. Regular food pellets with the same composition were given to control rats for the same period. Drug delivery via food chow in rats ensures a stable target engagement and removes any negative effects of chronic invasive dosing on overall animal welfare and experimental outcomes. Fluoxetine, also provided by H. Lundbeck A/S, was delivered *ad libitum* in the drinking water (160 mg/L) for 7 and 28 days. This dose was chosen to achieve >80% SERT occupancy with fluoxetine. The body weight, food intake, and fluid intake were measured regularly throughout the period of vortioxetine/fluoxetine administration. All procedures were approved by the Danish animal ethics committee (2012-15-2934-00254; C -sheet 1).

2.3. Tissue preparation and Golgi-Cox staining

Animals were anesthetized by an i.p. injection of 75 mg/kg phenobarbital sodium (Unikem A/S, Copenhagen, Denmark) in phosphate-buffered saline and euthanized by decapitation. Brains were immediately removed and split into two hemispheres. Golgi staining was performed using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Inc., Columbia MD) following the manufacturer's instructions.