Biased 5-HT1A receptor agonists F13714 and NLX-101 differentially affect pattern separation and neuronal plasticity in rats after acute and chronic treatment

van Hagen, B.T.J; van Goethem, N.P.; Nelissen, E.; Paes, D.; Koymans, K.; van Hoof, S.; Schreiber, R.; Varney, M.; Newman-Tancredi, A.; Prickaerts, J.

DOI
10.1016/j.mcn.2022.103719

Publication date
2022

Document Version
Final published version

Published in
Molecular and cellular neuroscience

Molecular and Cellular Neuroscience 120 (2022) 103719

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Biased 5-HT$_{1A}$ receptor agonists F13714 and NLX-101 differentially affect pattern separation and neuronal plasticity in rats after acute and chronic treatment

Britt T.J. van Hagen [a], Nick P. van Goethem [a], Ellis Nelissen [a], Dean Paes [a], Karin Koymans [b,c], Scott van Hoof [d,e], Rudy Schreiber [f], Mark Varney [g], Adrian Newman-Tancredi [g], Jos Prickaerts [a,*]

[a] Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands
[b] Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands
[c] Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 XH Amsterdam, the Netherlands
[d] Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, The Netherlands
[e] German Center for Neurodegenerative Diseases (DZNE) Berlin, 10117 Berlin, Germany
[f] Faculty of Psychology and Neuroscience, Section Neuropsychology and Psychopharmacology, Maastricht University, Maastricht, the Netherlands
[g] Neurolixis Inc., Dana Point, California, USA

ARTICLE INFO

Keywords:
5-HT$_{1A}$
NLX-101
F13714
hippocampus
Neurogenesis
Pattern separation

ABSTRACT

Pattern separation is a hippocampal process in which highly similar stimuli are recognized as separate representations, and deficits could lead to memory impairments in neuropsychiatric disorders such as schizophrenia. The 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) is believed to be involved in these hippocampal pattern separation processes. However, in the dorsal raphe nucleus (DRN), the 5-HT$_{1A}$R is expressed as a somatodendritic autoreceptor, negatively regulates serotonergic signaling, and could thereby counteract the effects of hippocampal postsynaptic 5-HT$_{1A}$ receptors. Therefore, this study aims to identify how pre- and post-synaptic 5-HT$_{1A}$R activity affects pattern separation. Object pattern separation (OPS) performance was measured in male Wistar rats after both acute and chronic treatment (i.p.) with 5-HT$_{1A}$R biased agonists F13714 (0.0025 mg/kg acutely, 0.02 mg/kg/day chronically) or NLX-101 (0.08 mg/kg acutely, 0.32 mg/kg/day chronically), which preferentially activate autoreceptors or postsynaptic receptors respectively, for 14 days. Body temperature - a functional correlate of hypothalamic 5-HT$_{1A}$R stimulation - was measured daily. Additionally, 5-HT$_{1A}$R density (DRN) and plasticity markers (hippocampus) were assessed. Acute treatment with F13714 impaired OPS performance, whereas chronic treatment normalized this, and a drop in body temperature was found from day 4 onwards. NLX-101 enhanced OPS performance acutely and chronically, and caused an acute drop in body temperature. Chronic NLX-101 treatment increased doublecortin positive neurons in the dorsal hippocampus, while chronic treatment with F13714 resulted in a downregulation of 5-HT$_{1A}$ autoreceptors, which likely reversed the acute impairment in OPS performance. Chronic treatment with NLX-101 appears to have therapeutic potential to improve brain plasticity and OPS performance.

Abbreviations

5-HT$_{1A}$R	5-hydroxytryptamine subtype 1A receptor
AD	Alzheimer's disease
BDNF	brain derived neurotropic factor
CA1	Cornu Ammonis region 1
CA3	Cornu Ammonis region 3
cAMP	Cyclic adenosine monophosphate
CIAS	cognitive impairments associated with schizophrenia
CREB	cAMP-response element binding
DCX	doublecortin
DG	dentate gyrus
DRN	Dorsal raphe nucleus
GCL	granular cell layer

(continued on next column)

(continued)

(continued on next page)

* Corresponding author: Universiteitssingel 50, 6229ER Maastricht, the Netherlands.
E-mail address: jos.prickaerts@maastrichtuniversity.nl (J. Prickaerts).

https://doi.org/10.1016/j.mcn.2022.103719
Received 7 October 2021; Received in revised form 16 February 2022; Accepted 7 March 2022
Available online 10 March 2022

(*continued*)

mPFC	medial prefrontal cortex
OPS	object pattern separation
PHC	parahippocampal cortex
PSD95	post-synaptic density protein 95
PTSD	post-traumatic stress disorder
PVN	paraventricular nucleus
SGZ	sub-granular zone

1. Introduction

Pattern separation is a type of memory processing that enables making separate representations from highly similar, but slightly different stimuli or events (Clelland et al., 2009). This is thought to be achieved by reducing the interference between overlapping patterns of activation so that similar patterns can be retrieved separately, enhancing memory accuracy. The underlying mechanism that supports cognitive pattern separation has not yet been unraveled completely, but evidence suggests that adult neurogenesis in the dentate gyrus (DG) of the hippocampus is crucial for pattern separation (Clelland et al., 2009; Franca et al., 2017). It is thought that by storing similar but new information in newborn cells, this information can be easily retrieved separately from older similar information, without interference of that particular information (Frankland et al., 2013). Increasing evidence suggests that pattern separation processes are affected in multiple psychiatric diseases, such as schizophrenia, dementia, PTSD and depression (Ally et al., 2013; Das et al., 2014; Eisch and Petrik, 2012; Kheirbek et al., 2012; Miller and Hen, 2015; Stark et al., 2013). It has been suggested that pattern separation dysfunction is the underlying cause for some of the symptoms displayed in schizophrenia and PTSD (Faghihi and Moustafa, 2015; Martinelli and Shergill, 2015; Schreiber and Newman-Tancredi, 2014). A targeted treatment to enhance pattern separation could therefore be of high value for the functional outcome and quality of life of patients.

The serotonin 1A receptor (5-HT_{1A}R) can exert differential effects on cognition-related neurotransmission, depending on the location of the receptors. Somatodendritic 5-HT_{1A}Rs are located on serotonergic neurons in the raphe nucleus and act as autoreceptors, negatively regulating serotonin release and its cortical projections upon activation. Post-synaptic 5-HT_{1A} receptors are distributed across brain stem, spinal cord, and cortical and limbic brain areas, with high numbers in the hippocampus innervating different types of neurons (Hensler, 2003; Rojas and Fiedler, 2016). 5-HT_{1A}R activity is an important regulator of cognitive processes and memory related plasticity (Seyedabadi et al., 2014) and post-synaptic 5-HT_{1A}R activation has been linked to increased adult neurogenesis (Arnold and Hagg, 2012; Soumier et al., 2010).

The differential effects of selective 5-HT_{1A}R activation on cognition, specifically on pattern separation processes, has been previously studied in our lab by investigating the effects of two 'biased' 5-HT_{1A}R agonists on rat performance in a spatial object pattern separation (OPS) task (van Goethem et al. (2015)). The OPS task has been designed by us to measure the ability of rodents to recognize small variations in spatial arrangement, utilizing pattern separation processes (van Goethem et al., 2018; van Hagen et al., 2015). The biased agonist NLX-101 preferentially activates postsynaptic receptors (Newman-Tancredi et al., 2009), while F13714 shows a preference for 5-HT_{1A} somatodendritic autoreceptors (Koek et al., 2001). These 'biased' agonists exert different cognitive effects upon binding to either the pre- or post-synaptic 5-HT1ARs and have low affinity for other binding sites (Assie et al., 2010; Assie et al., 2006; Llado-Pelfort et al., 2010; Newman-Tancredi et al., 2009; Vacher et al., 1998). Our previous study showed that acute administration of NLX-101 enhanced OPS performance of rats, while F13714 impaired OPS performance acutely, when compared to vehicle treated rats (van Goethem et al. (2015)). When both compounds were administered together these differential effects cancelled each other out. These findings illustrate the therapeutic potential of specifically targeting 5-HT_{1A}R sub-populations to affect pattern separation function, and their value as a scientific tool to separately study the function of different 5-HT_{1A}R sub-populations. Additionally, previous studies investigating the effect of NLX-101 administration on different aspects of memory and cognition showed a high efficacy in the alleviation of cognitive impairments, when compared to the more 'traditional' reference compounds phencyclidine and 8-OH-DPAT (Assie et al., 2010; Depoortere et al., 2010). Therefore, the selective targeting of specific receptor sub-populations using 'biased' agonists might prove a more effective treatment strategy for cognitive enhancement with higher effectivity than the (non-specific) treatments currently available.

The current study was designed to further elucidate the underlying mechanisms of differentially located 5-HT_{1A}R activity on pattern separation performance, by investigating the effects of chronic NLX-101 and F13714 treatment on OPS task performance and plasticity changes in the rat brain. A study by Assie et al. (2006) showed that F13714 induced rapid autoreceptor desensitization during chronic threatment, indicated by recovery of extracellular serotonin levels in the hippocampus. Other (non-biased) agonists showed desensitization of somatodendritic 5-HT1A autoreceptors on a neurochemical level after 7 days of treatment (Blier and Ward, 2003; Dong et al., 1997). The behavioral outcome in these studies was not affected by pre-synaptic desensitization.

The aim of this study is to investigate the underlying mechanism of pattern separation performance and how this is mediated by 5-HT_{1A}R activity. Animals were treated both acutely and chronically (14 days) with either F13714 or NLX-101 and OPS performance was assessed. Additionally, body temperature was measured daily as an extra mechanistic readout, since it is known that post-synaptic 5-HT1ARs in the hypothalamus regulate body temperature and agonistic stimulation of these 5-HT1ARs leads to a hypothermic response (Hensler, 2003; Rausch et al., 2006). Lastly, a series of memory related neuronal plasticity measurements were performed.

2. Methods

2.1. Animals

In total, 48 three-month-old male Wistar rats (Charles River Laboratories International, Inc., Sulzfeld, Germany) were used (average weight of 325.4 g at the beginning of the study). All experimental procedures were approved by the local animal ethical committee of Maastricht University (licensed animal ethical committee: Min.VWS, GZBIVVB981845) under the Dutch Experiments on Animals Act (EAA, amended 1996), in accordance with the European Directive (2010/63/EU) for animal experiments. These experiments were registered under the protocol number DEC2013-013. All experimental results are reported in accordance with ARRIVE guidelines (Percie du Sert et al., 2020).

Rats were housed individually in standard Tecniplast IVC system greenline cages, on a sawdust bedding, and with a 12/12 h reversed light/dark cycle (lights off from 7:00 h to 19:00 h). Animals were housed in a temperature- (22 ± 1 °C) and humidity- (55% ±10%) controlled environment. Food (maintenance chow, ssniff Spezialdiäten GmbH, Germany) and water were available ad libitum. Background noise was provided by a radio playing softly. All testing was performed during the dark phase of the light/dark cycle, under low illumination (20 lx). One of the animals died prior to the start of the experiment, therefore the entire experiment was performed with 47 rats.

2.2. Materials

F13714 (3-chloro-4-fluorophenyl-(4-fluoro-4-(((5-methyl-6-methylaminopyridin-2-ylmethyl)-amino)-methyl)-piperidin-1-yl-methanone) and NLX-101 (3-Chloro-4-fluorophenyl-(4-fluoro-4-(((5-

methylpyrimidin-2-ylmethyl)-amino)-methyl)-piperidin-1-yl)-methanone) fumarate salts were a kind donation from Neurolixis Inc. and were provided as a dry powder. Donkey serum for immunohistochemistry was purchased from Sigma Aldrich (D9663), and antibodies for immunolabelling were 1:100 goat anti-DCX purchased from Santa Cruz (SC-8066), 1:500 rabbit anti- 5-HT_{1A}R purchased from Genetex (GTX104703), 1:500 biotinylated donkey anti-rabbit purchased from Jackson Laboratories, and donkey anti-goat Alexa 488 purchased from Invitrogen. Additionally, the avidin-biotin enzyme complex ABC-kit was purchased from Vector Laboratories.

For western blotting, PVDF membranes, Odyssey blocking buffer, and secondary antibodies (1:10000 donkey anti-mouse IRDye 680, and 1:10000 goat anti-rabbit IRDye 800) were all purchased from Li-Cor Biosciences. Primary antibodies used were 1:1000 mouse anti-synaptophysin (MAB5258, Merk Millipore), 1:600 rabbit anti-BDNF (#F0110, Santa Cruz), 1:3000 mouse anti-CREB and 1:100 rabbit anti-pCREB (#9104 and #9198S, Cell Signaling Technologies), 1:2000 mouse anti-PSD95 (#0711 QED Bioscience), and 1:2000000 mouse anti-GAPDH (#10R-G109A, Fitzgerald).

2.3. Treatments

Animals were randomly assigned to one of the treatment conditions at the start of the study. 16 rats received NLX-101 treatment, 16 rats received F13714 and 15 rats were given vehicle (saline) treatment. All injections in this study were administered intra peritoneally (IP) with a volume of 2 ml/kg. As a baseline measurement for OPS performance we first aimed to replicate the acute administration effects on OPS performance found previously (van Goethem et al., 2015), with 0.08 mg/kg NLX-101 and 0.0025 mg/kg F13714 when given 30 min before T1 of the OPS. After the acute study, there was a two-week wash out period and animals stayed assigned to the same treatment groups. Chronic treatment consisted of daily injections (24 h in between) over a 14-day period with either 0.32 mg/kg NLX-101, 0.02 mg/kg F13714 or vehicle at the same time each morning (approx. 10 am). These dosages were chosen to accomplish maximum receptor occupancy over the 24 h period without inducing serotonergic symptoms (Assie et al., 2010) and refer to the weight of the salts. The drugs were dissolved in saline. All solutions were prepared fresh on the day of administration.

Of note, due to a limited time window to perform OPS testing and subsequent harvesting of brain material at the end of the experiment, not all animals could start the experiment on the same day. Therefore, the start of the treatment was divided over four consecutive days with equal distribution of each treatment condition. This has led to the advantage that possible fluctuations due to subtle day-differences have been balanced out over the behavioral and temperature data. The experimenter performing the treatments was blinded to the treatment conditions to prevent any bias during the course of the experiment.

2.3.1. Behavioral OPS task apparatus & objects

Spatial pattern separation performance was measured in the Object Pattern Separation task (van Hagen et al., 2015). The apparatus and procedures followed were similar to those previously described in detail by Van Goethem et al. (2018). In brief, OPS testing was performed in a circular arena with a diameter of 83 cm and 40 cm high. One half (facing the experimenter) consisted of see-through PVC and the other half of grey PVC. Two sets of identical objects were used, consisting of either two rectangular metal blocks (10.0 cm × 5.0 cm × 7.5 cm) containing two holes (diameter 1.9 cm), or two aluminium cubes with square bases and tapering tops (13.0 cm × 8.0 cm × 8.0 cm). The rats were unable to displace these objects. The arena was dimly lit by fluorescent red light and a small desk lamp, providing illumination of about 20 lx.

2.3.2. OPS testing procedure and habituation

The OPS task has been adapted from the object location task (Ennaceur and Delacour, 1988), to asses spatial pattern separation processes in rodents using voluntary exploration based on the novelty-preference paradigm. A detailed description of the entire protocol and rationale can be found in Van Goethem et al. (2018). In short, the task is performed over two 3 min trials in an arena, separated by a 1 h interval. In the first (acquisition) trial (T1), two identical objects were placed symmetrically on a horizontal line in the arena (position 1) to be explored by the rats. After the inter-trial-interval spent in the home cage, the second (test) trial (T2) contained the same set of objects, but now one of the objects was displaced by a small/intermediate separation from the previously explored location (position 3). Untreated rats showed an intermediate discrimination for this position. This is defined as an OPS performance in between that of completely similar (position 1, discrimination is equal to chance level) or highly different positions (position 5, discrimination is high). This allowed to measure both increases and decreases in pattern separation performance.

Object displacement variation (left or right object, to front or back) and different object-sets were divided in a balanced manner over conditions and varied across test-days for individual animals. After each trial, the objects and arena were cleaned with a 70% ethanol solution. Exploration times of each object were recoded manually by the experimenter on a desktop computer, using in-house developed software. Exploration was defined as directing the nose to the object from a distance no more than 2 cm or touching the object with the nose. Sitting on, or leaning to, an object was not scored as exploratory behavior. Before the start of the experiment, the rats were habituated to the experimenter, the set-up and injections. After habituation to vehicle injections, the rats were assigned to a fixed/lasting treatment condition and acute treatment was tested (divided over 2 days), followed by a two-week wash-out. Animals were tested on Day 15, 24 h after the last treatment, to not include acute additive effects.

2.4. Body temperature

Core body temperature was measured daily on the same time in the morning for each of the animals, to prevent individual differences due to circadian rhythm fluctuations. Measurements were always taken exactly 30 min after the daily treatment administration, using a digital rectal thermometer with an accuracy of 0.01 degree Celsius (Onbo Electronics Co. Ltd., Shenzhen, China). The thermometer was cleaned with paper towel and lubricated with Vaseline® (Unilever, the Netherlands) before each measurement.

The average temperature of each condition was calculated per treatment day, to show temperature changes over time. Furthermore, additional baseline temperature measurements were taken on day 7 and 14 of the chronic treatment, 30 min prior to injection.

2.4.1. Brain tissue collection

Brain samples from all animals were obtained ±1 h after OPS testing on day 15. For immunohistochemical (IHC) measurements, half of the animals (N = 8 per condition) were put under deep anesthesia (100 mg/kg sodium pentobarbital, IP) and transcardially perfused with PBS (0.1 M phosphate buffered saline, pH 7.4) followed by 4% paraformaldehyde (PFA). Entire brains were taken out and post-fixed overnight in 4% PFA, and subsequently washed in PBS for 24 h. The samples incubated in 10% sucrose (48 h), followed by 3 days in 20% sucrose. 30 μm thick coronal sections were cut using a cryostat (CM3010, Leica Biosystems) with both the dorsal hippocampus (−1.72 mm to −5.52 mm from Bregma) and the dorsal raphe nucleus (−6.96 mm −8.76 mm to Bregma) according to Paxinos and Watson (2007). Section were collected in series of every 8th section and stored at −80 °C until further analysis.

The other half of the animals (N = 8 for NLX-101 and F13714, N = 7 for vehicle treated animals) were used for western blot analysis. Animals were decapitated, the right and left hippocampus were dissected and divided in the dorsal- and ventral part before storage in separate tubes. The samples were snap-frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.4.2. Immunofluorescent labelling of doublecortin

Fluorescent labelling of doublecortin (DCX) was performed on hippocampal sections to quantify ongoing cell division, differentiation and migration of neuronal precursor cells in the sub-granular (SGZ) and granular cell layer (GCL) in the DG of the hippocampus (von Bohlen Und Halbach, 2007). Immunofluorescent labelling was performed on free floating hippocampal sections. Frozen sections were washed in TBS (0.1 M Tris–HCl, pH 7.4, 0.9% NaCl), transferred to glass containers with pre-heated sodium citrate solution (10 mM Sodium Citrate, 0.05% Tween 20 pH 6.0). The sections incubated for 20 min in an 80 °C water bath for antigen retrieval, followed by 20 min cool down at room temperature (RT) and washing in TBS (2 × 15 min) and TBS-T (TBS containing 0.3% Triton X-100; 15 min). Endogenous epitope blocking was performed for 1 h in 3% donkey serum (Sigma Aldrich # D9663), followed by overnight incubation at 4 °C with goat anti-DCX (1:100, Santa Cruz SC-8066) with 0.3% donkey serum in TBS-T. Washing in TBS-T, TBS and TBS-T (15 min) was performed followed by 2 h incubation with donkey anti-goat Alexa 488 (Invitrogen) at RT. After final washing steps (15 min TBS-T, 2 × 1 h TBS) slices were carefully mounted on gelatin-chromaluin coated glasses, coverslips were mounted with 80% glycerol.

2.4.3. 5-HT_{1A} receptor immune-labelling in the dorsal raphe nucleus

5-HT_{1A} receptors were visualized using DAB immunohistochemistry, in sections containing the DRN. Stored free-floating sections were washed in TBS (3 × 10 min). Subsequent washing was performed after each incubation step with TBS-T, TBS and TBS-T (10 min). Endogenous epitopes were blocked by 30 min incubation with 0.3% H_2O_2 in TBS, followed washing. Rabbit anti- 5-HT_{1A}R antibody (1:500, GTX104703, Genetex, Irvine, CA, USA) incubated overnight at 4 °C in TBS-T. Biotinylated donkey anti-rabbit (1:500, Jackson Laboratories, West Grove, PA, USA) incubated for 2 h at RT, followed by the avidin-biotin enzyme complex for 1 h (ABC-kit, Vector Laboratories, Burlingame, Calif, USA). The immunoperoxidase reaction was carried out using a DAB solution (0.01% 3,3'diaminobenzine, 0.025% NiCl2, 0.003% H_2O_2 in 0.05 M Tris–HCl, the reaction was stopped after ±10 min by rinsing in TBS (3 × 10 min). Sections were mounted on gelatin-chromaluin coated glasses and air-dried overnight. Dehydration was performed in ascending ethanol concentrations and coverslips were mounted over the sections using depex.

2.4.4. Protein gel electrophoresis

To compare relative protein quantities of memory related plasticity markers, western blots were performed with homogenized brain sample of the dorsal hippocampus tissue. Membranes were stained for; mature and pro- brain derived neurotropic factor (BDNF), the transcription factor cAMP response element binding protein (CREB) and its phosphorylated state pCREB, synaptophysin, and post-synaptic density protein 95 (PSD95).

The dissected dorsal hippocampus samples were homogenized using a lysis buffer (100 mM Tris, 200 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.05% Triton) with added phosphatase and protease inhibitors (protease inhibitor mix and phosphostop, Roche, Basel, Switzerland). Protein concentration was determined (BioRad concentration assay, Hercules, CA, USA) to load equal amounts of protein from each sample into a 10% SDS-polyacrylamide gel (14% for BDNF) and separate them through gel electrophoresis. Subsequently, proteins were transferred into a PVDF membrane (Li-Cor Biosciences, Lincoln, NE, USA). Membranes were blocked with 1:1 diluted Odyssey blocking buffer (Li-Cor Biosciences, Lincoln, NE, USA) in PBS. Primary antibody solutions incubated overnight at 4 °C, on separate blots for; rabbit anti-BDNF (1:600, #F0110, Santa Cruz, CA, USA); mouse anti-synaptophysin (1:1000, MAB5258, Merck Millipore, Burlington, MA, USA); mouse anti-CREB + rabbit anti-pCREB (1:3000, #9104; 1:100 #91988, Cell Signaling Technologies, Beverly, MA, USA) and mouse anti-PSD95 (1:2000, #0711, QED Bioscience, San Diego, CA, USA). Mouse anti-GAPDH (1:2000000, #10R-G109A, Fitzgerald, Huissen, NL) was used as a loading control. After washing in PBS and PBS-T (PBS + 0.1% Tween) secondary antibodies (1:10000 donkey anti-mouse IRDye680; 1:5000 goat anti-rabbit IRDye800, Li-Cor Biosciences, Lincoln, NE, USA) incubated for 1 h. Membranes were air dried and imaged with an Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE, USA).

2.5. Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2018). More details on power calculations for the different experimental procedures can be found in the Supplementary Methods.

2.5.1. Statistical analysis behavioral experiment (OPS task and temperature)

Total exploration times for each of the objects were calculated for both T1 and T2. Total time spent exploring both symmetrically placed objects in T1 ('a1' + 'a2' = 'e1') and total exploration times of the moved ('b') and stationary ('a3') object in T2 ('a3' + 'b' = 'e2') were calculated to analyze exploration differences between treatment conditions. The main performance measure was the discrimination index or 'd2', calculated through ('a3'-'b')/'e2', e.g. the difference in exploration time between objects relative to the total exploration in T2. See Akkerman et al. (2012a) for more detailed information on these measures. Total exploration times in T1 and T2 should be sufficient to reliably assess pattern separation memory. Therefore, animals that spent less than 9 s exploring both objects in T1 or T2 were excluded from the dataset (Akkerman et al., 2012a).

One-sample t-statistics were used to compare the mean d2 index for each condition to zero (i.e. chance level performance) (Akkerman et al., 2012b). A difference from chance level signified that the animals recognized the moved object in T2 as being in a different position than in T1. Differences in d2 between conditions were evaluated by one-way ANOVAs separate for each test day. When the overall ANOVA was significant, Bonferroni post-hoc tests were performed. Mean body temperature per condition was calculated separately for each day. A two-way repeated measures ANOVA for treatment and day was performed. Bonferroni's multiple comparisons were used to evaluate treatment effects on each day. To measure receptor activation changes within a treatment condition, a repeated measures ANOVA over all days was performed for treatment condition. If significant, LSD *t*-tests were performed to compare day one temperature (baseline response) to all subsequent days. To analyze differences in temperature before and after treatment, pre-treatment measurements taken on day 7 and 14 were compared to the corresponding post-treatment measurement for each condition, using a paired samples *t*-tests. Additional paired samples t-tests were performed between day 7 and 14, for both the pre-treatment and post-treatment measurements.

2.5.2. Imaging and statistical analysis of plasticity measures

A brief overview will be given of the statistical analysis. More details can be found in the Supplementary Methods. DCX labelled neurons were counted in the subgranular zone (SGZ) and granule cell layer (GCL) of the DG. Dorsal hippocampal sections from bregma-2.16 to −4.68 (Paxinos and Watson, 2007) were imaged with an Olympus BX50 microscope and DP70 camera (Olympus, Tokyo, Japan), quantification was performed using StereoInvestigator® (version 11, MBF biosciences, Williston, VT, USA). Both hemispheres were analyzed and included as one observation. DCX positive neurons were counted in the DG area at 40× magnification using the optical fractionator method. To control for volume differences, the total number of cells was divided by the estimated volume of each sample. The mean DCX ratios of the treatment conditions were compared by a one-way ANOVA, followed by Bonferroni's post-hoc comparisons to vehicle.

5-HT_{1A}R differences in optical density were quantified and compared between treatment conditions in the DRN. Using a bright-field AX70

microscope and XM10 camera (Olympus, Tokyo, Japan), multiple photographic images were obtained at 20× magnification. Lighting conditions and settings were kept constant during imaging for all sections. First, multiple obtained images from one section were stitched together using the ImageJ NIH (http://rsb.info.nih.gov/ij/) software extension MosaicJ®. Subsequently, the entire DRN per section could be delineated and analyzed in ImageJ as described elsewhere (Yagi et al., 2016). A minimum of three sections per animal were used (Madden and Zup, 2014). Mean ratios per animal were compared between treatment groups with a one-way ANOVA, followed by post-hoc Bonferroni tests between groups. Outliers were removed per condition using a Dixon test for outliers, with a significance level of $\alpha = 0.05$.

Western blot protein markers were quantified using background corrected grey value intensities obtained with ImageJ. To control for loading differences, target proteins were normalized by dividing them by the intensity of the corresponding GAPDH band. Outliers were removed per condition using the Dixon test for outliers. NLX-101 and F13714 treatment conditions were compared to vehicle using a one-way ANOVA. A α level of 0.05 was considered significant for all statistical comparisons.

3. Results

3.1. OPS performance

An overview of main OPS task outcome measures is listed in Table 1A and B. For acute treatment, the treatment conditions did not affect exploration times in the T1 (e1: $F_{2,44} = 0.68$, n.s.) and T2 (e2: $F_{2,44} = 2.50$, n.s.). Similar results were found for chronic treatment (e1: $F_{2,43} = 1.14$, n.s.; e2: $F_{2,43} = 0.77$, n.s.).

Acute treatment with either vehicle or NLX-101 resulted in an OPS performance different from zero (i.e. chance level performance), contrary to F13714 treatment (one-sample *t*-test, $P < 0.05$; Fig. 1A). Additionally, one-way ANOVA revealed that NLX-101 treated rats performed better than F13714 treated rats ($F_{2,44} = 4.30$; $P < 0.05$; Bonferroni post-hoc *t*-test $P < 0.05$). However, no significant difference was found between the d2 index of vehicle treated and both drug treated groups.

After 14 days of chronic treatment, both vehicle and NLX-101 rats showed an OPS performance different from chance, similar to acute treatment (one-sample t-test, $P < 0.05$; Fig. 1B). Additionally, contrary to acute treatment, rats treated chronically with F13714 also performed differently from zero. This indicates that acute treatment with F13714 initially impairs pattern separation ability, but is restored after chronic treatment.

Table 1
Mean OPS measures and their SEM for acute treatment conditions.

A Acute treatment	Vehicle	NLX-101	F13714
e1 (SEM)	36.44 (2.76)	32.87 (2.03)	34.44 (1.58)
e2 (SEM)	20.70 (1.93)	26.83 (1.94)	22.02 (2.23)
d2 (SEM)	0.20 (0.07) #	0.33 (0.08) ###	0.02 (0.08)

B Chronic treatment	Vehicle	NLX-101	F13714
e1 (SEM)	30.82 (2.70)	26.18 (2.58)	26.09 (2.14)
e2 (SEM)	25.59 (2.61)	21.45 (2.14)	24.02 (2.38)
d2 (SEM)	0.16 (0.07) #	0.27 (0.06) ###	0.17 (0.07) #

Mean OPS task outcome measures and their SEM for each of the treatment conditions; e1 = total exploration time during T1 (s), e2 = total exploration time during T2 (s), d2 = discrimination index. A difference between d2 and chance level performance are indicated by hashes (one-sample t-test, # $P < 0.05$; ### $P < 0.001$. A) Acute treatment OPS task Vehicle N = 15, NLX-101 (0.08 mg/kg IP) N = 16, F13714 (0.0025 mg/kg IP) N = 16. B) OPS task measures 24 h after the last chronic treatment. Vehicle N = 14, NLX-101 (0.32 mg/kg/day IP) N = 16, F13714 (0.02 mg/kg/day IP) N = 16.

3.2. Temperature measurements

Two-way repeated measures ANOVA for both treatment condition and treatment day revealed differences for treatment condition ($F_{2,44} = 143.20$, $P < 0.001$) and over time ($F_{8,347} = 3.18$, $P < 0.001$), as well as their interaction ($F_{26,572} = 5.83$, $P < 0.01$). Significant differences from the vehicle condition were evaluated separately for each treatment day using post-hoc Bonferroni *t*-tests (Fig. 2). Comparison to vehicle treatment showed that post-synaptic 5-HT_{1A}R activation by NLX-101 caused a lower body temperature ($P < 0.001$ for each day), an effect which gradually faded during chronic treatment, yet remaining significantly lower compared with vehicle. This is supported by a repeated measures ANOVA for the NLX-101 treated rats ($F_{13,195} = 10.29$, $P < 0.001$). For F13714, body temperature was similar to vehicle-treated rats for the first 4 days of treatment. However, from treatment day 5 onwards, F13714 treated animals display a lower body temperature compared to vehicle, indicating a hypothermic response, presumably due to hypothalamic 5-HT_{1A}R activation (Fig. 2).

Additionally, body temperature was measured 30 min prior to injections on day 7 and 14, and the differences between pre- and post-treatment body temperature was measured (Fig. 3). Only the vehicle treated group showed an increase in temperature between the pre- and post-treatment measurement on both day 7 and 14 (paired-samples t-test). Furthermore, the differences between pre-treatment temperatures on day 7 versus 14 and post-treatment temperatures on day 7 versus 14 were analyzed, which revealed an increase in day 14 pre- and post-treatment temperatures compared to day 7 within the NLX-101 group ($P < 0.05$), while no differences were found for F13714. Interestingly, the vehicle condition showed an increase in pre-treatment temperature between day 7 and 14, but no difference between both post-treatment temperatures.

3.3. Doublecortin positive cells in the DG

DCX positive cells were counted in the DG of the dorsal hippocampus to evaluate quantitative differences in adult born neurons between the conditions (Fig. 4). A one-way ANOVA between conditions ($F_{2,18} = 6.57$, $P < 0.01$) revealed that NLX-101 treatment increased the DCX cell ratio compared to vehicle (Bonferroni t-test, $P < 0.01$), while no effect was found for F13714 treatment (Fig. 4).

3.4. 5-HT_{1A}R density

A one-way ANOVA showed that 5-HT_{1A}R labelled DRN sections differed in density ratios between the different treatment conditions ($F_{2,14} = 7.12$, $P < 0.05$; Fig. 5A–C). Bonferroni's post-hoc tests revealed that both drug-treatment conditions have a lower 5-HT_{1A}R distribution compared to vehicle treatment ($P < 0.05$).

3.5. Plasticity markers

Relative quantities of several protein markers were evaluated in dorsal hippocampus tissue from chronically treated animals, the obtained results are summarized in Fig. 6. For BDNF, the amount of mature BDNF (mBDNF) was found to be increased ($F_{2,19} = 4.20$; $P < 0.05$) after treatment with NLX-101 (post-hoc Bonferroni correction, $P < 0.05$). Neither proBDNF ($F_{2,20} = 0.92$; n.s.) protein quantities nor the mBDNF/proBDNF ratio ($F_{2,20} = 2.41$; n.s.) was changed after treatment. For all of the other markers measured, i.e. PSD95, synaptophysin, and the pCREB/CREB ratio no significant differences were found for these protein quantities between the conditions (see Supplementary Fig. S1).

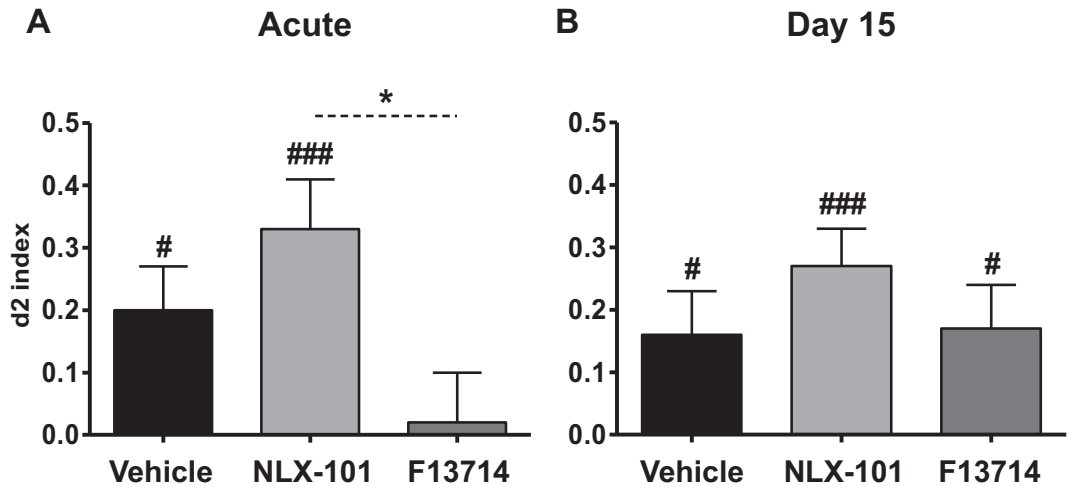

Fig. 1. Acute and chronic effects of NLX-101 and F13714 treatment on pattern separation (OPS) task performance in rats.
Mean OPS task scores are depicted as d2 index (+SEM) for each of the treatment conditions. A) d2 index after acute NLX-101 (0.08 mg/ kg IP), F13714 (0.0025 mg/ kg IP) and vehicle (saline) treatment. A difference between groups is indicated by an asterisk (post-hoc Bonferroni, * $P < 0.05$). Vehicle N = 15, NLX-101 N = 16, F13714 N = 16. B) Chronic treatment effects on OPS task d2 performance measured 24 h after the last treatment with either NLX-101 (0.32 mg/kg/day IP), F13714 (0.02 mg/kg/day IP) and vehicle (saline). Vehicle N = 15, NLX-101 N = 16, F13714 N = 16. A difference between d2 and chance level performance is indicated by hashes (one-sample t-test, # $P < 0.05$; ### $P < 0.001$).

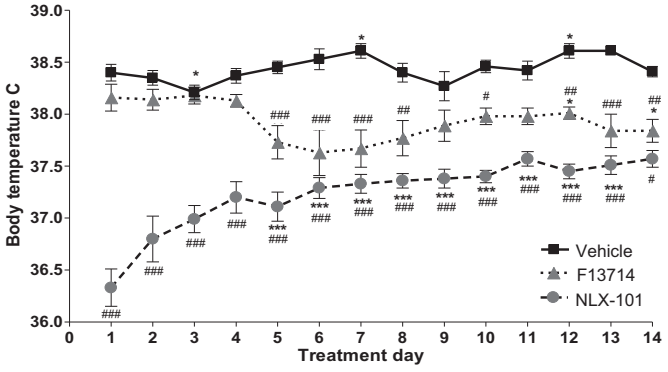

Fig. 2. Mean daily body temperature and SEM per day for each treatment condition.
The average body temperature (+SEM) in °C for each treatment condition is plotted for all subsequent days. N = 15 for vehicle and for both NLX-101 and F13714 N = 16 for each of the observations. Differences from vehicle on the same day are indicated by hashes (Bonferroni's t-tests, # $P < 0.05$; ## $P < 0.01$; ### $P < 0.001$). Differences compared to day 1 of the same treatment are indicated by asterisk (LSD t-tests, * $P < 0.05$; *** $P < 0.001$). Animals received i.p. injections for 14 consecutive days of vehicle, 0.32 mg/kg NLX-101 or 0.02 mg/kg F13714.

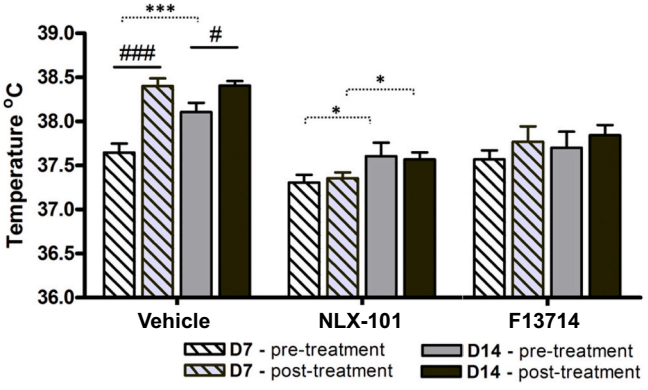

Fig. 3. Mean body temperature per condition, measured before and after treatment on day 7 and 14.
For each of the treatment conditions average body temperature (+SEM) measured 30 min before, and 30 min after treatment are depicted separately for both day 7 (D7, striped bars) and day 14 (D14, plain bars). Treatment condition is indicated on the x-axis, with the 4 different bars representing repeated measures in the same animals (NLX-101 and F13714, N = 16; Vehicle, N = 15). Differences between pre- and post-measurement per day are indicated by hashes (paired samples t-test, # $P < 0.05$; ### $P < 0.001$). Asterisks indicate a difference between day 7 and 14 measurements of a treatment condition (paired samples t-test, * $P < 0.05$; *** $P < 0.001$). Animals received i.p. injections for 14 consecutive days of vehicle, 0.32 mg/kg NLX-101 or 0.02 mg/kg F13714.

4. Discussion

4.1. Differential effects of chronic pre- and post-synaptic 5-HT$_{1A}$R stimulation on OPS performance

The OPS task results showed that acute treatment with F13714, which activates 5-HT$_{1A}$ autoreceptors, fully impaired pattern separation compared with OPS performance levels similar to zero (chance) and vehicle. These results are in line with a previous study performed by us, in which we investigated in depth the dose-response relationship between acute F13714 and NLX-101 treatment and OPS performance (van Goethem et al., 2015). As a follow-up to the study by van Goethem et al. (2015), the current study investigated the chronic effects of F13714 and NLX-101 treatment. The OPS task results show that the initial impairment in OPS performance after acute F13714 treatment was ameliorated after 14 days of chronic treatment: while acutely the F13714 treated animals showed poor OPS performance, this performance increased to vehicle levels after 14 day of chronic treatment. This shift in performance might be explained by desensitization of pre-synaptic 5-HT$_{1A}$Rs in the DRN due to the continuous stimulation of these receptors (Assie et al., 2006), resulting in a reduction of the initial inhibition of cortical 5-HT release, which lead to the acute impairment in OPS performance.

Similar to the study by van Goethem et al. (2015), acute treatment with NLX-101 improved OPS performance. Furthermore, NLX-101 treated animals performed significantly better than F13714 treated animals. OPS performance of NLX-101 treated animals remained stable after 14 days of chronic treatment, i.e. tachyphylaxis was not observed.

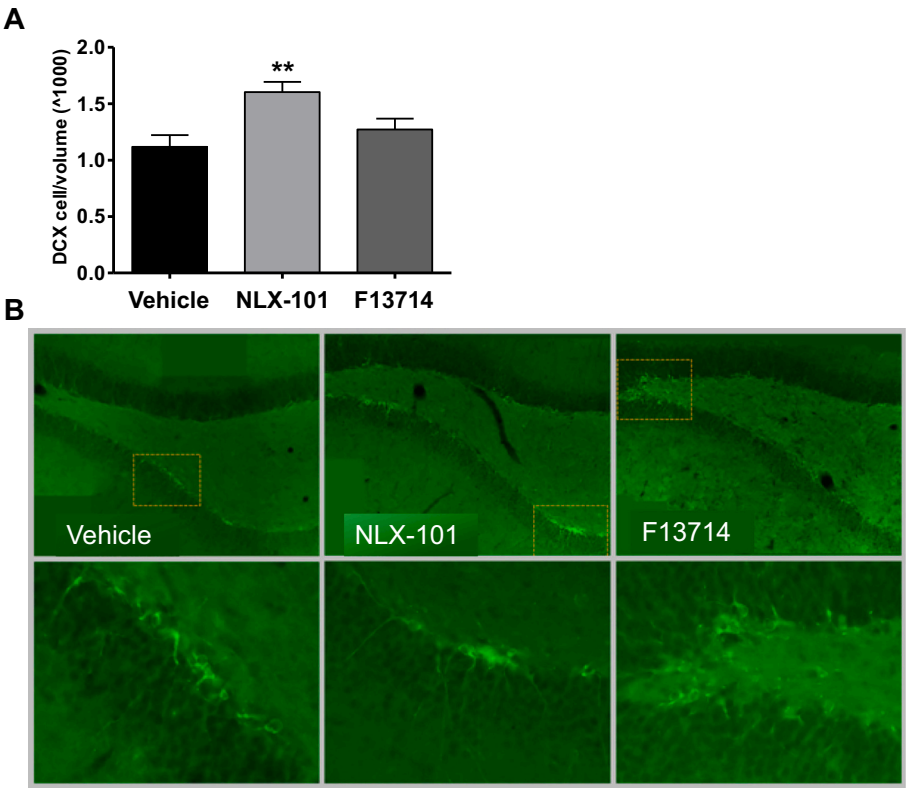

Fig. 4. Mean DCX ratio per condition and corresponding microscopy images.
A) Given is the mean ratio of estimated total DCX-positive cells in the DG divided by the total volume of the region analyzed, obtained from dorsal hippocampus sections of N = 8 chronically treated animals for each treatment condition. A difference from vehicle is indicated by asterisks (Bonferroni's multiple comparisons: ** $P < 0.01$). B) Example images of the analyzed DCX labelled sections from each treatment condition. Above: image at 10× magnification showing the granule cell layer of the DG. Below: close up of the 10× images indicated by the yellow box, showing DCX-positive cells in more detail. Animals received i.p. injections for 14 consecutive days of vehicle, 0.32 mg/kg NLX-101 or 0.02 mg/kg F13714.

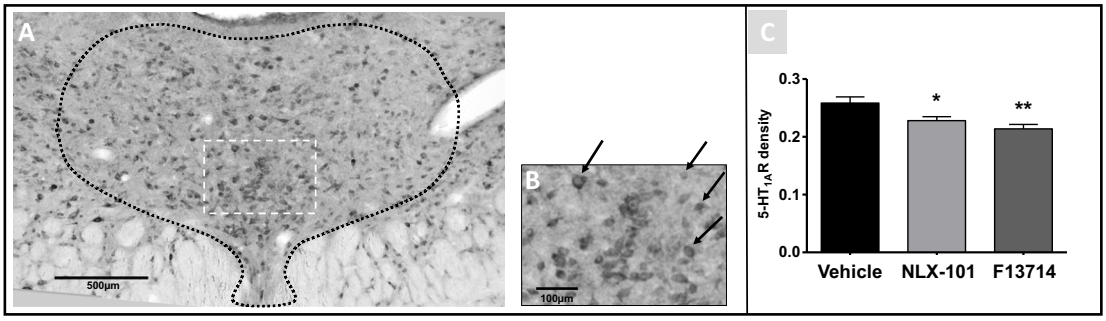

Fig. 5. Summary of 5-HT_{1A}R distribution measures in DRN.
A) Microscopically obtained image of a 5-HT_{1A}R labelled DRN section from a vehicle treated animal, with the region of interested used for analysis delineated by the black dotted line. B) Close up of image A (indicated by the white square), showing dense 5-HT_{1A}R presence across cells of the DRN. Arrows indicate examples of cells which are labelled entirely of high enough intensity (above threshold) to be included in the measurements. C) Graph illustrating the mean ratio between 5-HT_{1A}R positive labelled surface and the remaining surface of the region analyzed. A difference from vehicle is indicated with asterisks (Bonferroni's multiple comparisons: * $P < 0.05$; **$P < 0.01$). Animals received i.p. injections for 14 consecutive days of vehicle, 0.32 mg/kg NLX-101 or 0.02 mg/kg F13714.

This suggests that the potential desensitization of 5-HT_{1A} receptors was specific for autoreceptors in the DRN and did not extend to postsynaptic receptors in the hippocampus.

4.2. Desensitization in the DRN could be explained by reduced 5-HT_{1A} autoreceptor densities

DRN receptor density ratios showed that F13714 treated animals had a lower receptor density in the plasma membranes compared to vehicle-treated animals after 14 days of chronic treatment. This suggests an internalization of 5-HT_{1A} receptors, which could be a mechanism of desensitization that could potentially explain the differential effects of OPS performance in F13714 treated animals after acute versus chronic treatment. 5-HT_{1A} receptor internalization from the plasma membrane into the cytoplasm has previously been demonstrated in literature (Riad et al., 2001).

Unexpectedly, the density of pre-synaptic autoreceptors in the DRN was also decreased after chronic NLX-101 treatment. Binding studies of NLX-101 to membrane-bound 5-HT_{1A}Rs showed only little activation of 5-HT_{1A}Rs at pre-synaptic sites in the DRN for NLX-101 (Newman-Tancredi et al., 2009). Yet, at a dose of 0.32 mg/kg, NLX-101 was found to elicit some inhibition of serotonin release (Llado-Pelfort et al., 2010). Thus, this does not exclude the possibility of NLX-101 binding to pre-synaptic 5-HT_{1A}Rs in our study, resulting in a chronic, small activation of these presynaptic receptors in the DRN. This light, yet chronic activation, could still lead to a desensitization reflected in a reduced presence of these receptors in the membrane of the DRN. This could also explain why acute treatment with NLX-101 only had a subtle enhancing effect on OPS performance, since a low activation of 5-HT_{1A} autoreceptors might suppress the OPS performance enhancing post-synaptic

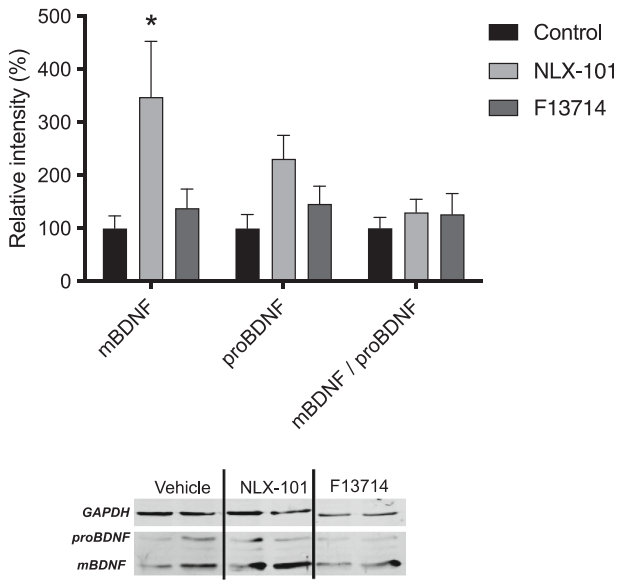

Fig. 6. Mean quantity percentages and SEM of BDNF per condition and the corresponding western blot.
Mean relative mature BDNF (mBDNF) and proBDNF quantity, and the mBDNF/proBDNF ratio in dorsal hippocampus tissue of rats subjected to chronic treatment with NLX-101 (N = 8), F13714 (N = 8) and vehicle (N = 7). of each condition. Mean differences are expressed percentages, with vehicle set to 100%. A difference from vehicle is indicated by asterisks (Bonferroni correction: * $P < 0.05$). Of note, full images of the cropped western blot examples can be found as supplementary Figs. S2–S5. Animals received i.p. injections for 14 consecutive days of vehicle, 0.32 mg/kg NLX-101 or 0.02 mg/kg F13714.

effects of NLX-101. This would also be consistent with the 'bell-shape' dose-response curve observed for OPS performance in our previous study (van Goethem et al., 2015). Indeed, this would also suggest that chronic treatment with NLX-101 should elicit stronger positive effects on OPS performance compared to acute treatment, due to the reduced autoreceptor density in the DRN. This was, however, not observed and can most likely be explained by a ceiling effect in the OPS task. In order to confirm that the observed results are indeed 5-HT_{1A} receptor specific, a combination treatment with 5-HT_{1A} receptor antagonist WAY-100635 may be an important addition for future research.

4.3. Differences in 5-HT_{1A} postsynaptic receptor responses to chronic stimulation

The core-body temperature measurements illustrate the differential effects of chronic stimulation by NLX-101 and F13714 on the hypothalamic 5-HT_{1A}R subpopulation over time. Initial activation of 5-HT_{1A} autoreceptors by F13714 did not alter core body temperature compared to vehicle. However, a drop in core body temperature could be detected after 4 days of F13714 treatment, probably reflecting increased activation of post-synaptic 5-HT_{1A}Rs in the PVN. This drop in body temperature indicates that there is a F13714-induced increase in serotonergic signaling in the PVN compared to the vehicle condition although the precise mechanism of this effect remains to be determined. In NLX-101 treated animals, a gradual increase of core-body temperature was observed after an initial drop in temperature. This indicates a possible desensitization occurring in the postsynaptic hypothalamic receptors. Previous studies have already suggested a functional desensitization of 5-HT_{1A}Rs in the PVN, mediated by (indirect) 5-HT_{2A} activation (Zhang et al., 2001; Zhang et al., 2004). As body temperature measurements after NLX-101 treatment remained lower than those after vehicle treatment during the entire chronic treatment period, it can be assumed that there is no full desensitization of 5-HT_{1A}Rs in the PVN. It is likely that such possible partial functional desensitization in the PVN could depend on post-translational modifications of the 5-HT_{1A} receptor, or via a cyclooxygenase-dependent metabolite of arachidonic acid, as previously suggested by others (Evans et al., 2001).

Comparison of the pre-treatment body temperature to the post-treatment body temperature measurements, taken on day 7 and 14 of treatment, revealed that the vehicle-treated animals showed an increase in temperature between pre- and post- administration measurements on both day 7 and 14. This rise in temperature is most likely caused by injection stress and anticipation stress for the daily post-treatment temperature measurements (Olivier et al., 2003). This response was likely being suppressed in both drug treatment conditions by the strong regulatory effect of PVN 5-HT_{1A}R activation.

4.4. Chronic NLX-101 treatment enhances hippocampal plasticity

NLX-101 treated animals showed a strong increase of doublecortin (DCX) positive cells in the subgranular zone (SGZ) of the dorsal hippocampus compared to vehicle after 14 days of chronic treatment. Expression of DCX occurs during active division by neuronal precursor cells and keeps on being expressed for 2–3 weeks by their daughter cells (von Bohlen Und Halbach, 2007). Therefore, DCX labels all immature neuronal cells which started differentiating over the entire course of the chronic treatment. This suggests that chronic NLX-101 treatment induced an increase in neurogenesis, which was previously found to be necessary in the DG for proper pattern separation function (Franca et al., 2017). Additionally, it has previously been demonstrated that both acute and chronic 5-HT_{1A}R activation can increase hippocampal neurogenesis by promoting precursor proliferation and survival of late differentiating cells, respectively (Huang and Herbert, 2005; Klempin et al., 2010; Soumier et al., 2010). Furthermore, 14-day chronic treatment with partial 5-HT1A agonist tandospirone in male Sprague-Dawley rats was shown to increase DCX-positive cells in the DG of the hippocampus similar to the effects of NLX-101 in the current study (Mori et al., 2014). Chronic treatment with F13714 did not affect the number of DCX positive cells. Despite an increase in OPS performance compared to acute F13714 treatment, chronic F13714 treatment only resulted in an OPS performance similar to vehicle. Therefore it is unsurprising that chronic F13714 treatment did not affect neurogenesis by measure of DCX positive cells.

Additionally, several protein markers related to neuronal plasticity and memory were quantified in dorsal hippocampal tissue and compared between conditions. PSD95, a marker for post-synaptic spine and synapse maturation, previously been found to mediate 5-HT_{1A}R synaptogenesis in the hippocampus (Dong et al., 1997), did not differ between treatment groups in our study. Similar results were found for synaptophysin, a protein present in all presynaptic synapses, and the pCREB/CREB ratio. The quantity of mBDNF was increased by NLX-101 treatment, whereas proBDNF, and the mBDNF/proBDNF ratio remained unaffected. Together, this indicates that F13714 did not affect synapse quantity. BDNF is a common growth factor that has been linked to neuronal plasticity through stimulation of neuronal proliferation, survival, migration, phenotypic differentiation, axonal and dendritic growth, and synapse formation (Martinowich and Lu, 2008). Increases in its expression have been found to be mediated by 5-HT signaling (Mattson et al., 2004). The observed increase in mBDNF quantity therefore indicates that NLX-101 treatment could have induced specific structural changes in synapse architecture or pruning, which could influence pattern separation performance after chronic treatment, next to neuronal proliferation and differentiation. This is supported by Aguiar et al. (2020) who showed that chronic NLX-101 treatment could rescue BDNF protein levels and increase dendritic length in the hippocampus of mice subjected to bilateral common carotid artery occlusion as a model for brain ischemia.

4.5. Conclusion and future perspectives

Taken together, this study underlines the complexity of 5-HT_{1A}R functioning, which appears to be highly dependent on the localization of the receptors within different brain structures. More research into these differential effects as well as different signaling cascades is needed to further elucidate the role of 5-HT_{1A}Rs. We can conclude that selective post-synaptic 5-HT_{1A}R stimulation by NLX-101 enhanced hippocampal serotonergic signaling and neuronal plasticity, with a positive effect on pattern separation performance itself, while F13714 initially impaired OPS performance, an effect that disappeared after 5-HT_{1A} autoreceptor desensitization in the DRN. It must be noted that the study focused on male rodents only, so future research should also investigate whether sex-differences could influence the effects of 5-HT_{1A} autoreceptor versus postsynaptic receptor activation. Overall, the present study provides compelling evidence of the differential regulation of 5-HT_{1A}Rs by two selective biased agonists targeting different receptor subpopulations. The data support the development of biased agonists that target post-synaptic 5-HT_{1A} receptors as potential treatments for disorders involving pattern separation deficits.

Declaration of competing interest

ANT and MV have a proprietary interest in NLX-101 but did not financially contribute to this study. All other authors declare no conflict of interest.

Acknowledgements

BvH was financially supported by the Heal initiative of Maastricht University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mcn.2022.103719.

References

1 Aguiar, R.P., Soares, L.M., Meyer, E., da Silveira, F.C., Milani, H., Newman-Tancredi, A., Varney, M., Prickaerts, J., Oliveira, R.M.W., 2020. Activation of 5-HT1A postsynaptic receptors by NLX-101 results in functional recovery and an increase in neuroplasticity in mice with brain ischemia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 99, 109832.
2 Akkerman, S., Blokland, A., Reneerkens, O., van Goethem, N.P., Bollen, E., Gijselaers, H.J., Lieben, C.K., Steinbusch, H.W., Prickaerts, J., 2012. Object recognition testing: methodological considerations on exploration and discrimination measures. Behav. Brain Res. 232, 335–347.
3 Akkerman, S., Prickaerts, J., Steinbusch, H.W., Blokland, A., 2012. Object recognition testing: statistical considerations. Behav. Brain Res. 232, 317–322.
4 Ally, B.A., Hussey, E.P., Ko, P.C., Molitor, R.J., 2013. Pattern separation and pattern completion in Alzheimer's disease: evidence of rapid forgetting in amnestic mild cognitive impairment. Hippocampus 23, 1246–1258.
5 Arnold, S.A., Hagg, T., 2012. Serotonin 1A receptor agonist increases species- and region-selective adult CNS proliferation, but not through CNTF. Neuropharmacology 63, 1238–1247.
7 Assie, M.B., Lomenech, H., Ravailhe, V., Faucillon, V., Newman-Tancredi, A., 2006. Rapid desensitization of somatodendritic 5-HT1A receptors by chronic administration of the high-efficacy 5-HT1A agonist, F13714: a microdialysis study in the rat. Br. J. Pharmacol. 149, 170–178.
6 Assie, M.B., Bardin, L., Auclair, A.L., Carilla-Durand, E., Depoortere, R., Koek, W., Kleven, M.S., Colpaert, F., Vacher, B., Newman-Tancredi, A., 2010. F15599, a highly selective post-synaptic 5-HT(1A) receptor agonist: in-vivo profile in behavioural models of antidepressant and serotonergic activity. Int. J. Neuropsychopharmacol. 13, 1285–1298.
8 Blier, P., Ward, N.M., 2003. Is there a role for 5-HT1A agonists in the treatment of depression? Biol. Psychiatry 53, 193–203.
9 Clelland, C.D., Choi, M., Romberg, C., Clemenson, G.D., Fragniere, A., Tyers, P., Jessberger, S., Saksida, L.M., Barker, R.A., Gage, F.H., Bussey, T.J., 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325, 210.
10 Curtis, M.J., Alexander, S., Cirino, G., Docherty, J.R., George, C.H., Giembycz, M.A., Hoyer, D., Insel, P.A., Izzo, A.A., Ji, Y., MacEwan, D.J., Sobey, C.G., Stanford, S.C., Teixeira, M.M., Wonnacott, S., Ahluwalia, A., 2018. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br. J. Pharmacol. 175, 987–993.
11 Das, T., Ivleva, E.I., Wagner, A.D., Stark, C.E., Tamminga, C.A., 2014. Loss of pattern separation performance in schizophrenia suggests dentate gyrus dysfunction. Schizophr. Res. 159, 193–197.
12 Depoortere, R., Auclair, A.L., Bardin, L., Colpaert, F.C., Vacher, B., Newman-Tancredi, A., 2010. F15599, a preferential post-synaptic 5-HT1A receptor agonist: activity in models of cognition in comparison with reference 5-HT1A receptor agonists. Eur. Neuropsychopharmacol. 20, 641–654.
13 Dong, J., de Montigny, C., Blier, P., 1997. Effect of acute and repeated versus sustained administration of the 5-HT1A receptor agonist ipsapirone: electrophysiological studies in the rat hippocampus and dorsal raphe. Naunyn Schmiedeberg's Arch. Pharmacol. 356, 303–311.
14 Eisch, A.J., Petrik, D., 2012. Depression and hippocampal neurogenesis: a road to remission? Science 338, 72–75.
15 Ennaceur, A., Delacour, J., 1988. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav. Brain Res. 31, 47–59.
16 Evans, K.L., Cropper, J.D., Berg, K.A., Clarke, W.P., 2001. Mechanisms of regulation of agonist efficacy at the 5-HT(1A) receptor by phospholipid-derived signaling components. J. Pharmacol. Exp. Ther. 297, 1025–1035.
17 Faghihi, F., Moustafa, A.A., 2015. A computational model of pattern separation efficiency in the dentate gyrus with implications in schizophrenia. Front. Syst. Neurosci. 9, 42.
18 Franca, T.F.A., Bitencourt, A.M., Maximilla, N.R., Barros, D.M., Monserrat, J.M., 2017. Hippocampal neurogenesis and pattern separation: a meta-analysis of behavioral data. Hippocampus 27, 937–950.
19 Frankland, P.W., Kohler, S., Josselyn, S.A., 2013. Hippocampal neurogenesis and forgetting. Trends Neurosci. 36, 497–503.
20 Hensler, J.G., 2003. Regulation of 5-HT1A receptor function in brain following agonist or antidepressant administration. Life Sci. 72, 1665–1682.
21 Huang, G.J., Herbert, J., 2005. The role of 5-HT1A receptors in the proliferation and survival of progenitor cells in the dentate gyrus of the adult hippocampus and their regulation by corticoids. Neuroscience 135, 803–813.
22 Kheirbek, M.A., Klemenhagen, K.C., Sahay, A., Hen, R., 2012. Neurogenesis and generalization: a new approach to stratify and treat anxiety disorders. Nat. Neurosci. 15, 1613–1620.
23 Klempin, F., Babu, H., De Pietri Tonel, D., Alarcon, E., Fabel, K., Kempermann, G., 2010. Oppositional effects of serotonin receptors 5-HT1a, 2, and 2c in the regulation of adult hippocampal neurogenesis. Front. Mol. Neurosci. 3.
24 Koek, W., Vacher, B., Cosi, C., Assié, M.-B., Patoiseau, J.-F., Pauwels, P.J., Colpaert, F.C., 2001. 5-HT1A receptor activation and antidepressant-like effects: F 13714 has high efficacy and marked antidepressant potential. Eur. J. Pharmacol. 420, 103–112.
25 Llado-Pelfort, L., Assie, M.B., Newman-Tancredi, A., Artigas, F., Celada, P., 2010. Preferential in vivo action of F15599, a novel 5-HT(1A) receptor agonist, at postsynaptic 5-HT(1A) receptors. Br. J. Pharmacol. 160, 1929–1940.
26 Madden, A.M., Zup, S.L., 2014. Effects of developmental hyperserotonemia on juvenile play behavior, oxytocin and serotonin receptor expression in the hypothalamus are age and sex dependent. Physiol. Behav. 128, 260–269.
27 Martinelli, C., Shergill, S.S., 2015. Clarifying the role of pattern separation in schizophrenia: the role of recognition and visual discrimination deficits. Schizophr. Res. 166, 328–333.
28 Martinowich, K., Lu, B., 2008. Interaction between BDNF and serotonin: role in mood disorders. Neuropsychopharmacology 33, 73–83.
29 Mattson, M.P., Maudsley, S., Martin, B., 2004. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci. 27, 589–594.
30 Miller, B.R., Hen, R., 2015. The current state of the neurogenic theory of depression and anxiety. Curr. Opin. Neurobiol. 30, 51–58.
31 Mori, M., Murata, Y., Matsuo, A., Takemoto, T., Mine, K., 2014. Chronic treatment with the 5-HT1A receptor partial agonist tandospirone increases hippocampal neurogenesis. Neurol. Ther. 3, 67–77.
32 Newman-Tancredi, A., Martel, J.C., Assie, M.B., Buritova, J., Lauressergues, E., Cosi, C., Heusler, P., Bruins Slot, L., Colpaert, F.C., Vacher, B., Cussac, D., 2009. Signal transduction and functional selectivity of F15599, a preferential post-synaptic 5-HT1A receptor agonist. Br. J. Pharmacol. 156, 338–353.
33 Olivier, B., Zethof, T., Pattij, T., van Boogaert, M., van Oorschot, R., Leahy, C., Oosting, R., Bouwknecht, A., Veening, J., van der Gugten, J., 2003. Stress-induced hyperthermia and anxiety: pharmacological validation. Eur. J. Pharmacol. 463, 117–132.
34 Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates in Stereotaxic Coordinates. Elsevier.
35 Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S. T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Wurbel, H., 2020. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. Br. J. Pharmacol. 177, 3617–3624.
36 Rausch, J.L., Johnson, M.E., Kasik, K.E., Stahl, S.M., 2006. Temperature regulation in depression: functional 5HT1A receptor adaptation differentiates antidepressant response. Neuropsychopharmacology 31, 2274–2280.
37 Riad, M., Watkins, K.C., Doucet, E., Hamon, M., Descarries, L., 2001. Agonist-induced internalization of serotonin-1a receptors in the dorsal raphe nucleus (autoreceptors) but not hippocampus (heteroreceptors). J. Neurosci. 21, 8378–8386.
38 Rojas, P.S., Fiedler, J.L., 2016. What do we really know about 5-HT1A receptor signaling in neuronal cells? Front. Cell. Neurosci. 10, 272.

39 Schreiber, R., Newman-Tancredi, A., 2014. Improving cognition in schizophrenia with antipsychotics that elicit neurogenesis through 5-HT(1A) receptor activation. Neurobiol. Learn. Mem. 110, 72–80.

40 Seyedabadi, M., Fakhfouri, G., Ramezani, V., Mehr, S.E., Rahimian, R., 2014. The role of serotonin in memory: interactions with neurotransmitters and downstream signaling. Exp. Brain Res. 232, 723–738.

41 Soumier, A., Banasr, M., Kerkerian-Le Goff, L., Daszuta, A., 2010. Region- and phase-dependent effects of 5-HT(1A) and 5-HT(2C) receptor activation on adult neurogenesis. Eur. Neuropsychopharmacol. 20, 336–345.

42 Stark, S.M., Yassa, M.A., Lacy, J.W., Stark, C.E., 2013. A task to assess behavioral pattern separation (BPS) in humans: data from healthy aging and mild cognitive impairment. Neuropsychologia 51, 2442–2449.

43 Vacher, B., Bonnaud, B., Funes, P., Jubault, N., Koek, W., Assié, M.-B., Cosi, C., 1998. Design and synthesis of a series of 6-Substituted-2-pyridinylmethylamine derivatives as novel, high-affinity, selective agonists at 5-HT1A receptors. J. Med. Chem. 41, 5070–5083.

44 van Goethem, N.P., Schreiber, R., Newman-Tancredi, A., Varney, M., Prickaerts, J., 2015. Divergent effects of the 'biased' 5-HT1 a receptor agonists F15599 and F13714 in a novel object pattern separation task. Br. J. Pharmacol. 172, 2532–2543.

45 van Goethem, N.P., van Hagen, B.T.J., Prickaerts, J., 2018. Assessing spatial pattern separation in rodents using the object pattern separation task. Nat. Protoc. 13, 1763–1792.

46 van Hagen, B.T., van Goethem, N.P., Lagatta, D.C., Prickaerts, J., 2015. The object pattern separation (OPS) task: a behavioral paradigm derived from the object recognition task. Behav. Brain Res. 285, 44–52.

47 von Bohlen Und Halbach, O., 2007. Immunohistological markers for staging neurogenesis in adult hippocampus. Cell Tissue Res. 329, 409–420.

48 Yagi, S., Chow, C., Lieblich, S.E., Galea, L.A., 2016. Sex and strategy use matters for pattern separation, adult neurogenesis, and immediate early gene expression in the hippocampus. Hippocampus 26, 87–101.

49 Zhang, Y., D'Souza, D., Raap, D.K., Garcia, F., Battaglia, G., Muma, N.A., Van de Kar, L.D., 2001. Characterization of the functional heterologous desensitization of hypothalamic 5-HT1A receptors after 5-HT2AReceptor activation. J. Neurosci. 21, 7919–7927.

50 Zhang, Y., Gray, T.S., D'Souza, D.N., Carrasco, G.A., Damjanoska, K.J., Dudas, B., Garcia, F., Zainelli, G.M., Sullivan Hanley, N.R., Battaglia, G., Muma, N.A., Van de Kar, L.D., 2004. Desensitization of 5-HT1A receptors by 5-HT2A receptors in neuroendocrine neurons in vivo. J. Pharmacol. Exp. Ther. 310, 59–66.