In conclusion, this study shows that fluoxetine chronic treatment results in an enhancement of the sensitivity to fluoxetine of STN neuron leading to an increment of neuron activity. This change in sensitivity may be involved in the extrapyramidal effects induced by fluoxetine since increased STN neuron activity results in an enhancement of basal ganglia output nuclei signal and leads to movement inhibition.

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P.1.g.012 The CB2 ligand AM630 (6-iodopravadoline) increases the content and activity of pro-apoptotic markers in mouse brain

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Purpose: CB1 receptors were shown to display constitutive activity; e.g. a tonic activation of Fas/FADD (Fas-associated death domain) receptor complex suggesting that endocannabinoids could induce pro-apoptotic actions in brain [1]. Moreover, the CB1 receptor agonist WIN55212–2 acutely reduced FADD and rimonabant (SR141716A), inactive by itself (neutral antagonist), antagonized this effect in mouse brain [1].

CB2 receptors also display constitutive activity in vitro, and the selective ligand AM630 has been classified as a CB2 receptor protean ligand; i.e. the compound can behave as an agonist, neutral antagonist or inverse agonist [2]. The aim of this work was to investigate the in vivo pharmacological nature of AM630 modulating pro-apoptotic FADD and other apoptotic factors in mouse brain.

Methods: Groups of male CD1 Swiss mice were acutely treated (i.p) with drug-vehicle (n = 14), AM630 (1 and 10 mg/kg, 1.5 h, n = 3–5), and JWH133 (a CB2 receptor agonist, 1 and 3 mg/kg, 1 h, n = 6–6). For comparison, other mice were acutely treated with the CB1 receptor antagonists rimonabant (3 mg/kg, 2 h, n = 5) and AM281 (10 mg/kg, 1.5 h, n = 8). The animals were killed by decapitation at the indicated times.

FADD, p-Ser191 FADD (associated with non-apoptotic actions), phosphorylated (p) and total (t) JNK (c-Jun-NH2-terminal protein kinase), cytochrome c, nuclear PARP-1 (polyADP-ribose-polymerase) and its main fragment were quantified in the cerebral cortex by Western immunoblot analyses with specific antibodies, and the content of beta-actin was used as a loading (negative) control [1].

Results: The CB2 ligand AM630, but not the CB2 agonist JWH133, markedly increase the content of FADD (72% and 172%, $p < 0.001$) and p-Ser191 FADD (61% and 178%, $p < 0.001$) in mouse brain cortex, without altering the ratio p-FADD/FADD when compared with that in controls. In contrast, the CB1 receptor antagonists rimonabant and AM281 did not significantly alter the content of brain FADD. Notably, JWH133 decrease (20%-40%, $p < 0.05$) and AM630 increase (115%-186%, $p<001$) the activation of pro-apoptotic JNK (ratio of p-JNK to t-JNK). Moreover, AM630, but not JWH133, also increased the content of pro-apoptotic cytochrone c (24%, $p < 0.01$). In line with these findings, the CB2 ligand AM630 increased the cleavage of PARP-1 (95%, $p < 0.01$), a marker of apoptosis. In contrast, the CB1 receptor antagonist AM281 did not alter the content of these pro-apoptotic factors in brain.

Conclusions: CB2 receptors appear to display constitutive activity in mouse brain cortex in vivo, as demonstrated in cell culture in vitro [2]. Thus, the CB2 agonist JWH133 downregulated pro-apoptotic JNK whereas the CB2 ligand AM630 upregulated the activity of this kinase.

Therefore, AM630 appears to behave as an inverse agonist at the CB2 receptor. AM630 also upregulated other pro-apoptotic factors such as FADD, cytochrome c and PARP-1 cleavage in mouse brain cortex, indicating that this drug is not a neutral antagonist at the CB2 receptor. In contrast, AM281, like rimonabant, appears to behave as a neutral antagonist at the CB1 receptor regulating these pro-apoptotic factors. The findings also suggest that the CB2 inverse agonist AM630 could induce abnormal brain cell death in vivo.

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P.1.g.013 Effects of vortioxetine versus paroxetine on polysomnography in man: a pharmacokinetic/pharmacodynamic study

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Purpose: The electroencephalogram (EEG) during human sleep is a sensitive measure of drug effects in the brain, and may be used to identify pharmacodynamic differences between compounds with different pharmacologic profiles. Selective serotonin (5-HT) reuptake inhibitors (SSRIs) and drugs that act at 5-HT receptors (including 5-HT_{1A}, 5-HT_3, 5-HT_7) give rise to acute detectable changes in sleep architecture, particularly in rapid eye movement (REM) sleep. Vortioxetine (Lu AA21004) is a novel investigational multimodal antidepressant for the treatment of major depressive disorder. Vortioxetine is a 5-HT_3, 5-HT_7 and 5-HT_{1D} receptor antagonist, a 5-HT_{1A} receptor agonist, a 5-HT_{1B} receptor partial agonist, and is also an inhibitor of the serotonin transporter (SERT) in vitro [1]. When this study was designed, it was expected that vortioxetine would affect REM sleep and that this effect would be different from that of a selective serotonin reuptake inhibitor (SSRI).

The purpose of this study was to compare the effect of vortioxetine, paroxetine and placebo after 3 days of dosing on sleep architecture, as measured by polysomnography (PSG).

Methods: This study was a randomised, double-blind, four-way crossover, placebo-controlled, multiple-dose clinical pharmacology study in healthy young men. A total of 24 subjects were randomised into the study and 19 subjects completed the study. The subjects received 20 mg vortioxetine (n = 20), 40 mg vortioxetine (n = 24), 20 mg paroxetine (n = 20), or placebo (n = 20) for 3 consecutive days in 4 different periods with at least 3 weeks between treatment periods. The dose of 40 mg vortioxetine for 3 days corresponds to a steady state plasma concentration with 20 mg.

PSG recordings were performed on the pre-dose night and on nights 1 and 3 of dosing in each period and were scored manually by an expert scorer blind to treatment, using standard methods. Blood samples were obtained for pharmacokinetic (PK) analysis. The plasma concentrations of vortioxetine and paroxetine during the PSG measurement were used to estimate SERT occupancies using published relationships between plasma concentration and SERT occupancy in healthy subjects [2,3].

Results: In the pharmacodynamic (PD) analysis both drugs suppressed REM sleep on Day 3. All 3 active treatments statistically significantly ($p < 0.0001$) increased REM onset latency (ROL) and decreased time in REM sleep (TREM). There was no obvious placebo effect on any of the PSG parameters. In the PK/PD analysis using an Emax model, significant relationships were found between REM onset latency (ROL) and vortioxetine/paroxetine exposure, and between time in REM sleep (TREM) and vortioxetine/paroxetine exposure. The relation between REM suppression parameters and SERT occupancy was significantly different between vortioxetine and paroxetine, despite the same SERT occupancy. At a given SERT occupancy, vortioxetine seemed to affect REM sleep to a lesser degree than paroxetine. This indicates that vortioxetine has a different clinical pharmacological profile than an SSRI.

Conclusion: By overlaying the estimated SERT occupancy and the effect on REM onset latency as functions of exposure to vortioxetine or paroxetine, the effects on REM suppression were clearly separated, indicating that vortioxetine and paroxetine have different pharmacological profiles in the human brain.

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P.1.g.014 Vortioxetine, a novel multimodal antidepressant, modulates GABA and glutamate neurotransmission via serotonergic mechanisms

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Purpose: Vortioxetine (Lu AA21004), an investigational multimodal antidepressant, acts as an antagonist at 5-HT$_3$, 5-HT$_7$ and 5-HT$_{1D}$ receptors, a partial agonist at 5-HT$_{1B}$ receptors, an agonist at 5-HT$_{1A}$ receptors and an inhibitor of the 5-HT transporter in recombinant cell lines [1,2].

Previous research demonstrates that vortioxetine has antidepressant activity and improves cognitive function as shown in behavioral models.

Published studies using selective ligands suggest that some of vortioxetine's receptor mechanisms can potentially modulate gamma-aminobutyric acid (GABA)ergic and glutamatergic neurotransmission. 5-HT$_3$ receptors are nearly exclusively expressed on GABA neurons, and 5-HT$_3$ receptor stimulation facilitates GABAergic neurotransmission, which may modulate glutamatergic neurotransmission downstream. 5-HT$_{1A}$ receptors also modulate GABAergic and glutamatergic neurotransmission. Here we examine experiments that suggest a portion of vortioxetine's effects may be mediated by effects on GABAergic and glutamatergic neurotransmission.

Methods: Executive function was evaluated in rats administered the NMDA receptor antagonist phencyclidine (5 mg/kg, b.i.d) for 7 days and acute vortioxetine was tested in the attentional set-shifting test (AST) after a 7-day washout period. The effects of vortioxetine on stress-induced reductions in hippocampal long-term potentiation (LTP) were examined in anesthetized male rats that had been exposed to an elevated platform for 30 min. In female rats, memory function was evaluated using the novel object recognition test (NOR) after serotonin depletion using 4-chloro-DL-phenylalanine methyl ester HCl (PCPA; 86 mg/kg/day, s.c., 4 days). Vortioxetine, the 5-HT$_3$ receptor antagonist ondansetron, or the 5-HT$_{1A}$ receptor agonist flesinoxan was administered 1hr before evaluation in NOR. Depression-like behavior was evaluated using the forced swim test (FST) in a progesterone-withdrawal (PWD) model, where female rats were administered progesterone (30 mg/kg in oil) on a 5d on, 2d off schedule for 3 weeks. Rats were tested 48hr after the final progesterone administration. Subacute vortioxetine, fluoxetine, duloxetine, or amitriptyline dosing and acute administration of ondansetron or flesinoxan were evaluated in the PWD model.

Results: In the AST, subchronic phencyclidine significantly impaired performance during the extradimensional shift (ED) and extradimensional reversal discriminations, and vortioxetine reversed these impairments at 3 and 10 mg/kg. Acute stress due to elevated platform exposure significantly reduced hippocampal LTP, which was reversed by acute 10 mg/kg vortioxetine. PCPA treatment induced profound deficits in NOR performance that were reversed by acute vortioxetine treatment above 0.1 mg/kg, as well as by ondanstron at 3 and 300 ug/kg or flesinoxan at 1 mg/kg. PWD, which induces alterations in GABAergic neurotransmission, caused a depression-like phenotype in the FST that was reversed by subacute vortioxetine or amitripyline but not fluoxetine or duloxetine.

Acute administration of ondansetron or flesinoxan significantly reduced immobility time in PWD rats.

Conclusions: The ability of vortioxetine to reverse phencyclidine-induced impairments in the AST, as well as acute stress-induced impairments in hippocampal LTP, supports the hypothesis that vortioxetine can modulate glutamatergic neurotransmission.

This idea is further supported by a putative role for 5-HT$_3$ and 5-HT$_{1A}$ receptors, both of which can modulate GABAergic and glutamatergic neurotransmission, in vortioxetine's effects in the PCPA and PWD models.

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