



# Insufficiency of ventral hippocampus to medial prefrontal cortex transmission explains antidepressant non-response

Mariusz Papp<sup>1</sup>, Piotr Gruca<sup>1</sup>, Magdalena Lason<sup>1</sup>, Ewa Litwa<sup>1</sup>, Wojciech Solecki<sup>2</sup> and Paul Willner<sup>3</sup>

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## Abstract

**Background:** There is extensive evidence that antidepressant drugs restore normal brain function by repairing damage to ventral hippocampus (vHPC) and medial prefrontal cortex (mPFC). While the damage is more extensive in hippocampus, the evidence of treatments, such as deep brain stimulation, suggests that functional changes in prefrontal cortex may be more critical. We hypothesized that antidepressant non-response may result from an insufficiency of transmission from vHPC to mPFC.

**Method:** Antidepressant non-responsive Wistar Kyoto (WKY) rats were subjected to chronic mild stress (CMS), then treated with chronic daily administration of the antidepressant drug venlafaxine (VEN) and/or repeated weekly optogenetic stimulation (OGS) of afferents to mPFC originating from vHPC or dorsal HPC (dHPC).

**Results:** As in many previous studies, CMS decreased sucrose intake, open-arm entries on the elevated plus maze (EPM), and novel object recognition (NOR). Neither VEN nor vHPC–mPFC OGS alone was effective in reversing the effects of CMS, but the combination of chronic VEN and repeated OGS restored normal behaviour on all three measures. dHPC–mPFC OGS restored normal behaviour in the EPM and NOR test irrespective of concomitant VEN treatment, and had no effect on sucrose intake.

**Conclusions:** The synergism between VEN and vHPC–mPFC OGS supports the hypothesis that the antidepressant non-responsiveness of WKY rats results from a failure of antidepressant treatment fully to restore transmission in the vHPC–mPFC pathway.

## Keywords

Ventral hippocampus, medial prefrontal cortex, venlafaxine, optogenetic stimulation, WKY rat

## Introduction

Major depression is projected to become the leading cause of global disease burden by 2030 (Lépine and Briley, 2011). The most severely disabled groups are those with treatment-resistant depression (TRD) (Greden, 2001), who comprise more than half of all depressed patients (Nemeroff, 2007; Thase, 2011; Thomas et al., 2013). After half a century in which there was very limited increase in the efficacy of antidepressant drugs, hope for treatment-resistant patients was kindled by the discovery of novel treatments that bring about rapid and sometimes lasting improvements in a high proportion of TRD patients, including the N-methyl-D-aspartate (NMDA)-receptor antagonist ketamine (Berman et al., 2000; DiazGranados et al., 2010; Zarate et al., 2006), and high-frequency electrical stimulation of the anterior cingulate cortex (ACC) and certain other brain areas (deep brain stimulation: DBS) (Delahoye and Holtzheimer, 2014; Hamani et al., 2011; McGrath et al., 2014; Mayberg, 2009). These discoveries also enabled the development of animal models of TRD, which had been blocked by the fact that responsiveness to antidepressant drug treatment has been considered to be an essential feature of a valid animal model of depression (Willner, 1984), severely limiting their usefulness in relation to TRD (Hendrie et al., 2013; Willner and Belzung, 2015).

In the chronic mild stress (CMS) procedure, the most widely used animal model of depression (Antoniuk et al., 2019; Willner,

2017), rats or mice display a wide range of behavioural and physiological changes characteristic of depression, which respond to chronic treatment with antidepressant drugs (Hill et al., 2012; Willner, 1997, 2017). Like TRD patients, rodents subjected to CMS show a rapid reversal of depression-related behaviours following ketamine treatment (Maciel et al., 2018; Papp et al., 2017; Tornese et al., 2019; Wen et al., 2019) or DBS of the medial prefrontal cortex (mPFC) (Dourmes et al., 2013; Hamani et al., 2012; Lim et al., 2015; Veerakumar et al., 2014). The Wistar Kyoto (WKY) rat has long been considered to be resistant to antidepressant drug treatment, largely on the basis of acute studies using the forced swim test (Lahmame et al., 1997; Lopez-Rubalcava and Lucki, 2000; Tejani-Butt et al., 2003). When tested in the CMS model, WKY rats failed to recover following chronic treatment with different antidepressant drugs, but nevertheless did show

<sup>1</sup>Maj Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland

<sup>2</sup>Department of Neurobiology and Neuropsychology, Institute of Applied Psychology, Jagiellonian University, Krakow, Poland

<sup>3</sup>Department of Psychology, Swansea University, Swansea, UK

## Corresponding author:

Mariusz Papp, Maj Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Street, Krakow 31-343, Poland.

Email: nfpapp@cyfronet.pl

recovery on a battery of behavioural tests (sucrose intake, novel object recognition (NOR) and the elevated plus maze (EPM)) following subchronic ketamine treatment or acute DBS of the mPFC (Papp et al., 2018, 2020; Willner et al., 2019). Hence, CMS in the WKY rat provides a validated model of TRD (Aleksandrova et al., 2017).

In antidepressant-responsive rat strains there is evidence suggesting a role for the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype of glutamate receptor in the mechanism of action of both antidepressant drugs (Ampuero et al., 2010; Barbon et al., 2011; Martínez-Turrillas et al., 2002; Neis et al., 2016; Park et al., 2018; Svenningsson et al., 2007) and novel treatments such as ketamine (Du et al., 2006; Maeng et al., 2008) and DBS (Jiménez-Sánchez et al., 2016a, 2016b). Consistent with these observations, we recently confirmed, using the CMS model, that intra-PFC administration of the selective AMPA-receptor antagonist NBQX (Shimizu-Sasamata et al., 1996) blocked the antidepressant effect of VEN in Wistar rats (Papp et al., 2020). NBQX also blocked the antidepressant effects of both DBS and optogenetic stimulation (OGS: Fuchikami et al., 2015) in WKY rats when administered at the same site in the mPFC (Papp et al., 2020). These results, along with antidepressant effects, in WKY rats, of intra-PFC administration of the AMPA-receptor positive allosteric modulator CX-516 (Arai et al., 2002; Papp et al., 2020), suggest that modulation of AMPA receptors in the mPFC represents a final common pathway for the action of antidepressants in Wistar rats, and of DBS and OGS in antidepressant non-responsive WKY rats.

The common action of NBQX to block the antidepressant actions of VEN in Wistar rats and DBS or OGS in WKY rats has the important implication that antidepressant resistance in WKY rats is likely to result from problems on the afferent side that prevent antidepressant drugs from activating the mPFC. The ventral hippocampus (vHPC)–mPFC pathway is a strong candidate to mediate such an effect. Stress (STR)-induced inactivation of the vHPC, with a consequent loss of vHPC–mPFC transmission, has been proposed as the basis of depressive psychopathology (e.g. Willner et al., 2014), and one study has reported that activation of this pathway was both necessary and sufficient for the antidepressant-like effect of ketamine in the mouse-forced swim test (Carreno et al., 2016). The restoration of mPFC afferent activity by antidepressant drugs may be compromised in WKY rats by differences in hippocampal dynamics: a genomic screening study found that the ratio of vHPC to dHPC expression of depression-related genes was lower in WKY rats, relative to drug-responsive Wistar rats, for 11 of the 22 genes examined (Papp et al., 2019). We therefore hypothesized that antidepressant resistance in WKY rats might be caused by insufficiency of vHPC–mPFC transmission.

In order to investigate this hypothesis, we implemented the CMS model in WKY rats, using a standard battery of behavioural tests, and tested the effects, alone and in combination, of chronic daily venlafaxine (VEN) together with repeated weekly OGS of the terminals of afferents from vHPC to mPFC. In order to evaluate anatomical specificity, we also tested the effects of OGS of the terminals of afferents from dorsal HPC (dHPC) to mPFC. In an initial experiment (previously reported in part: Papp et al., 2020), we examined the effect of acute OGS of the vHPC–mPFC pathway, so as to be confident that OGS alone would not create antidepressant effects that would obscure any potentiation of effects of VEN.

## Methods

### Subjects

A total of 128 male WKY rats (Charles River, Germany), aged 5 weeks and weighing 120 g on arrival were housed singly with free access to food (standard animal feed FRF1, AnimaLab, Poznan, Poland) and water, and maintained on a 12-h light/dark cycle (lights on at 08.00h) in conditions of constant temperature ( $22 \pm 20^\circ\text{C}$ ) and humidity ( $50 \pm 5\%$ ), with standard bedding (Middi LTE E-002 Abedo sawdust, AnimaLab, Poznan, Poland). At the time of the final baseline test before the onset of CMS (see below), mean body weights were 300 g. All procedures used conformed to the rules and principles of EEC Directive 86/609 and were approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

### Experimental design

Two experiments were conducted. In both experiments, STR animals were subjected to CMS (STR) throughout (see below for details), whereas controls (CONs) were left undisturbed in their home cages, except for weekly sucrose intake tests, operative procedures and standard husbandry applied to both groups.

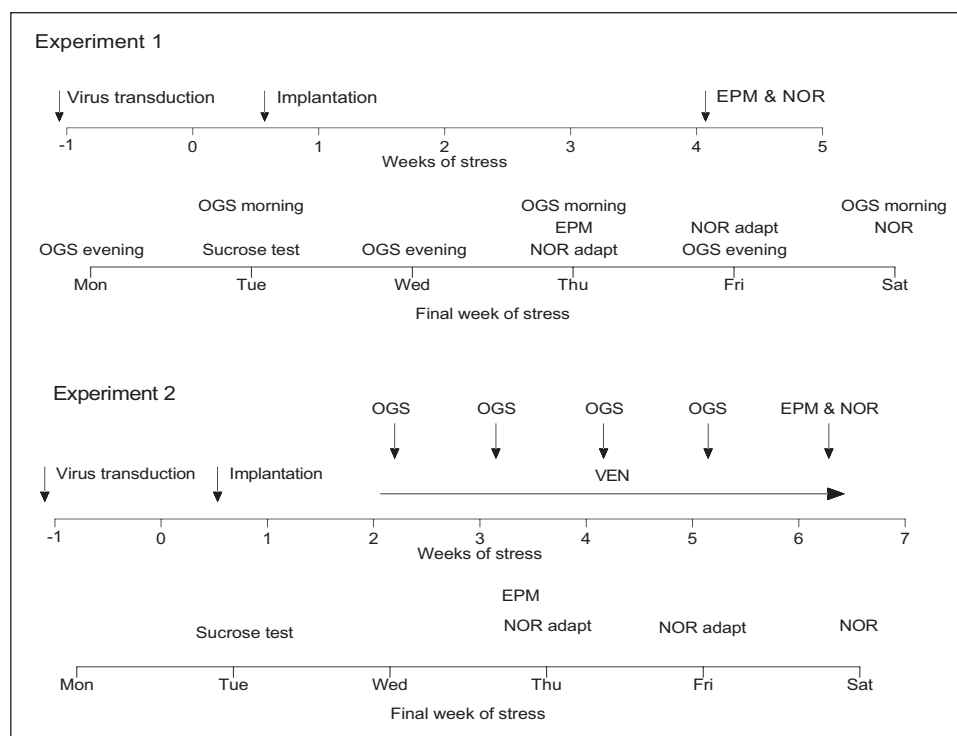
Experiment 1 ( $N=32$ ) tested the effect of acute OGS of the vHPC–mPFC pathway, or sham stimulation, in WKY rats subjected to CMS or non-STR CONs ( $n=8/\text{group}$ ), following 4 weeks of STR. Two 2-h sessions of OGS or sham stimulation were administered, the night before and immediately preceding each of three behavioural tests (sucrose intake; EPM (Pellow and File, 1986) and NOR (Ennaceur and Delacour, 1988); Figure 1).

Experiment 2 ( $N=96$ ) asked whether repeated OGS of either the vHPC–mPFC pathway or the dHPC–mPFC pathway would overcome the antidepressant non-response of WKY rats. After 2 weeks of CMS, CON ( $n=32$ ) and STR ( $n=64$ ) groups received daily injections, for 4 weeks and 3 days, of either saline (SAL) or VEN: 10 mg/kg i.p.; Carbosynth Ltd, Compton, Berkshire, UK; dissolved in 0.9% sterile SAL). The 10 mg/kg dose of VEN was used in all of our previous studies with this drug and was fully efficacious in Wistar rats (e.g. Papp et al., 2020; Willner et al., 2019).

Animals in all groups were administered either OGS or sham stimulation, on four weekly occasions, approximately 2 h prior to VEN administration on days 1, 8, 15, and 22. The final OGS session was 6 days before the final sucrose test (week 6 of STR), followed by a session on the EPM on day 2 of week 7, and the exposure trial in the NOR test on day 4 of week 7 (Figure 1). In all, there were six CON groups ( $n=8/\text{group}$ ): four groups (CON or STR treated with SAL or VEN) that received sham stimulation, and two non-STR OGS groups treated with SAL or VEN. In each CON group, subgroups ( $n=4$ ) were infected in either vHPC or dHPC; these subgroups were combined for analysis and presentation ( $n=8/\text{group}$ ). The active OGS treatment was applied to four STR groups ( $n=12/\text{group}$ ), treated with SAL or VEN and infected in vHPC or dHPC.

### Surgical and stimulation procedures

**Operative procedures.** Animals were anaesthetized with pentobarbital (60 mg/kg, i.p.) and placed in a stereotaxic apparatus



**Figure 1.** Experimental time lines.

(Stoelting Co., Wood Dale, IL, USA) set with bregma 1 mm higher than lambda. Anaesthesia was deep enough that the animals did not respond to tail pinch, and no top-ups were needed. During surgery, lidocaine (Lidocain 10%, EGIS Pharmaceuticals PLC, Budapest, Hungary) was sprayed on the uncovered skull. After surgery, the animals were moved for 24 h to a separate room heated to 24°C, they received 0.2 mg/kg meloxicam (Boehringer Ingelheim, Germany) for 3 days, and ampicillin (Ampicilin TZF, Polfa, Warsaw, Poland) was applied to the wound for up to 3 days if signs of inflammation (rarely observed) such as oedema or oozing of fluid were present.

**Viral infection.** During the week before the penultimate baseline sucrose test animals that were to receive OGS were administered adeno-associated viruses (AAVs) inducing channelrhodopsin-2 (ChR2) and EYFP gene expression non-selectively in all neurons (AAV5-hSyn-ChR2-EYFP). Rats with virally delivered EYFP only (AAV5-hSyn-EYFP) were used as sham CONs. AAVs were obtained from the University of North Carolina Viral Core.

For viral infusion, a small skin incision at the top of the scalp and a small hole in the skull were made above the left vHPC (experiments 1 and 2) or dHPC (experiment 2 only), followed by AAV infusion (0.1 µl/min; total volume 0.5 µl;  $4.4 \times 10^{12}$  virus molecules/ml) at coordinates AP -5.3, L +5.5, DV -7.5 (vHPC) or AP -4.16, L +2.8, DV -2.6 (dHPC), according to the atlas of Paxinos and Watson (1998). Infusions were made using an infusion pump and 2-µl Hamilton syringes. After 5 min, the skin was sutured, and the animals were transferred to their home cages.

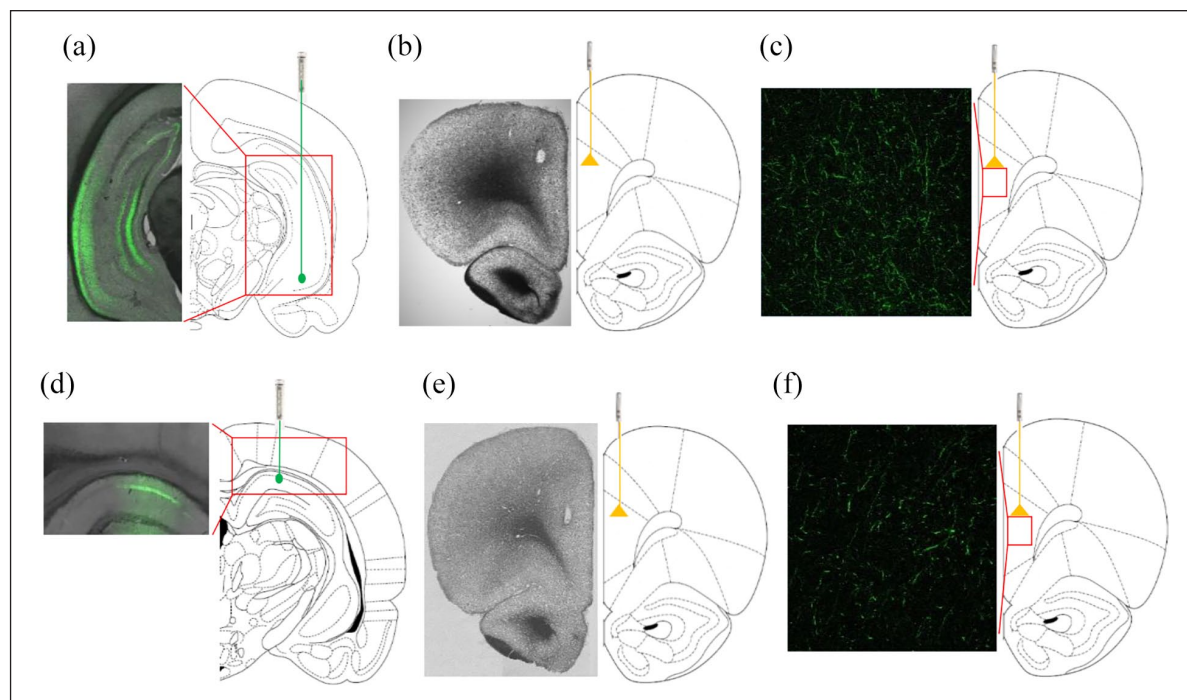
**Laser implantation.** Three (experiment 1) or two (experiment 2) weeks after virus transduction, an optical fibre (model

DFC\_200/230-0.48\_5mm\_ZF2.5(G)\_FLT, Doric Lenses Inc., Quebec, Canada) was implanted into the left vm-PFC, at the level of the prelimbic (PL) cortex (AP +3.0 mm, L +0.7 mm, DV -3.5 mm, according to the atlas of Paxinos and Watson, 1998). The implant was connected to a plastic pedestal and was fixed to the skull with dental cement (Adhesor Carboline, SpofaDental, Jicin, Czech Republic).

**Optogenetic stimulation.** Beginning 2 weeks after implantation of the optical fibre, animals received a total of six 60-min sessions of stimulation with blue light pulses (473 nm, 15 ms light pulses at 20 Hz; 1 min on and 1 min off for 30 cycles, the same parameters as used in our previous OGS experiments: Papp et al., 2020). In unpublished preliminary experiments, we did not find stronger effects at higher frequencies of OGS.

For each session, the rats were connected to a laser source (473 nm, with power density of approximately 5 mW/mm<sup>2</sup> at the fibre tip) via fibre-optic patchcord (Ø200 µm core, 0.37 NA, Doric Lenses Inc., Quebec, Canada), fibre-optic rotary joint (Doric Lenses Inc., Quebec, Canada) and fibre-optic patchcord (Ø200 µm core, 0.22 NA, CNI Optoelectronics Tech. Co., Ltd., Changchun, China). OGS was applied in a separate room in the animals' home cages. Before OGS administration, the animals were adapted to the OGS apparatus for 5 min on 2 consecutive days.

In experiment 1, two 1-h OGS sessions were conducted before each of the final sucrose intake test, the EPM, and the exposure session (T1) in the NOR test (Figure 1(a)), one on the previous evening and one in the morning ending 15 min before the test. The stimulation parameters and the schedule of OGS administration were based on the study of Fuchikami et al. (2015)



**Figure 2.** Histology: (a) AAV5-hSyn-CHR2-EYFP virus transduction (green) in the ventral hippocampus (vHPC), (b) location of the optical fibre in prelimbic (PL) PFC, (c) AAV5-hSyn-CHR2-EYFP virus infection (green) in terminals of the vHPC–medial prefrontal cortex (mPFC) pathway following virus transduction in the vHPC at coordinates AP  $-5.3$ , L  $+5.5$ , DV  $-7.5$  (vHPC) according to the atlas of Paxinos and Watson (1998), (d) AAV5-hSyn-CHR2-EYFP virus transduction (green) in the dorsal hippocampus (dHPC) at coordinates AP  $-4.16$ , L  $+2.8$ , DV  $-2.6$  (dHPC) according to the atlas of Paxinos and Watson (1998), (e) location of the optical fibre in PL PFC, and (f) AAV5-hSyn-CHR2-EYFP virus infection (green) in terminals of the dHPC–mPFC pathway following virus transduction in the dHPC.

and our earlier study (Papp et al., 2020). Animals in experiment 2 received a total of 4 weekly 1-h OGS sessions (Figure 1(b)).

**Verification of implants and virus infection.** At the end of each experiment, animals were sacrificed by decapitation, and the correct placement of the optical fibre and effectiveness of the virus transduction were verified in frozen coronal brain sections ( $40\mu\text{m}$ ) with the use of confocal microscopy Leica TCS SP8 WLL (Leica Microsystems, Mannheim, Germany; magnification  $10\times$ ). Representative infection and placement data are shown in Figure 2.

### Behavioural procedures

**Chronic mild stress.** The CMS procedure was conducted according to a standard protocol (Papp, 2012) used in many previous studies. Briefly, after 3 weeks of habituation to laboratory and housing conditions, the animals were trained to consume a 1% sucrose solution in six baseline tests conducted once weekly in the home cage. After 14h food and water deprivation, the animals were presented with a freshly prepared 1% sucrose solution for 1 h. Sucrose intake was calculated by weighing bottles before and after the test. Subsequently, sucrose consumption was monitored once weekly, under similar conditions, until the end of the study.

On the basis of their intakes in the final pre-STR baseline test, the animals were divided into two matched groups: CON and STR. The STR animals were then exposed to a CMS procedure.

Each week of the STR regime consisted of: two periods of food or water deprivation, two periods of 45-degree cage tilt, two periods of intermittent illumination (light on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low intensity stroboscopic illumination (150 flashes/min) and three periods of no STR. The duration of all stressors was 10–14 h, and they were applied individually and continuously, day and night. The CON animals were housed in separate rooms and were deprived of food and water for 14 h before each sucrose test, but otherwise food and water were freely available.

In experiment 2, after 2 weeks of STR each group (i.e. STR and CMS) was split into subgroups, matched for sucrose consumption, which were administered different treatments under continuing CMS exposure, as described above. SAL or VEN were administered at approximately 10.00 h, and all behavioural tests were carried out 24 h following the previous drug injections.

**Elevated plus maze.** The animals were tested at random in a wooden apparatus comprising two open ( $50\text{cm} \times 11\text{cm}$ ) and two non-transparent closed ( $50\text{m} \times 11\text{m} \times 40\text{cm}$ ) arms, elevated 50 cm above the floor and illuminated by two 25 W bulbs located beneath the open arms. The animals were placed individually in the centre of the apparatus, and the number of entries of all four feet into open and closed arms and the time spent in each arm were recorded manually in a 5-min test by a trained observer who was blind to treatments. Two rats were observed simultaneously via a mirror located 1.5 m

above the apparatus. A % open-arm entries (%OE) score was calculated according to the following formula: number of open-arm entries divided by total number of entries multiplied by 100. The apparatus was cleaned with warm water between animals.

**Novel object recognition test.** The animals were tested at random in an opaque circular wooden open field (100 cm in diameter, 35 cm high, floor divided into painted 16-cm squares). After a period of 2 days adaptation (10 min daily), the animals were allowed to explore two identical cylinder-shaped white objects (7 cm in diameter, 11 cm high) for the time required to complete 15 s of exploration of both objects (T1 session). In the retention trial (T2 session) conducted 1 h later, one of the objects presented previously was replaced by a novel prism-shaped black object (5 cm wide, 14 cm high). The objects were placed 50 cm apart and 20 cm from the wall, and their right/left location was counterbalanced in a semi-random order. Rats were returned to the open field for 5 min, and the duration of exploration of each object (i.e. sitting in close proximity to the objects, sniffing or touching them) was recorded by a trained observer who was blind to treatments. Two rats were observed simultaneously via a mirror located 1.5 m above the apparatus. A NOR index was calculated according to the following formula: time of novel object exploration minus time of familiar object exploration, divided by total exploration time (novel plus familiar objects). During the test session the number of line crossings was also recorded as a measure of locomotor activity. The apparatus and objects were cleaned with warm water between animals.

### Statistical analysis

The effects of acute vHPC OGS were analyzed by two-way analysis of variance (ANOVA; SHAM vs. OGS; CON vs. CMS).

Four considerations guided the analysis strategy for the chronic study:

1. The design of the study was unbalanced, with a single OGS CON group serving for both vHPC and dHPC OGS STR groups, making the study unsuitable for overall factorial analysis of variance (ANOVA).
2. Factorial ANOVA was also unsuitable for the overall data set because it would not test the main comparison of interest, the effect of the combination of OGS + VEN relative to untreated (SAL + SHAM) animals;
3. We had a set of specific predictions regarding the reversal of CMS effects that VEN alone would be ineffective (based on previous studies), and repeated OGS alone would not be effective (based on the results of the acute study: below), but the combination of VEN and repeated vHPC–mPFC OGS would reverse CMS effects. (We were agnostic with respect to the combination of VEN with dHPC OGS.)
4. The critical question was whether the combination of VEN and vHPC–mPFC OGS would return behaviour to levels seen in untreated (SAL + SHAM) animals, creating a risk of type 2 but not type 1 errors.

We therefore first used a series of *t*-tests for each of the three experimental variables that we expected would be impaired by CMS (sucrose intake at week 6; % open-arm time on the EPM; recognition index in the NOR test), examining the effect of STR

versus CON in each of the six experimental conditions (using the same CON group for each of the vHPC and dHPC STR groups). In order to guard against type 2 errors, we adopted a conservative criterion of  $p > 0.2$  for a nonsignificant effect. We also examined the effect of all treatments on three further variables that are typically unaffected by CMS, also using *t*-tests. (Although there was a potential risk of type 1 error in these analyses, in practice all were nonsignificant.)

We next examined the effect of CMS (STR vs. CON) in the vHPC combination group (vHPC–mPFC OGS + VEN) versus the no treatment group (SAL + SHAM) by means of two-way ANOVAs on data from the EPM (% open-arm time) and NOR test (recognition index), and a three-way ANOVA, including the repeated-measures factor Weeks of treatment, for the sucrose intake data, with planned comparisons to test the a priori hypotheses.

Finally, in light of the results obtained, we conducted exploratory analyses of the effects of dHPC–mPFC OGS on the three variables of interest (two-way ANOVA: CON vs. STR, OGS vs. SHAM).

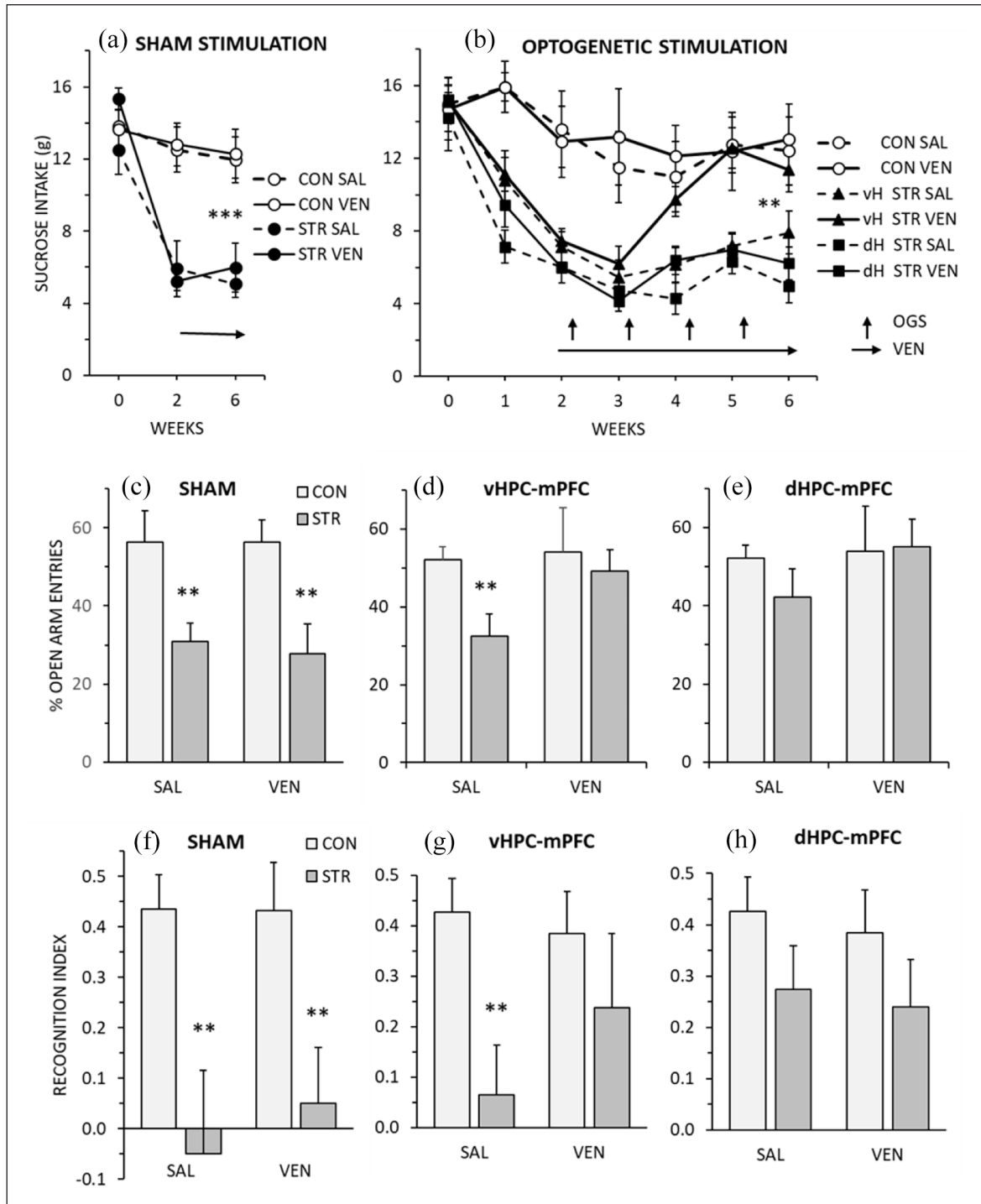
## Results

In both experiments, chronic exposure to CMS caused a decrease in sucrose consumption, open-arm entries on the EPM, and NOR memory, as observed in many previous studies (Figure 3, Supplemental Table S1). Acute OGS of the vHPC–mPFC pathway (experiment 1) had no effect on any of these measures (Supplemental Figure S1).

### *Repeated optogenetic stimulation of the vHPC–mPFC pathway enables an antidepressant response in antidepressant-refractory WKY rats*

Both chronic administration of VEN in sham-stimulated animals and repeated OGS of the vHPC–mPFC pathway in SAL-treated animals were ineffective in reversing the suppression of sucrose drinking by CMS (Figure 3(a) and (b); Supplemental Table S1). However, in VEN-treated STR animals, repeated OGS of the vHPC–mPFC pathway caused a gradual restoration of sucrose intake to control levels (Figure 3(b): three-way interaction:  $F(4,128)=2.83$ ,  $p < 0.05$ ; weeks  $\times$  treatments interaction in STR animals,  $F(4,72)=6.36$ ,  $p < 0.001$ ). Sucrose intake was significantly elevated in treated STR animals relative to the SAL-SHAM group in weeks 4, 5 and 6 of STR (i.e. weeks 2, 3 and 4 of treatment) ( $t(18)=4.69$ ,  $5.46$ ,  $3.78$ ;  $p < 0.001$ ), with no significant difference between combination-treated stressed animals and their respective non-stressed CONs (week 6:  $t(18)=0.764$ ,  $p=0.455$ ).

Similarly, neither chronic VEN alone nor repeated OGS alone reversed the anxiogenic effect of CMS on performance in the EPM (Figure 3(c) and (d); Supplemental Table S1), but performance was restored to control levels in stressed animals treated with the combination of chronic VEN and repeated vHPC–mPFC OGS (Figure 3(d)). The two-way interaction was nonsignificant ( $F(1,44)=2.55$ ), but a planned comparison showed that the effect of the combination treatment in STR animals was significant relative to the no-treatment group ( $t(18)=2.31$ ,  $p < 0.05$ ).



**Figure 3.** Effects of chronic venlafaxine (VEN) and repeated optogenetic stimulation (OGS), alone and in combination, on sucrose intake (a and b), open-arm entries on the elevated plus maze (d and e) and novel object recognition (f-h), in control (CON) and stressed (STR) groups. Animals received either sham stimulation in the medial prefrontal cortex (mPFC) (a, c and f), or OGS of terminals in mPFC (b, d, e, g and h), following viral transfection in either ventral hippocampus (vHPC) or dorsal hippocampus (dHPC). The effects of vHPC-mPFC and dHPC-mPFC OGS are shown together in B and separately in (d, e, g and h), which show the same non-STR CON groups. Horizontal arrows: daily VEN treatment; vertical arrows, weekly OGS. \* $p < 0.05$ , \*\* $p < 0.02$  and \*\*\* $p < 0.002$  for effects of STR versus CON. (In (b) the \*\* at week 6 is illustrative: the actual significance levels were \* $p < 0.05$  for the vHPC-OGS-SAL group and \*\*\* $p < 0.002$  for the two dHPC-OGS groups: see Supplemental Table S1.) The combination of vHPC-mPFC OGS and VEN overcame the effect of STR in all of the sucrose intake (b), EPM (d) and NOR (g) tests. dHPC-mPFC OGS reversed the effect of STR in the EPM (e) and NOR (h) tests in both SAL- and VEN-treated animals, but had no effect on sucrose intake (b) in either group.

Very similar results were seen in the NOR test (Figure 3(f)–(h)): the suppression of object recognition memory by CMS was unaffected by chronic VEN alone or repeated vHPC–mPFC OGS alone (Figure 3(f) and (g); Supplemental Table S1), but was restored to control levels by the combination treatment (Figure 3(g)). In this case, the two-way interaction was nonsignificant ( $F(1,44)=2.0$ ) as was the difference between treated and non-treated STR animals, but planned comparisons showed that when the non-treated group showed a pronounced effect of STR ( $t(18)=2.72, p < 0.02$ ), the treated group did not differ significantly from non-STR CONs ( $t(18)=0.756, p=0.46$ ).

Neither VEN nor OGS, alone or in combination, significantly affected either closed-arm entries, a measure of locomotor activity, or exploratory and locomotor activities in the NOR test (Supplemental Figure S2 and Table S2).

### *Repeated optogenetic stimulation of the dHPC–mPFC pathway reverses some effects of CMS but does not interact with antidepressant drug treatment*

Repeated OGS of the dHPC–mPFC pathway, either with or without concomitant chronic VEN treatment, did not elicit a recovery of sucrose intake in STR animals (Figure 3(b); Supplemental Table S1). However, OGS did reverse the effects of CMS in the EPM (Figure 3(e)) and the NOR test (Figure 3(h)) (OGS  $\times$  STR interaction:  $F(1,68)=4.97$  and  $4.25, p < 0.05$ ). These effects were seen equally in SAL- and VEN-treated animals.

OGS of the dHPC–mPFC pathway did not affect either closed-arm entries on the EPM, or exploratory or locomotor activities in the NOR test (Supplemental Figure S2 and Table S2).

## Discussion

The acute experiment established that unlike OGS of the identical site in the mPFC (Papp et al., 2020), acute OGS of the vHPC–mPFC pathway did not elicit an antidepressant-like response. This is in some respect a curious result because HPC afferents to mPFC are glutamatergic and AMPA receptor mediated (Jay et al., 1992; Parent et al., 2010), so might be expected to resemble the effects of other glutamatergic manipulations (DBS, OGS, CX-516: Papp et al., 2020) acting via mPFC AMPA receptors at the same site. It is likely that stimulation of vHPC afferents provides a weaker burst of glutamate release, relative to cell-body stimulation by OGS or DBS of mPFC. Alternatively, the restricted subset of cells activated by HPC afferents (Figure 2(c)) may differ from those responsible for the antidepressant-like effects of OGS of mPFC.

Repeated weekly OGS of HPC afferents to mPFC, with the final stimulation a week before the behavioural tests also failed to elicit antidepressant-like effects. By contrast, combined treatment with repeated OGS of vHPC afferents did elicit an antidepressant-like effect in VEN-resistant WKY rats. The effect cannot be explained by light stimulation per se because this was identical in all animals: they differed only in the viral construct expressed in HPC afferent terminals (EYFP in sham animals) or the source of infection (vHPC or dHPC). The effects were seen in

all three behavioural tests, and the effects on sucrose intake in particular (Figure 3(a) and (b)) show a remarkable specificity.

Repeated OGS of dHPC afferents to mPFC had anxiolytic (EPM) and pro-cognitive (NOR test) effects in animals subjected to CMS, but there was no interaction with VEN, and no restoration of sucrose intake. The effect on object recognition is perhaps less surprising than the effect on anxiety. The dHPC is known to be involved in aspects of learning and memory, albeit its role in NOR specifically is less certain. An early study reported that NOR was disrupted by lesions to the vHPC, but not by lesions restricted to the dHPC (Broadbent et al., 2004). However, although there are some exceptions (e.g. Moreno-Castilla et al., 2017; Yu et al., 2018), a significant body of studies has found that performance in the NOR test can be affected by pharmacological or chemogenetic manipulation of dHPC (Baker and Kim, 2002; Cohen and Stackman, 2015; Cohen et al., 2013; Gross et al., 2021; Hammond et al., 2004; Sánchez-Sarasa et al., 2021; Tuscher et al., 2018). Thus, there is a basis for understanding the effect of dHPC OGS to reverse the suppressive effect of CMS on NOR. But this is less true for the reversal of CMS-induced anxiogenesis by dHPC OGS. The weight of data shows an involvement of vHPC but not dHPC in anxiety (Bannerman et al., 2004; Barkus et al., 2010). And while there is evidence of dHPC involvement in some anxiety tests, they do not include a first trial on the EPM (Abela et al., 2020; File et al., 2000), though again, there are some exceptions (e.g. Solati et al., 2010; Zhang et al., 2014). In brief, although the effect of dHPC to mPFC OGS in the NOR test is compatible with the results of earlier studies, the effect on performance in the EPM is somewhat unexpected. Both effects remain to be explained.

There is a more reliable context within which to understand the effects of OGS of vHPC afferents to mPFC. STR activates the amygdala, which exerts excitatory control over the hypothalamic–pituitary–adrenal (HPA) axis, causing an increase in circulating glucocorticoids, which act in a positive feedback manner to further stimulate the amygdala (Duvarci and Pare, 2007). The HPC holds this system in check by exerting inhibitory control over the amygdala and the HPA axis. But under conditions of chronic STR the abnormally high levels of circulating glucocorticoids cause neurotoxic damage to the HPC, resulting in decreased inhibitory control of the HPA axis and further neurotoxicity (de Kloet et al., 2005; Holsboer and Ising, 2010; Willner et al., 2013). It is well established that the vHPC is the region of the brain that is most susceptible to STR-induced neurotoxicity, reflecting the fact that dentate gyrus has the highest density of glucocorticoid receptors. Through a variety of cellular mechanisms, prolonged exposure to STR (Magarinos et al., 1996) or high levels of glucocorticoids (Woolley et al., 1990) causes atrophy of apical dendrites in the dentate gyrus, and ultimately, granular cell death (Sapolsky, 2000). Exposure to CMS is sufficient to cause loss of granule cells (Jayatissa et al., 2008, 2010). STR also causes a profound suppression of neurogenesis, involving both the early cell proliferation phases, and the later phases of neuronal survival and the genesis of functional synapses (Tanti and Belzung, 2013a, 2013b; Wong and Herbert, 2004).

These cellular effects of STR have visible morphological consequences: meta-analyses of structural brain imaging studies have found a reliable decrease in hippocampal volume in patients suffering from major depression (Campbell et al., 2004; Videbech and Ravnkilde, 2004), comparable to the decrease seen in

Cushing's disease (Sapolsky, 2000), and the same effect is seen in rats exposed to CMS (Delgado y Palacios et al., 2011). The major factor in these volumetric changes, in both preclinical studies of prolonged STR exposure (Tata and Anderson, 2010) and post-mortem studies of depressed patients (Czeh and Lucassen, 2007), is thought to be changes in hippocampal morphology and loss of dendrites. After recovery from depression, patients with a history of recurrent depression continue to show a decreased hippocampal volume (Cole et al., 2010; Neumeister et al., 2005). The degree of hippocampal shrinkage in depressed patients is directly proportional to the number and duration of prior depressive episodes, and to the total duration of illness, which are factors associated with resistance to antidepressant treatment (Cole et al., 2010; Colla et al., 2007; Sheline et al., 2003; Tata and Anderson, 2010). A decrease in hippocampal volume is also seen in adults who experienced childhood trauma (Heim et al., 2008; Pechtel and Pizzagalli, 2011; Vythilingam et al., 2002), another group who have a dysregulated HPA axis (McCrary et al., 2012; Raymond et al., 2018) and are resistant to antidepressant treatment (Nanni et al., 2012; Perna et al., 2021; Targum and Nemeroff, 2019). Maternally separated rats, an animal model of early life STR, show a similar HPA dysregulation when adult (Rentesi et al., 2010), with decreases in hippocampal cell proliferation and synaptic plasticity (Aisa et al., 2009). A genomic screening study comparing WKY and Wistar rats suggested that functional activity in the vHPC may be suppressed in WKY rats (Papp et al., 2019), which also show numerous neurobiological abnormalities, including decreased synaptic plasticity and HPA dysregulation (Aleksandrova et al., 2017). Taken together, these results suggest that, as in treatment-resistant patients, the antidepressant resistance of WKY rats may reflect a compromised vHPC.

While the hippocampus is the brain area that is most sensitive to the neurotoxic effects of STR, prolonged exposure to high levels of glucocorticoids can also cause damage in many other brain regions, particularly the PFC, where neurodegenerative changes include microglial activation (Hinwood et al., 2012), atrophy of pyramidal neurons (Liu and Aghajanian, 2008), dendritic retraction (Dias-Ferreira et al., 2009; McEwen, 2010; Shansky et al., 2009), suppression of cell proliferation (Czeh et al., 2007) and reduction of synaptic proteins such as PSD95, and synapsin I (Li et al., 2011). There is a strong argument that these effects in PFC may be secondary to the damage to vHPC, which, both directly and via increased activity of the amygdala, disinhibits the HPA axis leading to widespread further neurotoxicity (Leuner and Shors, 2013; Willner et al., 2013, 2014). Chronic treatment with antidepressant drugs reverses both dendritic atrophy and suppression of neurogenesis in the HPC (Bessa et al., 2009; Hanson et al., 2011; Samuels and Hen, 2011; Wiborg, 2013), as well as microglial activation, dendritic atrophy, loss of spines, suppression of cell proliferation and decreased expression of synaptic proteins in the PFC (Bessa et al., 2009; Czeh et al., 2007; Djordjevic et al., 2012; Rossetti et al., 2016; Song et al., 2019). Critically, recovery from STR-induced behavioural impairments is dependent on a restoration of neurogenesis in the vHPC (Mateus-Pinheiro et al., 2013; Santarelli et al., 2003; Surget et al., 2008, 2011), indicating the primacy of antidepressant effects in vHPC for functional recovery. Also pointing to the importance of vHPC

in antidepressant effects, a single administration of ketamine increased spine density 1 h later in the vHPC but not in the dHPC (Fraga et al., 2020).

Stress impairs not only the functioning of vHPC and mPFC, but also the functional connectivity between these two structures (Godsil et al., 2013; Jay et al., 2004), as also reported in depressed patients (Genzel et al., 2015). The importance of intact vHPC to mPFC transmission is suggested by a study showing that depression-related behavioural effects of CMS in rats were reversed by high-frequency electrical stimulation of the vHPC (Jett et al., 2015), and further data showing that optogenetic or chemogenetic activation of the vHPC to mPFC pathway elicited antidepressant-like effects in the forced swim test (Carreno et al., 2016). A similar effect of vHPC electrical stimulation on behaviour in the forced swim test has also been reported in WKY rats (Kanzari et al., 2018). Conversely, either functional disconnection or optogenetic inhibition of this pathway blocked the sustained antidepressant-like effect of ketamine in the forced swim test, which strongly suggests that increased transmission from vHPC to mPFC may be essential for the antidepressant effects of ketamine (Carreno et al., 2016). Whether this holds true of conventional antidepressants has not previously been investigated. However, there is a suggestion that this may be the case from a study showing that long-term potentiation (LTP) at mPFC synapses following tetanic stimulation of vHPC in anaesthetized rats was increased by chronic, but not acute, treatment with the selective serotonin reuptake inhibitor (SSRI) fluvoxamine (Ohashi et al., 2002).

In the present study, neither acute nor repeated weekly OGS of vHPC afferent terminals in mPFC reversed behavioural effects of CMS in VEN-resistant WKY rats. However, the combination of daily VEN and weekly OGS fully reversed the effects of CMS in behavioural tests carried out a week later, with the effect on sucrose intake apparent a week after the second OGS session. We speculate that both VEN and OGS may have slow-onset sub-threshold effects on synaptic plasticity in the mPFC, whereas the combination produces a sufficient change to affect behaviour. The concept of a threshold for antidepressant effects is pertinent because, in the situation of continued STR throughout the period of drug treatment, there is a dynamic balance between the potential restorative effect of treatment and the ongoing neurotoxic effects of concurrent chronic STR.

There is evidence that AMPA receptors in mPFC represent a common pathway for antidepressant effects of VEN in Wistar rats and of DBS or OGS in WKY rats (Papp et al., 2020). The effects may also involve dopaminergic transmission in the mPFC, as LTP is dependent on intact transmission in the mesocortical DA system, which is impaired by STR (Jay et al., 2004), whereas a DA D2 receptor antagonist blocked the behavioural recovery from CMS in VEN-treated Wistar rats (Papp et al., 2018). One potential mechanism is that VEN acts in the HPC of WKY rats to effect a partial restoration of function in the vHPC–mPFC pathway and OGS boosts the signal in mPFC to a level sufficient to restore synaptic functioning to full capacity. Alternatively, OGS of mPFC afferents may act via the indirect descending mPFC–vHPC feedback pathway to amplify the effect of VEN in the HPC, leading to a full restoration of function in the ascending pathway. Or it may be that, in WKY rats, both treatments act within the PFC, with no restoration of function in the vHPC to mPFC pathway. Such effects could represent direct drug actions



within the PFC, or a suppression of the input to mPFC from the basolateral amygdala, in both cases bypassing the HPC. However, considering that antidepressant effects of ketamine and DBS of mPFC are associated with numerous neurochemical effects in the HPC of WKY rats (Akinfiresoye and Tizabi, 2013; Papp et al., 2019; Tizabi et al., 2012), as also reported following chronic antidepressant drug treatment despite the absence of behavioural recovery (Durand et al., 1999; Jeannotte et al., 2009; Pollier et al., 2000), this third hypothesis seems unlikely.

## Conclusions

The results confirm that the antidepressant non-responsiveness of the WKY rat appears to reflect an insufficiency of transmission in the vHPC to mPFC pathway following chronic antidepressant treatment, which can be completely overcome by concurrent occasional OGS of afferent terminals in the mPFC. It remains to be established whether the behavioural recovery from CMS in antidepressant-refractory rats is mediated by full restoration of functional connectivity between vHPC and mPFC or by a synergism between partial recovery of connectivity and local actions within the mPFC – or indeed, by other interactions that do not involve restoration of vHPC–mPFC connectivity. We hypothesize that in general, antidepressant non-response reflects a failure of antidepressant treatment to elicit effects in the brain that are sufficient to cross a threshold needed to exceed ongoing neurotoxicity, preventing the initiation of processes leading to recovery. We acknowledge that we have tested only a single antidepressant drug and need to confirm the generality of the effect.

Previous studies have suggested hippocampal involvement in resistance to antidepressant drugs. This study provides definitive evidence that hippocampal insufficiency – and specifically, insufficiency of vHPC to mPFC transmission – is responsible for antidepressant non-response, and that it is possible to kick-start a process that overcomes the deficit and renders the recipient treatment-responsive.

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## ORCID iD

Mariusz Papp  <https://orcid.org/0000-0003-1282-3939>

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