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Histamine-deficient mice do not respond to the antidepressant-like effects of oleoylethanolamide

Alessia Costa [a], Claudia Cristiano [b], Tommaso Cassano [c], Cristina Anna Gallelli [d], Silvana Gaetani [d], Carla Ghelardini [a], Patrizio Blandina [a], Antonio Calignano [b], M. Beatrice Passani [e, *, 1], Gustavo Provensi [a, 1]

[a] Dipartimento di Neurofarba Universitá di Firenze, Viale Pieraccini 6, 50139 Firenze (I), Italy
[b] Dipartimento di Farmacia, Scuola di Medicina, Universitá di Napoli Federico II, Via Domenico Montesano 49, 80131, Napoli (I), Italy
[c] Dipartimento di Medicina Clinica e Sperimentale Universitá di Foggia, Via Luigi Pinto 1, 71122 Foggia (I), Italy
[d] Dipartmento di Fisiologia e Farmacologia "V. Erspamer", Sapienza Universitá di Roma, Piazzale Aldo Moro 5, 00185, Roma (I), Italy
[e] Dipartimento di Scienze della Salute Universitá di Firenze, Viale Pieraccini 6, 50139 Firenze, Italy

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ABSTRACT

It has been suggested that the bioactive lipid mediator oleoylethanolamide (OEA), a potent agonist of the peroxisome proliferator-activated receptor-alpha (PPAR-α) possesses anti-depressant-like effects in several preclinical models. We recently demonstrated that several of OEA's behavioural actions require the integrity of the brain histaminergic system, and that an intact histaminergic neurotransmission is specifically required for selective serotonin re-uptake inhibitors to exert their anti-depressant-like effect. The purpose of our study was to test if OEA requires the integrity of the histaminergic neurotransmission to exert its antidepressant-like effects. Immobility time in the tail suspension test was measured to assess OEA's potential (10 mg/kg i.p.) as an antidepressant drug in histidine decarboxylase null ($HDC^{-/-}$) mice and $HDC^{+/+}$ littermates, as well as in PPAR-$\alpha^{+/+}$ and PPAR-$\alpha^{-/-}$ mice. CREB phosphorylation was evaluated using Western blot analysis in hippocampal and cortical homogenates, as pCREB is considered partially responsible for the efficacy of antidepressants. Serotonin release from ventral hippocampi of $HDC^{+/+}$ and $HDC^{-/-}$ mice was measured with in-vivo microdialysis, following OEA administration. OEA decreased immobility time and increased brain pCREB levels in $HDC^{+/+}$ mice, whereas it was ineffective in $HDC^{-/-}$ mice. Comparable results were obtained in PPAR-$\alpha^{+/+}$ and PPAR-$\alpha^{-/-}$ mice. Microdialysis revealed a dysregulation of serotonin release induced by OEA in $HDC^{-/-}$ mice.

Our observations corroborate our hypothesis that brain histamine and signals transmitted by OEA interact to elaborate appropriate behaviours and may be the basis for the efficacy of OEA as an antidepressant-like compound.

1. Introduction

Oleoylethanolamide (OEA) is an endocannabinoid analogue with multiple, diverse effects on peripheral organs and the brain, mainly mediated by the activation of peroxisome proliferator-activated receptor-α (PPAR-α) (Fu et al., 2005; Schwartz et al., 2008). Recently it was reported that administration of OEA is effective in a mouse model of depression by using several protocols (Jin et al., 2015). The anti-depressant effect of OEA seems to be related to the regulation of BDNF in the hippocampus and cortex (Jin et al., 2015) and to increased levels of serotonin and norepinephrine in brain homogenates (Yua et al., 2015). A selective agonist of PPAR-α as well produced anti-depressant-like activity by promoting pCREB/BDNF signaling cascade in the hippocampus and medial prefrontal cortex (Jiang et al., 2015). Furthermore, recent evidence reports that OEA exerts neuroprotective effects, prevents a depressive-like behaviour after ethanol binge administration (Antón et al., 2017), and participates in the control of reward-related behaviour (Bilbao et al., 2013).

We recently demonstrated that the activation of the brain histaminergic system is necessary for the full expression of some

* Corresponding author.
E-mail address: beatrice.passani@unifi.it (M.B. Passani).
[1] Equal contributor.

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behavioural effects of OEA. The synthesis of this lipid mediator is driven by the nutritional status in a tissue specific manner (Fu et al., 2007) and serves as a hypophagic signal by indirectly activating central oxytonergic (Gaetani et al., 2010; Romano et al., 2013, 2017) and histaminergic (Provensi et al., 2014; Umehara et al., 2016) neural pathways. Also, we found that the activation of histaminergic afferents to the amygdala has a permissive role for the memory-enhancing effects of OEA (Provensi et al., 2017). Histaminergic neurons are confined to the posterior hypothalamus and innervate virtually the whole brain and partake in the regulation of homeostatic and cognitive processes by selective activation of specific circuits according to the required response (Blandina et al., 2012; Fujita et al., 2017; Munari et al., 2013). Preclinical studies conducted in our laboratory demonstrated that an intact histaminergic neurotransmission is specifically required for selective serotonin re-uptake inhibitors (SSRI) such as citalopram and paroxetine to exert their anti-depressant-like effect, as shown by reduced immobility of mice in the tail suspension test (Munari et al., 2015). The role of histamine and its receptors in animal models of depression has been addressed recently (Bahi et al., 2014) implicating the disinhibition of histamine release in the hippocampus as a mechanism of antidepressant-like effects of the histamine H_3 receptor antagonists clobenpropit (Femenía et al., 2015). Indeed, dysfunctions of the hippocampal formation such as hypermetabolism (Fitzgerald et al., 2008) or volume reduction (Posener et al., 2003) have been associated with depressive symptoms. Furthermore, previous studies demonstrated that several forms of stress reduce CREB and BDNF levels in the hippocampus and medial prefrontal cortex (mPFC), whereas chronic antidepressant treatment reverses these molecular changes (Blendy, 2006; Castrén and Rantamäki, 2010; Razzoli et al., 2011).

Given this premise and the mounting evidence of an interplay between histamine and OEA, we supposed that the latter required the integrity of the histaminergic neurotransmission to exert its antidepressant-like effects. We addressed this question by using genetically modified mice that do not produce histamine, (histidine-decarboxylase null mice, HDC$^{-/-}$) and their wild type littermates (HDC$^{+/+}$), or pharmacologically silencing HDC with intracerebral infusion of the irreversible HDC inhibitor α-fluoromethylhistidine (α-FMHis, Garbarg et al., 1980) and subjecting them to the TST. We also tested the efficacy of OEA in PPAR-α$^{-/-}$ mice to understand the participation of this receptor in the behavioural effects of OEA. We adopted both a repeated and sub-chronic protocol of OEA administration and, as a neurochemical readout, we measured CREB phosphorylation in the hippocampi and frontal cortices of experimental animals. Finally, prompted by the observation that OEA restores serotonin and noradrenaline levels in stressed mice (Yua et al., 2015), we measured extracellular levels of serotonin in the hippocampi of HDC$^{-/-}$ and wild type littermates sub-chronically treated with OEA or vehicle.

2. Experimental procedures

2.1. Animals

All animals were housed in macrolon cages in temperature-controlled rooms (20–24 °C), allowed free access to food and water, and kept on a 12-h-light/-dark cycle (light started at 7:00 a.m.). Male.

CD1 mice (25–30 g body weight, Harlan, Italy) were used along with male inbred HDC$^{+/+}$ and HDC$^{-/-}$ mice of 11–13 weeks of age and bred in the Centre for Laboratory Animals, Universitá di Firenze (I) and housed in a dedicated room. The genotype of HDC$^{-/-}$ mice was determined according to the polymerase chain reaction (PCR) protocol described by Provensi et al. (2014).

Male PPAR-α$^{+/+}$ and PPAR-α$^{-/-}$ (B6.129S4-SvJae-Ppar-atm1Gonz) mice (25–30 g body weight, Jackson Laboratories), were bred in the animal facility of the Dipartimento di Farmacia, Universitá di Napoli Federico II, and the colony was established and maintained by heterozygous crossing. Mice were genotyped as described on the supplier webpage (http://jaxmice.jax.org), using the RedExtract kit (Sigma–Aldrich, Italy).

All the experiments were performed in strict compliance with the EEC recommendations for the care and use of laboratory animals (2010/63/EU) and were approved by the Animal Care Committee of the Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, Sezione di Farmacologia e Tossicologia, Universitá di Firenze (I). Ethical policy of the Universitá di Firenze complies with the Guide for the Care and Use of Laboratory Animals of the Council Directive of the European Community (2010/63/EU) and the Italian Decreto Legislativo 26 (13/03/2014). Every effort was made to minimize animal suffering and to reduce the number of animals used. Animals were handled for at least 4 days before experiments begun, to let them acclimatise to human contact.

2.2. Tail suspension test

The Tail suspension test (TST), which is widely used to assess the antidepressant activity of compounds, was carried out as previously described (Munari et al., 2015). Briefly, 1 h after the last i.p. injection of saline or drugs, mice were individually suspended by the tail to a horizontal ring stand bar (distance from floor = 30 cm) using adhesive tape (distance from tip of tail = 2 cm). Usually, mice demonstrated several escape-oriented behaviours interspersed with brief bouts of immobility. A 4-min test session was scored by a trained observer who was unaware of the treatment and genotype. Seconds spent immobile were recorded and quantified by an experimenter unaware of the treatment. All experimental sessions were carried out between 9:00 a.m. and 2:00 p..m.. Mice were randomly assigned to treatment conditions and tested in a counterbalanced order. In the repeated treatments, OEA (5 and 10 mg/kg) or imipramine (10 mg/kg) were administered i.p. 24, 5 and 1 h before the TST, for a total of 3 injections. In sub-chronic treatments, compounds were injected i.p. once a day for 7 consecutive days and 1 h before exposure to the TST on the 8th day. Control animals were injected with vehicle following the same schedule.

2.3. Locomotor activity

Mice were placed in a 55 cm × 60 cm plastic arena free to explore the environment for 5 min. Locomotion was recorder with a camera and the distance travelled was measured on line using the Smart 2.5 software. The tests were carried out between 9:00 am and 2:00 p.m. The next day the same mice were exposed to the TST.

2.4. Surgical procedures

To infuse α-FMHis i.c.v., mice were anesthetized with 5% isoflurane in humidified O_2 and positioned in a stereotaxic frame (Stoelting) according to Provensi et al. (2016). A stainless-steel cannula (7 mm in length, outer diameter 0.5 mm, and inner diameter 0.25 mm) was implanted in the lateral ventricle and fixed to the skull using dental cement. The following coordinates were used according to the mouse brain atlas (Paxinos and Franklin, 1997): AP -0.3; L ± 1; DV -1. After 7 days of recovery, α-FMHis was infused into the ventricle. A stainless-steel injection microneedle was connected through a polyethylene catheter to a 1000-μL Hamilton precision syringe and then lowered into the lateral cerebral ventricle (dorsoventral, DV 2.4 mm). α-FMHis was

delivered via an infusion pump (5 μL) within 5 min. After infusion, the needle was left in place for an additional minute. For microdialysis experiments mice were implanted with one guide cannula in the ventral hippocampus (vHIPP) according to the following coordinates from bregma (Paxinos and Franklin, 1997): AP = −3.0, L = +3.0, DV = −1.8. A surgical screw served as an anchor, and the cannula was fixed to the skull with acrylic dental cement.

2.5. Microdialysis

Microdialysis was performed 48 h after surgery to allow for mice recovery, housed one per cage. After removal of the stylet from the guide cannulae, the microdialysis probes (CMA/7 7/2 Cuprofane; molecular mass cutoff 6000 Da; CMA Microdialysis) were inserted and the dialyzing membrane protruded 2 mm from the tip of the cannula. Probes were perfused with Krebs–Ringer phosphate (KRP) buffer at a flow rate of 1 μL/min using a microperfusion pump (Mod CMA/100; Carnegie Medicine, Stockholm, Sweden). The constituents of the buffer were (in mM) 147 NaCl, 2.4 $CaCl_2$, 4.0 KCl, pH 7.0, containing 0.5 μM citalopram to improve 5-HT detectability. In our protocol, OEA was sub-chronically administered (10 mg/kg i.p., once a day for 8 days) and, 24 h after the last OEA injection, the microdialysis probe was inserted into the cannula and 30-min dialysates were collected after a 2 h stabilization period, in order to reach a steady state levels of the extracellular basal release of the 5-HT in the vHIPP of all mice. Spontaneous release was defined as the average value of the first four-30 min fractions collected during KRP solution perfusion. All subsequent fractions were expressed as pg/ml.

2.6. Determination of 5-HT in the ventral hippocampus

The dialysates were kept at −80 °C until analysis. HPLC was performed as described (Munari et al., 2015). Briefly, 5-HT levels were assayed by microbore HPLC using a SphereClone 150-mm × 2-mm column (3-μm packing). Detection was accomplished with a Unijet cell (BAS) with a 6-mm-diameter glassy carbon electrode at +650 mV vs an Ag/AgCl reference electrode connected to an electrochemical amperometric detector (INTRO, Antec Leyden, Netherlands). The chromatographic conditions were: (1) a mobile phase composed of 85 mM of sodium acetate, 0.34 mM Ethylenediaminetetraacetic acid (EDTA), 15 mM sodium chloride, 0.81 mM of octanesulphonic acid sodium salt, 6% methanol (vol/vol), pH = 4.85; (2) a rate flow of 800 μL/min; and (3) a total runtime of 35 min. A set of standards containing various concentrations of the analyte was prepared in 0.1 M percloric acid solution. The calibration curves were analysed by linear regression and the retention times of standards were used to identify peaks; peak areas were used to extrapolate neurotransmitters levels, calculated as pmol/30 min.

2.7. Western-blotting analysis

The analysis was performed according to Munari et al. (2015). Hippocampi and cortices were homogenized in 0.2 mL ice-cold lysis buffer (50 mM TrisHCl, pH 7.5, 1 M NaCl, 100 mM ethylene glycol tetraacetic acid, 50 mM Ethylenediaminetetraacetic acid, 100 mM Sodium Pyrophosphate, 40 mM Para-Nitrophenylphosphate, 100 mM Na3VO4, 57 mM Phenyl-methyl-sulphonyl fluoride, 2 mg/mL leupeptin, 1.7 mg/mL aprotinin, 10% SDS) using a pestle, sonicated briefly, and centrifuged at 12 000 rpm at 4 °C for 15 min. The supernatant was collected and protein concentration was determined by Pierce BSA (Thermo Scientific). Samples were diluted in a mix of lysis buffer and loading buffer 2× (1 M TrisHCl, pH 6.8, 100 mM DL-dithiothreitol (DTT), 10% glycerol, 1% bromophenol blue, and 2% sodium dodecyl sulphate (SDS)) and boiled for 10 min at 95 °C. Aliquots containing 40 μg total proteins were separated on 8% (SDS)-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Blots were blocked in Tris-buffered saline, pH 7.6, containing 0.1% of Tween 20 (TBS-T) and 5% skimmed milk (Bio-Rad Laboratories) for 2 h at room temperature and then incubated overnight, on different days, with monoclonal antibodies against pCREB (pCREB-Ser133) (1:1000, catalogue no. 9198) and CREB (1:1000, catalogue no. 9197, both from Cell Signaling Technology), that were dissolved in TBS-T with 5% bovine serum albumin. Membranes were then washed 3 times with TBS-T and incubated for 120 min at room temperature in TBS-T with 1% skimmed milk containing anti-rabbit peroxidase-conjugated secondary antibody (1:5000, catalogue no. 7074, Cell Signaling Technology). After washing in TBS-T 3 times, enhanced chemiluminescence reaction (Luminata Crescendo, Millipore) was used to visualize the peroxidase-coated bands. The bands were quantified by densitometry analysis using an ImageQuant 350 imager and ImageQuant TL software (Perkin Elmer). pCREB densities were divided by their respective CREB densities within each sample to obtain pCREB/CREB ratio values and were averaged for each treatment group.

2.8. Chemicals

α-FMHis (synthesized at Johnson & Johnson Laboratories, a kind gift of Dr. Nicholas Carruthers) was dissolved in saline. OEA (Tocris Bioscience, UK) was dissolved in saline/polyethylene glycol/Tween80 (90/5/5, v/v), whereas imipramine (Sigma) was dissolved in saline. All other reagents and solvents were of high performance liquid chromatography (HPLC) grade or the highest grade available (Sigma).

2.9. Statistical analysis

Statistical significance for immobility time and Western blot analyses was determined using two-way ANOVA followed by Bonferroni's post hoc test. Microdialysis data were analysed by two-way ANOVA for repeated measures, with “time” and “treatment” as the two factors, followed by Dunnett's and Bonferroni's post hoc test for multiple comparisons. 5-HT basal values calculated as marginal means of the first four dialysates samples were statistically analysed by two-way ANOVA, with “genotype” and “treatment” as the two factors. Bonferroni's post hoc test was used as a post hoc test to perform multiple comparisons. The level of significance was set at $p < 0.05$. Values that were outside the mean ± two deviation standards were discarded.

3. Results

3.1. Effect of OEA on the tail suspension test

We assessed the possible antidepressant-like effect of repeated or sub-chronic OEA treatment in the TST response of $HDC^{-/-}$ mice and their wild type littermates $HDC^{+/+}$. Imipramine was used a positive control. Fig. 1A shows the effect of different doses of OEA (5 and 10 mg/kg) administered 3 times in 24 h. We adopted this protocol previously (Munari et al., 2015) because three injections of the antidepressant citalopram during 24 h were shown to be sufficient to produce long-lasting neurochemical changes that are commonly observed with chronic administration of antidepressants (Mombereau et al., 2010). OEA decreased immobility time in $HDC^{+/+}$ mice at both doses used, whereas it was ineffective in $HDC^{-/-}$. Data were subjected to a 2-way ANOVA to examine the effect of genotype and repeated OEA treatment on immobility time.

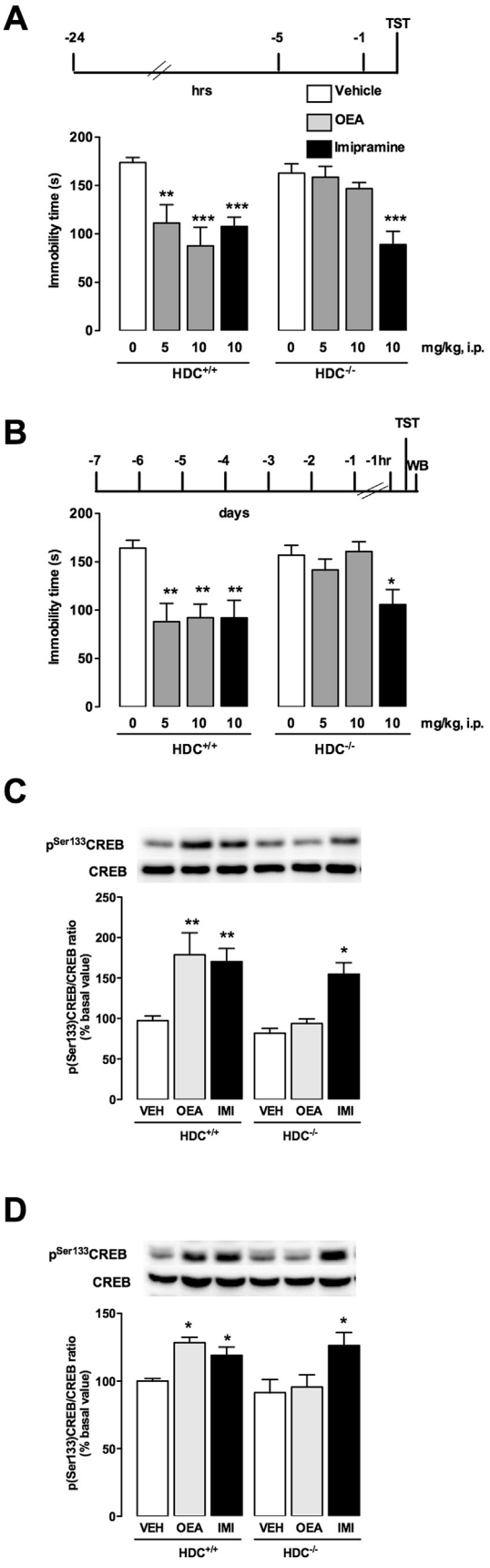

Fig. 1. Behavioural and neurochemical effects of OEA in mice genetically deprived of histamine. Schematic representations of the protocols used to evaluate vehicle, OEA or imipramine effects on the tail suspension test (TST) are shown at the top of panels A (repeated) and B (subchronic). Vehicle (VEH), OEA or imipramine were delivered i.p. 1 hr before the TST. In both protocols, OEA reduced immobility time of $HDC^{+/+}$ but not of $HDC^{-/-}$ mice. The tricyclic antidepressant imipramine decreased immobility time of both genotypes. Shown are means ± SEM of 8–12 mice for each group; *P < 0.05, **P < 0.01 and ***P < 0.001 vs VEH within each genotype. Subchronic administrations of OEA increased pCREB/CREB ratio in the hippocampus (C) and cortex (D) of $HDC^{+/+}$ but not of $HDC^{-/-}$ mice, as shown by Western-blot analysis. Imipramine was effective in both genotypes. Shown are means ± SEM of 8–10 mice for each group; *P < 0.05, **P < 0.01 vs VEH within each genotype.

An overall significant difference was found between groups ($F_{(genotype\ x\ treatment)\ 3,69} = 5.026$, $p < 0.001$); $F_{(treatment)\ 3,69} = 12.85$, $p < 0.0001$; ($F_{(genotype)1,69} = 4.446$ $p < 0.05$). Bonferroni's post hoc test showed that both doses of OEA significantly decreased the immobility time of $HDC^{+/+}$ mice exposed to the TST (5 mg/kg, $p < 0.01$; 10 mg/kg, $p < 0.001$), whereas no significant effects were observed in $HDC^{-/-}$ mice. Imipramine decreased the immobility time of both genotypes ($HDC^{+/+}$ and $HDC^{-/-}$, $p < 0.001$; Fig. 1A). We also investigated the effect of OEA on the TST in CD1 mice that received i.c.v. injections of α-FMHis, an irreversible HDC inhibitor, to verify if the absence of brain histamine is indeed responsible for the lack of OEA's effect in $HDC^{-/-}$mice (Fig. 2). In analogy with $HDC^{-/-}$ mice, OEA did not modify the immobility time of α-FMHis-treated mice, whereas imipramine remained effective regardless of the presence of brain histamine ($F_{(icv\ x\ treatment)\ 3,79} = 4.707$, $p < 0.01$); $F_{(treatment)\ 3,79} = 43.62$, $p < 0.0001$; $F_{(icv)\ 1,79} = 5.007$, $p < 0.05$). Significant differences for OEA treatments by Bonferroni's post hoc test were $p < 0.001$.

OEA sub-chronic treatment produced similar behavioural results. We adopted the sub-chronic administration protocol to exclude that mice developed tolerance to the behavioural effect of OEA. Either OEA or imipramine were administered i.p. for 8 consecutive days, and the last injection 1 h before the test (Fig. 1B). A 2-way ANOVA showed significant differences between groups ($F_{(genotype\ x\ treatment)\ 3,57} = 3.362$, $p < 0.05$); $F_{(treatment)\ 3,57} = 7.418$, $p < 0.001$; ($F_{(genotype)1,57} = 10.34$ $p < 0.01$). Bonferroni's post hoc analysis revealed that OEA treatment at all doses tested significantly reduced the immobility time of $HDC^{+/+}$ mice (5 mg/kg and 10 mg/kg OEA, $p < 0.01$), whereas no effect on immobility time was observed in $HDC^{-/-}$ mice. Imipramine reduced the immobility time of both genotypes ($HDC^{+/+}$, $p < 0.01$; in $HDC^{-/-}$, $p < 0.05$).

3.2. Effect of OEA on CREB phosphorylation of $HDC^{+/+}$ and $HDC^{-/-}$ mice hippocampus and prefrontal cortex

Administration of antidepressants increases CREB phosphorylation in the hippocampus, supporting the hypothesis that pCREB is at least in part responsible for the efficacy of these medications (Blendy, 2006). Using the sub-chronic regimen, we compared the effect of OEA (10 mg/kg) imipramine (10 mg/kg) and vehicle-treated $HDC^{+/+}$ and $HDC^{-/-}$ mice. Two-way ANOVA showed an overall significant difference between groups ($F_{(genotype\ x\ treatment)\ 2,21} = 4.14$, $p < 0.05$; $F_{(treatment)\ 2,21} = 14.05$, $p = 0.0001$; $F_{(genotype)\ 1,21} = 11.56$, $p < 0.05$). Bonferroni's post hoc test found significant differences between groups (Fig. 1C–D). OEA significantly increased pCREB in the hippocampus of $HDC^{+/+}$ mice compared with vehicle treated animals (Bonferroni's post hoc test $p < 0.01$; Fig. 1C). However, OEA treatment was ineffective in $HDC^{-/-}$ mice (Fig. 1C), as pCREB levels were not different from those of vehicle-treated mice. Similar to the effect in the hippocampus, OEA increased pCREB levels in the frontal cortex of $HDC^{+/+}$ mice ($p < 0.05$; Fig. 1D), but not in the cortex of $HDC^{-/-}$ mice (2-way ANOVA, $F_{(genotype\ x\ treatment)\ 2,24} = 3.78$, $p < 0.05$; $F_{(treatment)\ 2,24} = 6.78$, $p < 0.01$; $F_{(genotype)1,24} = 3.57$, $p = 0.07$). Imipramine administered at 10 mg/kg for 8 days increased pCREB in the

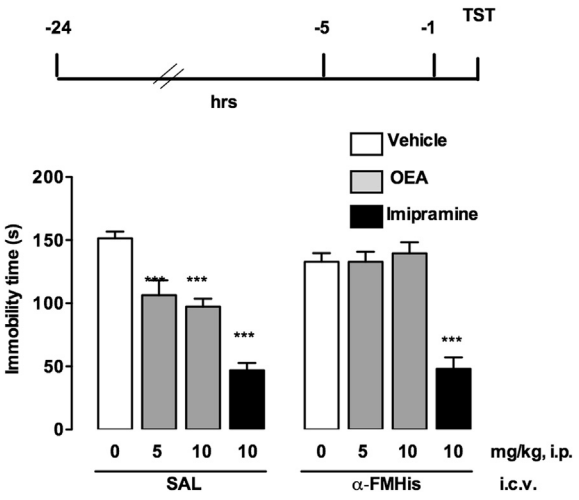

Fig. 2. Behavioural effects of OEA in mice acutely deprived of brain histamine. Schematic representation of the protocol used to evaluate saline, OEA or imipramine effects on the tail suspension test (TST) is shown at the top of the figure. CD-1 mice were injected i.c.v. with alpha-fluoromethylhistidine (α-FMHis) 24 h before the TST. Vehicle (VEH), OEA or imipramine were i.p. 1 hr before testing. Represented are means ± SEM of 8–10 mice., ***P < 0.001 vs VEH.

hippocampus and cortex of both genotypes with respect to controls ($p < 0.05$).

3.3. PPAR-α is necessary for the effects of OEA on TST and CREB phosphorylation

To explore if PPAR-α is required for the antidepressant-like effect of OEA, PPAR-$\alpha^{+/+}$ and PPAR-$\alpha^{-/-}$ mice were tested in the TST after repeated or sub-chronic OEA (10 mg/kg) treatments as shown in Fig. 3A–B. A 2-way ANOVA was conducted to examine the effect of genotype and repeated (3 × in 24 h) treatment on immobility time that revealed an overall significant difference between groups ($F_{(genotype\ x\ treatment)\ 1,29} = 5.925$, $p < 0.05$); ($F_{(treatment)\ 1,29} = 11.80$, $p < 0.01$; ($F_{(genotype)1,29} = 0.788$ $p = 0.38$). Bonferroni post-hoc test showed that OEA repeated treatment significantly decreased the immobility time of PPAR-$\alpha^{+/+}$ mice ($p < 0.001$), but not of PPAR-$\alpha^{-/-}$ mice. Qualitatively similar results were obtained with a sub-chronic treatment (1 × 8 days; Fig. 3B). There was a statistically significant interaction between the effect of genotype and treatment ($F_{(genotype\ x\ treatment)\ 1,27} = 9.147$; $p = 0.0054$), but there were no significant effects of single variables ($F_{(treatment)\ 1,\ 27} = 2.943$, $p = 0.09$; $F_{(genotype)\ 1,27} = 1.206$; $p = 0.28$). Bonferroni's post hoc analysis showed that OEA was effective at reducing immobility time only in PPAR-$\alpha^{+/+}$ ($p < 0.05$). Using the sub-chronic regimen, we compared the effect of OEA (10 mg/kg) and vehicle treatment on CREB phosphorylation in PPAR-$\alpha^{+/+}$ and PPAR-$\alpha^{-/-}$ mice. In the hippocampus, 2-way ANOVA showed an overall significant effect on interaction and treatment ($F_{(genotype\ x\ treatment)\ 1,30} = 10.9$, $p < 0.05$; $F_{(treatment)\ 1,30} = 23.39$, $p = 0.001$), but not on genotype ($F_{(genotype)\ 1,30} = 1.33$, $p = 0.4$). OEA increased significantly pCREB in the hippocampus of PPAR-$\alpha^{+/+}$ mice compared with vehicle treated animals (Bonferroni's post hoc test $p < 0.01$; Fig. 3C), whereas no effect was observed in PPAR-$\alpha^{-/-}$ mice. In the cortices two-way ANOVA showed an overall significant effect on interaction and treatment ($F_{(genotype\ x\ treatment)\ 1,29} = 95.991$ $p < 0.05$; $F_{(treatment)\ 1,29} = 4.631$, $p < 0.01$), but not on genotype ($F_{(genotype)\ 1,29} = 2.047$ $p = 0.16$). OEA increased pCREB in cortical homogenates of PPAR-$\alpha^{+/+}$ mice as revealed by Bonferroni's post hoc test ($p < 0.01$).

3.4. General motility

To exclude possible effects of the various treatments and genotypes on spontaneous locomotor activity that may have affected the immobility time in the TST, mice were exposed to the open field and motor activity recorded for 5 min. No differences were observed between experimental groups in the repeated treatment nor in the sub-chronic treatment (Fig. S1).

3.5. Functional response of the hippocampal 5-HT system of HDC$^{+/+}$ and HDC$^{-/-}$ mice

To address the impact of sub-chronic OEA treatment on the serotonergic transmission we performed a microdialysis experiment in the vHIPP of both HDC$^{+/+}$ and HDC$^{-/-}$ mice. In this experiment both the basal 5-HT tone and the impulse-driven 5-HT release were estimated by perfusing the probes first with KRP buffer (basal condition), and then with K^+-enriched KRP buffer (containing KCl 100 mM) for 30 min (stimulated condition; Fig. 4A and B). The overall basal concentrations of extracellular 5-HT (calculated as the marginal means of the first four dialysates) measured in the presence of 0.5 μM citalopram in the perfusion medium are reported in Fig. 4 C. Two-way ANOVA analysis of basal 5-HT levels in the vHIPP of HDC$^{+/+}$ and HDC$^{-/-}$ mice revealed a significant main effect of genotype ($F_{1,17} = 6.195$ $p < 0.05$), whereas no significant main effect of treatment ($F_{1,17} = 0.245$, $p = 0.629$) and interaction ($F_{1,17} = 0.493$, $p = 0.495$) was observed. Post hoc test showed that sub-chronic OEA treatment significantly decreased (−26%) the 5-HT basal output of HDC$^{-/-}$ mice compared to OEA-treated HDC$^{+/+}$ mice ($p < 0.05$; Fig. 4C). K^+-stimulation significantly increased the extracellular 5-HT release in the vHIPP of HDC$^{+/+}$ mice treated with either vehicle or OEA (two-way ANOVA for repeated measures: $F_{time\ (1,9)} = 7.012$ $p < 0.05$; $F_{treatment\ (1,9)} = 3.118$ $p = 0.121$; $F_{time\ x\ treatment\ (1,9)} = 1.104$ $p = 0.349$). In particular, 30 min after high K^+ retrodialysis administration, 5-HT reached 654 and 840 pg/ml in vehicle- (87% of basal values, $p < 0,05$) and OEA-treated (136% of basal values, $p < 0,05$) HDC$^{+/+}$ mice, respectively (Fig. 4A).

Conversely, statistical analysis of 5-HT output under stimulation condition in the HDC$^{-/-}$ mice revealed a significant main effect of time ($F_{1,8} = 7.695$ $p < 0.05$) and treatment ($F_{1,8} = 7.870$ $p < 0.05$), but not time-by-treatment interaction ($F_{1,8} = 2.039$ $p = 0.184$). Post hoc analysis (Dunnett's test) showed a greater response to K^+ stimulation in HDC$^{-/-}$ mice treated with OEA compared to the vehicle-treated HDC$^{-/-}$ mice (Fig. 4B). In fact, in the vHIPP of OEA-treated HDC$^{-/-}$ mice the 5-HT levels reached 1122 pg/ml (327% of basal values, $p < 0,05$) 30 min after the beginning of K^+-stimulation, whereas, at the same time-point, extracellular 5-HT levels peaked at 574 pg/ml (93% of basal values, $p < 0,05$) in vehicle-treated HDC$^{-/-}$ mice. Moreover, Bonferroni's post hoc test showed a statistically significant difference between vehicle- and OEA-treated HDC$^{-/-}$ mice 30 min after high K^+ retrodialysis administration ($p < 0.05$).

4. Discussion

The first intuition that OEA may have antidepressant-like properties dates back to the work by the group of Piomelli (Bortolato et al., 2007) who showed that a fatty acid amide hydrolase (FAAH) inhibitor increased the cerebral levels of the endocannabinoids anandamide, OEA and palmitoylethanolamide and corrected the alterations associated with chronic mild stress, a validated rat model of depression. The finding that OEA activates the central oxytonergic system (Gaetani et al., 2010) and the well-known role of this system in major depressive disorders (Romano

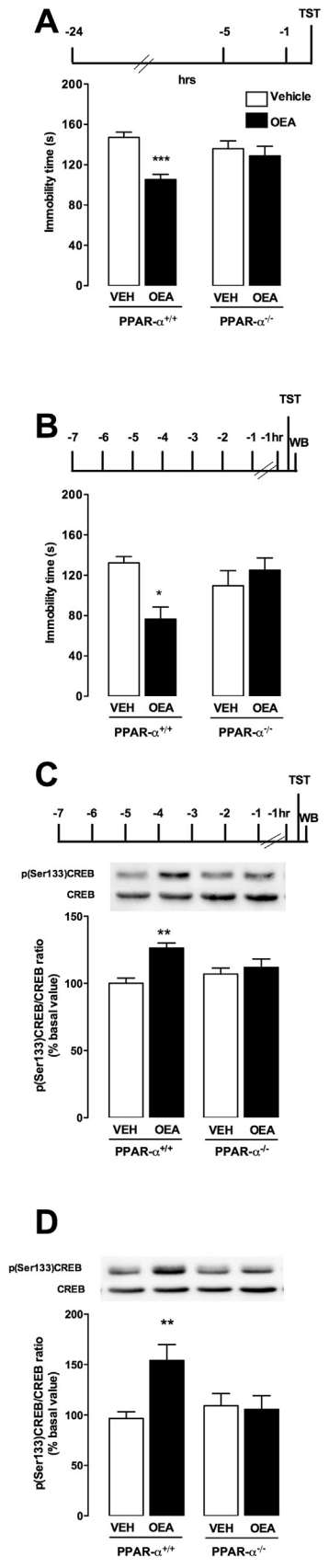

Fig. 3. Behavioural and neurochemical effects of OEA in mice genetically deprived of PPAR-α. Schematic representations of the protocols used to evaluate vehicle or OEA effects on the tail suspension test (TST) are shown at the top of panels A (repeated) and B (subchronic). Vehicle (VEH) and OEA were delivered i.p. 1 hr before the TST. In both protocols, OEA reduced the immobility time of PPAR-$\alpha^{+/+}$ but not of PPAR-$\alpha^{-/-}$ mice. Shown are means ± SEM of 7–10 mice for each group; *P < 0.05, ***P < 0.001 vs VEH within each genotype (Bonferroni's post hoc test). Subchronic administrations of OEA increased pCREB/CREB ratio in the hippocampus (C) and cortex (D) of PPAR-$\alpha^{+/+}$ but not of PPAR-$\alpha^{-/-}$ mice, as shown by Western-blot analysis. Shown are means ± SEM of 7–8 mice for each group; *P < 0.05, **P < 0.01 vs VEH within each genotype (Bonferroni's post hoc test).

et al., 2016) further supported this hypothesis.

More recently, OEA was found to decrease the immobility time of mice exposed to the TST (Jiang et al., 2015), a test that evaluates stress-coping behaviour of mice, rather than depression-associated behaviour, and to normalize several activities and neurochemical parameters affected by chronic mild stress (Jin et al., 2015). In the past years, we provided evidence of a neurobiological pathway between OEA and brain histamine (Provensi et al., 2014, 2017) and here, we examined if OEA requires the integrity of the brain histamine system to exert its antidepressant-like effect as well. We used the TST, as all antidepressants effectively reduce immobility in this test (Lucki et al., 2001), without attempting to reproduce a model of chronic stress or depression. We found that OEA decreased the immobility time of $HDC^{+/+}$ mice, but did not decrease that of $HDC^{-/-}$ mice, nor it affected the immobility time of mice with pharmacological depletion of brain histamine. Accordingly, OEA augmented pCREB in the hippocampus and cortex of $HDC^{+/+}$ mice, but not in the brain of $HDC^{-/-}$ mice. Creb phosphorylation is one of the molecular mechanisms associated to the efficacy of SSRI treatment (Carlezon et al., 2005) and is required for TST response to citalopram (Gur et al., 2007).The tricyclic antidepressant imipramine, however, was effective in both parameters irrespective of brain histamine availability. In this respect, OEA behaved exactly like SSRIs, as we earlier found that an intact histaminergic neurotransmission is required specifically for citalopram and paroxetine (but not the TCA imipramine, nor the selective NA reuptake inhibitor reboxetine) to reduce the immobility time in the TST and to modulate pCREB expression (Munari et al., 2015).

The ability to enhance serotonergic transmission is a feature common to SSRIs, therefore to assess if OEA also shares this characteristic we measured 5-HT outflow in the vHIPP, a region primarily involved in regulating stress, emotion, and anxiety (Bannerman et al., 2003), of both $HDC^{+/+}$ and $HDC^{-/-}$ mice. We found that sub-chronic OEA administrations did not enhance 5-HT outflow of $HDC^{+/+}$ compared to vehicle treated mice, as assessed by *in vivo* microdialysis. Surprisingly, though, OEA significantly decreased 5-HT basal outflow in the vHIPP of $HDC^{-/-}$ mice compared to OEA-treated $HDC^{+/+}$ mice. The basal activity of the serotonergic transmission in the hippocampus of $HDC^{-/-}$ mice treated with vehicle did not appeared different form that of $HDC^{+/+}$ mice, which is in agreement with our previous observation that $HDC^{-/-}$ mice do not show desensitization of $5\text{-}HT_{1A}$ receptors, nor insensitivity to SSRI administration (Munari et al., 2015). However, OEA-treated $HDC^{-/-}$ mice appeared 'supersensitive' to the acute K^+ challenge, with respect to $HDC^{+/+}$ mice. We cannot offer at this point an explanation for this response, nor we can assert that the dysregulation of 5-HT release induced by OEA in $HDC^{-/-}$ mice contributes to the inefficacy in the TST. Our experiments though, do not agree with the reported 5-HT increase in whole mice brain homogenates (Yua et al., 2015). However, we propose a neuronal circuitry responsible for the results reported here, based on our previous and actual observations. We previously demonstrated that OEA increases histamine release and neuronal activation in a brain region- and behavioural status-dependent manner (Provensi et al., 2014, 2017). OEA, though, does not increase c-fos expression in selected brain regions of $HDC^{-/-}$ mice except for the nucleus of the

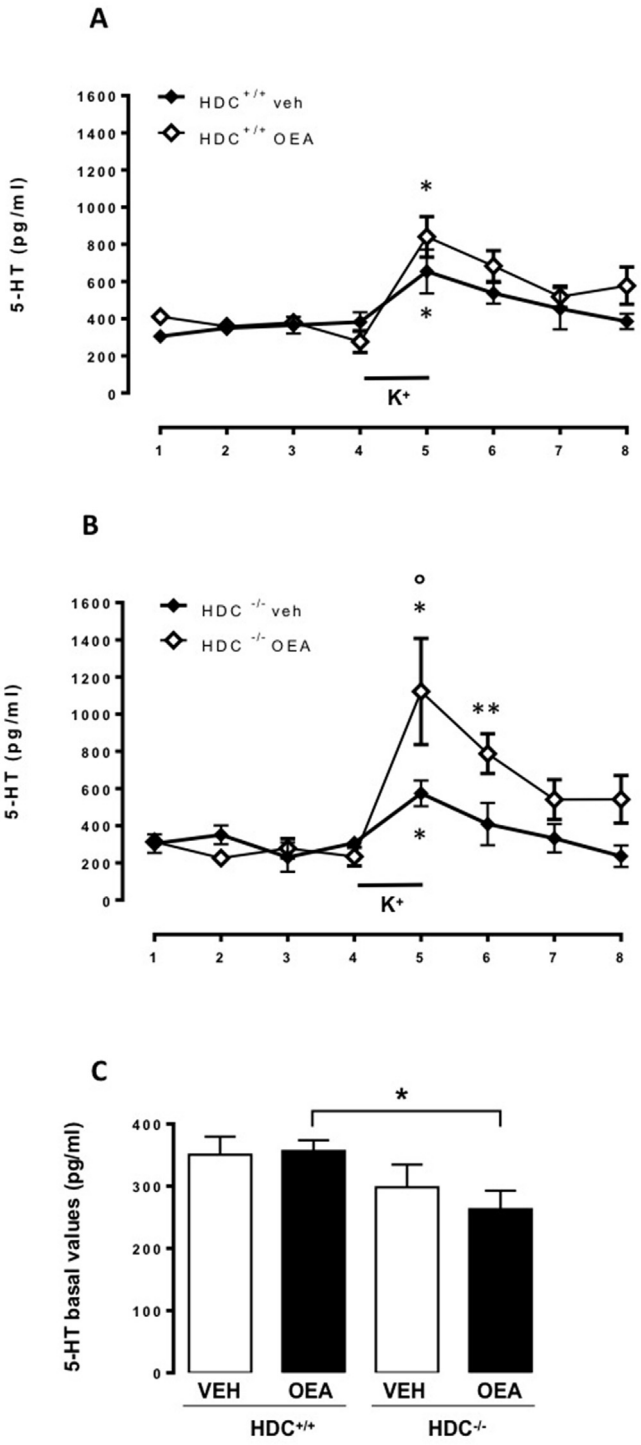

Fig. 4. Effects of vehicle (veh) or OEA administration on the basal and K^+-stimulated (horizontal black line) 5-HT extracellular levels in the vHippo of $HDC^{+/+}$ (**A**) and $HDC^{-/-}$ (**B**) freely moving mice. Data are expressed as mean ± S.E.M. *P < 0.05; **P < 0.01 versus basal mean within the same group (Dunnett's multiple comparison test). P < 0.05 versus $HDC^{-/-}$ veh mice (Bonferroni's test for between groups comparisons). Basal 5-HT extracellular levels (**C,** calculated as marginal means of the first four dialysate samples) in the vHippo of $HDC^{+/+}$ and $HDC^{-/-}$ mice treated with either vehicle or OEA during microdialysis experiment. Data are expressed as mean ± SEM. *P < 0.05 versus $HDC^{+/+}$ in the same treatment group (Tukey's test; N = 5–4).

solitary tract (NST), the primary brainstem area activated by vagal afferents relaying OEA signaling from the periphery (Umehara et al., 2016). We therefore believe that OEA-induced NST activation precedes the stimulation of the histaminergic system, strongly suggesting that in $HDC^{-/-}$ mice the NST activated by OEA sends signals to the TMN via presumably noradrenergic fibers (Provensi et al., 2014), but histaminergic neurons are not available to relay information further along histaminergic pathways. One of these pathways leads to the activation of H_1 and H_2 receptors in the hippocampus, a region responsible of the antidepressant-like responses induced by increased histamine release (Femenía et al., 2015). Here we demonstrated that in the hippocampus of $HDC^{+/+}$mice OEA increased pCREB, a feature common to other PPAR-α agonists such as fenofibrate with antidepressant like effects (Jiang et al., 2017), but not in $HDC^{-/-}$ mice, although the CREB intracellular machinery is not impaired in these animals (Munari et al., 2015). In this respect, OEA and SSRI appear to interact with the histaminergic system in a different manner; as we previously proposed (Munari et al., 2015), the inefficacy of SSRIs in the TST and CREB phosphorylation of $HDC^{-/-}$ mice could be attributed to the disruption of a raphe-TMN loop, whereas OEA appears to bypass the serotonergic system, as it does not significantly affect 5-HT release from the hippocampus of normal mice.

It has been proposed that OEA may act via other receptors, such as the vanilloid receptor TRPV1 (Wang et al., 2005), or the GPR119 receptor (Overton et al., 2006) to control food intake, and via as yet unknown receptors to reduce pain (Suardíaz et al., 2007). Our results demonstrate that activation of PPAR-α mediates the behavioural and neurochemical actions of OEA, as OEA did not reduce the immobility, nor increased pCREB in PPAR-$\alpha^{-/-}$ mice, suggesting that these nuclear receptors play an important role in the properties of OEA here reported.

Neuronal histamine has been described in several species, from Drosophila (reviewed in Nässel, 1999) to humans (reviewed in Panula and Nuutinen 2013); the behavioural effects of OEA as well, which is normally secreted by enterocytes after a fatty meal, have been described in lesser vertebrate species (Astarita et al., 2006) and in humans (Schaefer et al., 2014). The observations that we present here corroborate our hypothesis that these two phylogenetically ancient systems interact and that the central histaminergic system, at least in rodents, receives signals coming from peripheral organs and transmitted by OEA to elaborate the appropriate behaviour and homeostatic responses.

Conflicts of interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuropharm.2018.03.033.

References

Antón, M., Alén, F., Gómez de Heras, R., Serrano, A., Pavón, F.J., Leza, J.C., García-Bueno, B., Rodríguez de Fonseca, F., Orio, L., 2017. Oleoylethanolamide prevents neuroimmune HMGB1/TLR4/NF-kB danger signaling in rat frontal cortex and depressive-like behavior induced by ethanol binge administration. Addict. Biol. 22, 724–741.

Astarita, G., Rourke, B.C., Andersen, J.B., Fu, J., Kim, J.H., Bennett, A.F., Hicks, J.W., Piomelli, D., 2006. Postprandial increase of oleoylethanolamide mobilization in small intestine of the Burmese python (Python molurus). Am. J. Physiol. Regul. Integr. Comp. Physiol. 290, R1407–R1412.

Bahi, A., Schwed, J.S., Walter, M., Stark, H., Sadek, B., 2014. Anxiolytic and antidepressant-like activities of the novel and potent non-imidazole histamine H_3 receptor antagonist ST-1283. Drug Des. Devel. Ther. 8, 627–637.

Bannerman, D.M., Grubb, M., Deacon, R.M., Yee, B.K., Feldon, J., Rawlins, J.N., 2003. Ventral hippocampal lesions affect anxiety but not spatial learning. Behav. Brain Res. 139, 197–213.

Bilbao, A., Blanco, E., Luque-Rojas, M.J., Suárez, J., Palomino, A., Vida, M., Araos, P., Bermúdez-Silva, F.J., 2013. Oleoylethanolamide dose-dependently attenuates cocaine-induced behaviours through a PPARreceptor–independent mechanism. Addiction Biol. 18, 78–87.

Blandina, P., Munari, L., Provensi, G., Passani, M.B., 2012. Histamine neurons in the tuberomamillary nucleus: a whole center or distinct subpopulations? Front Syst. Neurosci. 6, 33. https://doi.org/10.3389/fnsys.2012.00033.

Blendy, J.A., 2006. The role of CREB in depression and antidepressant treatment. Biol. Psychiatr. 59, 1144–1150.

Bortolato, M., Mangieri, R.A., Fu, J., Kim, J.H., Arguello, O., Duranti, A., Tontini, A., Mor, M., Tarzia, G., Piomelli, D., 2007. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol. Psychiatr. 62, 1103–1110.

Carlezon, W., Duman, R., Nestler, E., 2005. The many faces of CREB. Trends Neurosci. 28, 436–445.

Castrén, E., Rantamäki, T., 2010. The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity. Dev Neurobiol. 70, 289–297.

Femenía, T., Magara, S., DuPont, C.M., Lindskog, M., 2015. Hippocampal-dependent antidepressant action of the H3 receptor antagonist clobenpropit in a rat model of depression. Int. J. Neuropsychopharmacol. 18, 1–11.

Fitzgerald, P.B., Laird, A.R., Maller, J., Daskalakis, Z.J., 2008. A meta-analytic study of changes in brain activation in depression. Hum. Brain Mapp. 29, 683–695.

Fu, J., Oveisi, F., Gaetani, S., Lin, E., Piomelli, D., 2005. Oleoylethanolamide, an endogenous PPAR-alpha agonist, lowers body weight and hyperlipidemia in obese rats. Neuropharmacology 48, 1147–1153.

Fu, J., Astarita, G., Gaetani, S., Kim, J., Cravatt, B.F., Mackie, K., Piomelli, D., 2007. Food intake regulates oleoylethanolamide formation and degradation in the proximal small intestine. J. Biol. Chem. 282, 1518–1528.

Fujita, A., Bonnavion, P., Wilson, M.H., Mickelsen, L.E., Bloit, J., de Lecea, L., Jackson, A.C., 2017. Hypothalamic tuberomammillary nucleus neurons: electrophysiological diversity and essential role in arousal stability. J. Neurosci. 37, 9574–9592.

Gaetani, S., Fu, J., Cassano, T., Di Pasquale, P., Romano, A., Righetti, L., Cianci, S., Laconca, L., Giannini, E., Scaccianoce, S., Mairesse, J., Cuomo, V., Piomelli, D., 2010. The fat-induced satiety factor oleoylethanolamide suppresses feeding through central release of oxytocin. J. Neurosci. 30, 8096–8101.

Garbarg, M., Barbin, G., Rodergas, E., Schwartz, J.C., 1980. Inhibition of histamine synthesis in brain by alpha-fluoromethylhistidine, a new irreversible inhibitor: in vitro and in vivo studies. J. Neurochem. 35, 1045–1052.

Gur, T., Conti, A., Holden, J., Bechtholt, A., Hill, T., Lucki, I., Malberg, J., Blendy, J., 2007. cAMP response element-binding protein deficiency allows for increased neurogenesis and a rapid onset of antidepressant response. J. Neurosci. 27, 7860–7868.

Jiang, B., Huang, C., Zhu, Q., Tong, L.-J., Zhang, W., 2015. WY14643 produces antidepressant-like effects in mice via the BDNF signaling pathway. Psychopharmacology 232, 629–1642.

Jiang, B., Wang, Y.J., Wang, H., Song, L., Huang, C., Zhu, Q., Wu, F., Zhang, W., 2017. Antidepressant-like effects of fenofibrate in mice via the hippocampal brain-derived neurotrophic factor signalling pathway. Br. J. Pharmacol. 174, 177–194.

Jin, P., Yu, H.-L., Tian-Lan, Zhang, Z., Quan, Z.-S., 2015. Antidepressant-like effects of oleoylethanolamide in a mouse model of chronic unpredictable mild stress. Pharmacol. Biochem. Behav. 133, 146–154.

Lucki, I., Dalvi, A., Mayorga, A., 2001. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology 155, 315–322.

Mombereau, C., Gur, T., Onksen, J., Blendy, J., 2010. Differential effects of acute and repeated citalopram in mouse models of anxiety and depression. Int. J. Neuropsychopharmacol. 13, 321–334.

Munari, L., Provensi, G., Passani, M.B., Blandina, P., 2013. Selective brain region activation by histamine H_3 receptor antagonist/inverse agonist ABT-239 enhances acetylcholine and histamine release and increases c-Fos expression. Neuropharmacology 70, 131–140.

Munari, L., Provensi, G., Passani, M.B., Galeotti, N., Cassano, T., Benetti, F., Corradetti, R., Blandina, P., 2015. Brain histamine is crucial for selective serotonin reuptake inhibitors' behavioral and neurochemical effects. Int. J. Neuropsychopharmacol. 18 (10) pyv045.

Nässel, D.R., 1999. Histamine in the brain of insects: a review. Microsc. Res. Tech. 44, 121–136.

Overton, H.A., Babbs, A.J., Doel, S.M., Fyfe, M.C., Gardner, L.S., Griffin, G., Jackson, H.C., Procter, M.J., Rasamison, C.M., Tang-Christensen, M., Widdowson, P.S., Williams, G.M., Reynet, C., 2006. Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. Cell Metabol. 3, 167–175.

Panula, P., Nuutinen, S., 2013. The histaminergic network in the brain: basic organization and role in disease. Nat. Rev. Neurosci. 14, 472–487.

Paxinos, G., Franklin, K.B.J., 1997. The Mouse Brain. Academic Press, New York.

Posener, J.A., et al., 2003. High-dimensional mapping of the hippocampus in depression. Am. J. Psychiatr. 160, 83–89.

Provensi, G., Coccurello, R., Umehara, H., Munari, L., Giacovazzo, G., Galeotti, N., Nosi, D., Gaetani, S., Romano, A., Moles, A., Blandina, P., Passani, M.B., 2014. Satiety factor oleoylethanolamide recruits the brain histaminergic system to inhibit food intake. Proc Natl Acad Sci 111, 11527–11532.

Provensi, G., Costa, A., Passani, M.B., Blandina, P., 2016. Donepezil, an acetylcholine esterase inhibitor, and ABT-239, a histamine H3 receptor antagonist/inverse agonist, require the integrity of brain histamine system to exert biochemical and procognitive effects in the mouse. Neuropharmacology 109, 139–147.

Provensi, G., Fabbri, R., Munari, L., Costa, A., Baldi, E., Bucherelli, C., Blandina Pand Passani, M.B., 2017. Histaminergic neurotransmission as a gateway for the cognitive effect of oleoylethanolamide in contextual fear conditioning. Int. J. Neuropsychopharmacol. 20, 392–399.

Razzoli, M., Domenici, E., Carboni, L., Rantamaki, T., Lindholm, J., Castrén, E., Arban, R., 2011. A role for BDNF/TrkB signaling in behavioral and physiological consequences of social defeat stress. Gene Brain Behav. 10, 424–433.

Romano, A., Cassano, T., Tempesta, B., Cianci, S., Dipasquale, P., Coccurello, R., Cuomo, V., Gaetani, S., 2013. The satiety signal oleoylethanolamide stimulates oxytocin neurosecretion from rat hypothalamic neurons. Peptides 49, 21–26.

Romano, A., Tempesta, B., Micioni Di Bonaventura, M.V., Gaetani, S., 2016. From autism to eating disorders and more: the role of oxytocin in neuropsychiatric disorders. Front. Neurosci. 9, 497.

Romano, A., Gallelli, C.A., Koczwara, J.B., Braegger, F.E., Vitalone, A., Falchi, M., Micioni Di Bonaventura, M.V., Cifani, C., Cassano, T., Lutz, T.A., Gaetani, S., 2017. Role of the area postrema in the hypophagic effects of oleoylethanolamide. Pharmacol. Res. 122, 20–34.

Schaefer, C., Enning, F., Mueller, J.K., Bumb, J.M., Rohleder, C., Odorfer, T.M., Klosterkötter, J., Hellmich, M., Koethe, D., Schmahl, C., Bohus, M., Leweke, F.M., 2014. Fatty acid ethanolamide levels are altered in borderline personality and complex posttraumatic stress disorders. Eur. Arch. Psychiatr. Clin. Neurosci. 264, 459–463.

Schwartz, G.J., Fu, J., Astarita, G., Li, X., Gaetani, S., Campolongo, P., Cuomo, V., Piomelli, D., 2008. The lipid messenger OEA links dietary fat intake to satiety. Cell Metabol. 8, 281–288.

Suardíaz, M., Estivill-Torrús, G., Goicoechea, C., Bilbao, A., Rodríguez de Fonseca, F., 2007. Analgesic properties of oleoylethanolamide (OEA) in visceral and inflammatory pain. Pain 133, 99–110.

Umehara, H., Fabbri, R., Provensi, G., Passani, M.B., 2016. The hypophagic factor oleoylethanolamide differentially increases c-fos expression in appetite regulating centres in the brain of wild type and histamine deficient mice. Pharmacol. Res. 11, 100–107.

Wang, X., Miyares, R.L., Ahern, G.P., 2005. Oleoylethanolamide excites vagal sensory neurones, induces visceral pain and reduces short-term food intake in mice via capsaicin receptor TRPV1. J. Physiol. 564, 541–547.

Yua, H.-L., Suna, L.-P., Li, M.-M., Quan, Z.-S., 2015. Involvement of norepinephrine and serotonin system in antidepressant-like effects of oleoylethanolamide in the mice models of behavior despair. Neurosci. Lett. 593, 24–28.