reuptake inhibitor) antidepressants in preclinical rodent models relevant for antidepressant activity and cognitive functioning.

Methods: In vivo extracellular unitary recordings were obtained from rat dorsal raphe nucleus (DRN) 5-HT, locus coeruleus (LC) NE and ventral tegmentum (VTA) dopamine (DA) neurons after acute (i.v.) and repeated (s.c. via osmotic minipumps) administration.

Assessment of antidepressant activity was performed using the mouse forced swim test and a rat progesterone withdrawal model [3]. The latter model assessed effect after acute as well as 2-weeks dosing. Anxiolytic activity was assessed in the rat social interaction test after acute dosing.

Assessment of cognitive function used quantitative EEG measures and a novel object recognition memory task in normal and 5-HT depleted rats.

Results: The recovery of DRN 5-HT neuronal firing and desensitization of somatodendritic 5-HT_{1A} autoreceptors was faster with vortioxetine than with the SSRI fluoxetine (1 day vs. 14 days) and occurred at a lower SERT occupancy. LC NE and VTA DA neuronal firing was largely unaffected by vortioxetine, whereas SSRIs or SNRIs will decrease the firing rate in these nuclei over time. Vortioxetine showed antidepressant-like activity in BALBc mice in the forced swim test, as did the SSRI fluoxetine. However, vortioxetine was effective at a lower SERT occupancy than required for fluoxetine. Vortioxetine, but not the SSRI escitalopram or the SNRI duloxetine, was active in a progesterone withdrawal model of depression under acute and chronic dosing conditions.

Furthermore, vortioxetine but not the SSRI paroxetine showed anxiolytic like activity in the rat social interaction test. qEEG analyses of the active awake state demonstrated increases across power bands with vortioxetine, but not with duloxetine or escitalopram, supporting a role for vortioxetine in modulating cortical networks recruited during cognitive behaviour. Similar to literature reports for SSRIs, vortioxetine restored time-induced memory deficits in normal rats, but only vortioxetine, not escitalopram or duloxetine, restored memory deficits induced by 5-HT depletion.

Conclusion: The effects of vortioxetine in preclinical rodent models relevant for antidepressant and anxiolytic activity and cognitive functioning differ from those of SSRIs and SNRIs. This indicates that vortioxetine's receptor mechanisms are critical for its pharmacological activity and warrants further investigation of the clinical impact of vortioxetine's mechanisms of action.

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P.2.e.003 Role of 5-HT_3 receptors in the mechanism of action of the investigational antidepressant vortioxetine

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Objective: Vortioxetine (Lu AA21004) is an investigational antidepressant [1] under clinical development for the treatment of major depressive disorder. In cell studies, vortioxetine is a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist, and inhibitor of the serotonin (5-HT) transporter (SERT) [2]. Analyses of target occupancies in the rodent brain and SERT occupancy studies from human PET studies support a dose dependent occupancy of all these targets at clinical doses of vortioxetine (Areberg et al., Basic Clin Pharmacol Toxicol, 2012; Mørk et al., J Pharmacol Exp Ther, 2012; Pehrson et al., Eur Neuropsychopharmacol, 2013). Vortioxetine increases the extracellular monoamine concentration in the forebrain to a greater extent than the selective serotonin reuptake inhibitors (SSRIs) [2]. This difference is likely due to the additional pharmacological activities of vortioxetine at 5-HT receptors, which may prevent local and distal self-inhibitory mechanisms. The aim of the present study was to explore the role played by 5-HT_3 receptors, for which vortioxetine shows high affinity (Ki = 3.7 nM). 5-HT_3 receptors are present in the forebrain on GABAergic interneurons that control pyramidal neuron activity in the hippocampus and the prefrontal cortex (PFC) [3]. 5-HT_3 receptor blockade may, therefore, alter the functional connectivity between the PFC and the midbrain, increasing the PFC excitatory tone onto serotonergic neurons.

Methods: We examined the effects of vortioxetine and the selective 5-HT_3 receptor antagonist ondansetron on the firing activity of medial PFC pyramidal neurons projecting to the midbrain in anesthetized male Wistar rats, using extracellular recordings. After obtaining a stable baseline recording for 5 min, drugs were administered i.v. and recordings were made for an additional 22–40 min. Electrode localization was performed by histological verification in frozen brain sections.

Results: Vortioxetine (0.1–1.6 mg/kg i.v., cumulative doses) dose-dependently increased the discharge rate of pyramidal neurons in the medial PFC, with a maximal effect at 0.4 mg/kg i.v. (297% of baseline), which persisted for >13 min after the last injection. The administration of the selective 5-HT_3 receptor antagonist ondansetron (0.16–1.28 mg/kg i.v., cumulative doses) evoked a dose-dependent increase of pyramidal discharge in 7 out of the 11 neurons recorded. The maximum effect was achieved at 1.28 mg/kg i.v. and the discharge persisted for >20 min after administration of the last dose.

Conclusions: Vortioxetine dose-dependently increased the discharge rate of pyramidal neurons in the mPFC. Preliminary experiments suggest that this effect is not due to SERT blockade. The enhancing effect of ondansetron was observed in a high proportion of recorded pyramidal neurons, despite the presence of 5-HT_3 receptors in a limited PFC interneuron population [3]. Overall, the present data indicate that 5-HT_3 receptors exert a tight control of pyramidal neuron activity and may warrant the investigation of vortioxetine's potential to treat clinical conditions associated with cognitive dysfunction. Further experiments will examine the effect

of simultaneous SERT and 5-HT_3receptor blockade on pyramidal neuron discharge.

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P.2.e.004 Vortioxetine (Lu AA21004) disinhibits pyramidal cell output and enhances theta rhythms and long-term plasticity in the hippocampus

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Purpose: Vortioxetine, an investigational multimodal antidepressant, is a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and 5-HT transporter inhibitor. Vortioxetine has been shown to enhance memory performance in several preclinical rat models, including novel object recognition, spontaneous alternation and contextual fear conditioning. Here we investigate the mechanistic basis for these effects by testing the effect of vortioxetine on synaptic transmission, theta rhythm, and long-term potentiation (LTP), a cellular correlate of learning and memory, in the hippocampus.

Methods: Hippocampal slices were prepared from male Sprague Dawley rats. Spontaneous inhibitory post-synaptic currents (sIPSCs) were recorded in CA1 pyramidal cells using whole cell patch recordings; LTP was recorded in the CA1 area using field potential recordings. LTP was induced through theta-burst stimulation of Schaffer collaterals (four trains of four pulses at 100 Hz separated by 200 ms) and recorded for one hour after induction. sIPSCs were recorded using patch electrodes filled with a cesium based internal solution. sIPSCs were pharmacologically isolated by adding NMDA and AMPA/kainate receptor antagonists to the recording buffer. Serotonin (5-HT, 100 μM) and the 5-HT_3 receptor agonist mCPPG (20 μM) were locally applied to brain slice surfaces. Vortioxetine (20 μM), escitalopram (2 μM), the 5-HT_{2A}/5-HT_{2C} receptor agonist DOI (2 μM), and the 5-HT_7 receptor agonist AS-19 (100 nM) were added to the extracellular recording buffer. Theta rhythms were recorded in vivo using electroencephalographic (EEG) recordings via a telemetric system (Data Sciences Int.). A multi-day Latin square, cross-over design was used (n = 9 rats per condition). Vehicle, vortioxetine and escitalopram were dosed between 9 and 10 am (lights on at 6 am) and analyzed from 90 min pre-dose until 4 hr post-dose.

Results: Local application of 5-HT increased the amplitude and frequency of sIPSCs in hippocampal slices. The increase in sIPSCs did not depend on the activation of 5-HT_{2A}/5-HT_{2C} and 5-HT_7 receptors, but was mediated by the stimulation of 5-HT_3 receptors on inhibitory γ-butyric acid (GABA) interneurons. Vortioxetine blocked the 5-HT-induced increase in sIPSCs, most likely through blockade of 5-HT_3 receptors on interneurons. Interneurons expressing 5-HT_3 receptors have been shown to regulate the strength of theta rhythms and LTP by controlling output of pyramidal cells [1].

Vortioxetine (at 5 and 10 mg/kg) significantly increased theta power during the active wake state in vivo. In addition, vortioxetine enhanced theta-burst LTP in hippocampal slices, most likely by decreasing the inhibition of pyramidal cells. The selective 5-HT reuptake inhibitor escitalopram, in comparison, had no effect on theta rhythms or on LTP.

Conclusion: Vortioxetine decreased 5-HT-induced inhibitory drive of pyramidal cells and enhanced theta rhythm and LTP in the hippocampus, mostly likely by antagonizing 5-HT_3 receptors.

These properties may contribute to the memory-enhancing effects of vortioxetine in preclinical rat models.

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P.2.e.005 Expression profiling of the lateral habenula in the chronic mild stress rat model of depression

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Purpose: Increased neuronal activity of the lateral habenula (LHb) has been implicated in the etiology of major depressive disorder (MDD), while functional inhibition of the LHb show antidepressant effects in a clinical case and in animal models of depression. The LHb has efferents to the monoaminergic system, indicating a role for the LHb in the monoaminergic imbalance observed in depressed individuals.

The present study was aimed to clarify molecular aberrations in the LHb during depressive-like states and after antidepressant treatment in the search for novel disease targets and treatment regimens.

Chronic mild stress (CMS) is a valid rat model of depression. Chronic exposure to mild and unpredictable stressors induces an anhedonic-like state, which is monitored as a reduced intake of a sucrose solution.

Anhedonic-like behavior can be reversed by chronic treatment with antidepressant drugs [1].

In our hands, the CMS model has additional features that enhance its validity. Thus rats show a graduated response to stress; a substantial fraction of animals submitted to stress are resilient and do not become anhedonia-like, but do have stress-induced cognitive impairments [2].

Furthermore antidepressant administration reverses stress-induced anhedonia only in approximately 50% of the treated animals, which mirrors clinical treatment refraction and is thus adding additional translational value to the CMS model.

Methods: The CMS protocol was applied as described, i.e. rats were exposed to a number of different micro-stressors and sucrose intake was used as a read out on reward sensitivity i.e. reflecting the hedonic status. We compared different phenotypes from the chronic mild stress (CMS) model of depression using chronic administration with the selective serotonin reuptake inhibitor (SSRI)