Rapid antidepressant effects of 5-HT$_{1A}$ Receptor biased agonists

Máster Oficial de la Universidad de Cantabria en

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Abstract

Major depressive disorder (MDD), is a common and severe mood disorder. Globally, ~ 300 million people of all ages suffer from MDD (WHO 2021), which is the leading cause of disability in 2021. Approximately one third of the patients have inappropriate responses or no response at all to treatment. Even though some response is present, all available antidepressant drugs need to be administered for weeks or months to produce a meaningful clinical improvement. Therefore, finding novel and rapid-acting antidepressant treatments is a global priority. In this regard, the finding that ketamine –a dissociative anesthetic- had a rapid and sustained antidepressant effect in depressed people and the subsequent approval of intranasal esketamine for treatment-resistant depression (TRD) has changed the view of depression therapy from monoaminergic to glutamatergic drugs. It is postulated that ketamine's antidepressant activity results from its inhibition of the γ-aminobutyric acid (GABA) interneurons and the consequent disinhibition of cortical glutamatergic systems.

Because selective activation of postsynaptic 5-HT_{1A} receptors induces a rapid antidepressant response, it was thought that novel serotonin 5-HT_{1A} receptor biased agonists could be one of such possible approaches. In the present work, we have studied the behavioral, biochemical and molecular aspects involved in the antidepressant-like effects of NLX-101 (formerly known as F15599). The results show that NLX-101 (0.16 mg/kg) reduced the immobility in the forced swim test when measured 30 min (albeit not 24 h and 7 days) after drug administration. Systemic administration of NLX-101 increased the extracellular concentration of dopamine and glutamate in the medial prefrontal cortex (mPFC). No changes were observed in the prefrontal output of noradrenaline and serotonin (5-HT). NLX-101 also produced a rapid increase in the synthesis of phospho-mTOR 30 min after drug administration.

Altogether, our results suggest that NLX-101 has a rapid antidepressant-like response possibly by reducing the activity of GABA interneurons, thus disinhibiting the release of glutamate by the pyramidal neuron and increasing the production of phospho-mTOR.

Introduction

Major depressive disorder (MDD), is a common and severe mood disorder characterized by a pathologically low mood (hypothymia) and negative esteem about oneself, one's status in the real world, and one's future (Shadrina et al. 2018) that has a duration of two weeks or more. Globally, ~ 300 million people of all ages suffer from MDD (WHO 2021). MDD has a prevalence of 10% - 20% and is the leading cause of disability in 2021. In addition, MDD is the second leading cause of death due to suicide in 15-29-year-old people (WHO 2021). It is estimated that near 800,000 people die due to suicide every year.

Different factors are involved in the development of this disorder, some are hereditary and some are stress-related factors. Indeed, repeated stress can induce a decrease in signaling of brain-derived neurotrophic factor (BDNF) and mechanistic target of rapamycin complex 1 (mTORC1) as well as loss of dendritic spines, which have been associated with the development of MDD. Also, different areas of the brain, such as hippocampus, cerebral cortex, amygdala, nucleus accumbens and hypothalamus are involved in the stress response and the pathogenesis of MDD.

Despite the prevalence of the illness, the great majority of medications developed have been based upon the so-called monoaminergic hypothesis, thus increasing the level of monoamines in the brain. The first antidepressants that were developed around the 1950s were tricyclic drugs, which inhibit the reuptake of NA and/or 5-HT, thus increasing their synaptic availability, and augmenting their action on postsynaptic receptors (Harvey et al. 2010).

Some years later, monoamine receptor antagonists and enzyme inhibitors were also developed as alternate mechanisms to enhance synaptic availability of monoamines (Di Giovanni et al. 2016). The main enzyme inhibitors used were monoamine oxidase (MAO) inhibitors (MAOI). These compounds increase synaptic concentration of monoamines by inhibiting their metabolism. Tricyclic and MAOI antidepressant drugs were somehow effective in treating MDD. However, they also bear a bad profile of adverse effects that compromise adherence to treatment. For this main reason, further investigations lead to the development of drugs that selectively block the reuptake of serotonin (selective serotonin reuptake inhibitors [SSRIs] and selective noradrenaline reuptake inhibitors [SNRIs]). These drugs possess a better profile of adverse effects, which make adherence to treatment improved and therefore have been widely

and primarily used to treat MDD ever since.

Other drugs, i.e the so-called atypical antidepressants were introduced later and had different mechanisms of action. For example, mianserin (antagonist of α_1- and α_2-adrenergic receptors) or trazodone (5-HT reuptake inhibitor and 5-HT_{2A} receptor antagonist).

Regardless of the widespread use of such different antidepressant drugs, all of them had the same problem, i.e. while the pharmacological effect was immediate, the therapeutic effect only appeared after chronic treatment that lasted weeks or even months. Furthermore, there is an important percentage of the population that do not respond to treatment, which is estimated to be approximately one third of the patients. Therefore, finding novel and rapid-acting antidepressant treatments is a global priority.

In this regard, the finding that ketamine –a dissociative anesthetic- had a rapid and sustained antidepressant effect in depressed people and the subsequent approval of intranasal esketamine for treatment-resistant depression (TRD) has changed the view of depression therapy from monoaminergic to glutamatergic drugs. It is postulated that ketamine's antidepressant activity results from blockade of glutamatergic NMDA receptors in γ-aminobutyric acid (GABA) interneurons and the consequent disinhibition of cortical glutamatergic systems. In support of this mechanism of action it has been demonstrated that the administration of MK-801, another NMDA receptor antagonist similar to ketamine, predominately decreases the activity of putative GABA interneurons but, at a delayed rate, increases the firing rate of the majority of pyramidal neurons (Homayoun and Moghaddam, 2007).

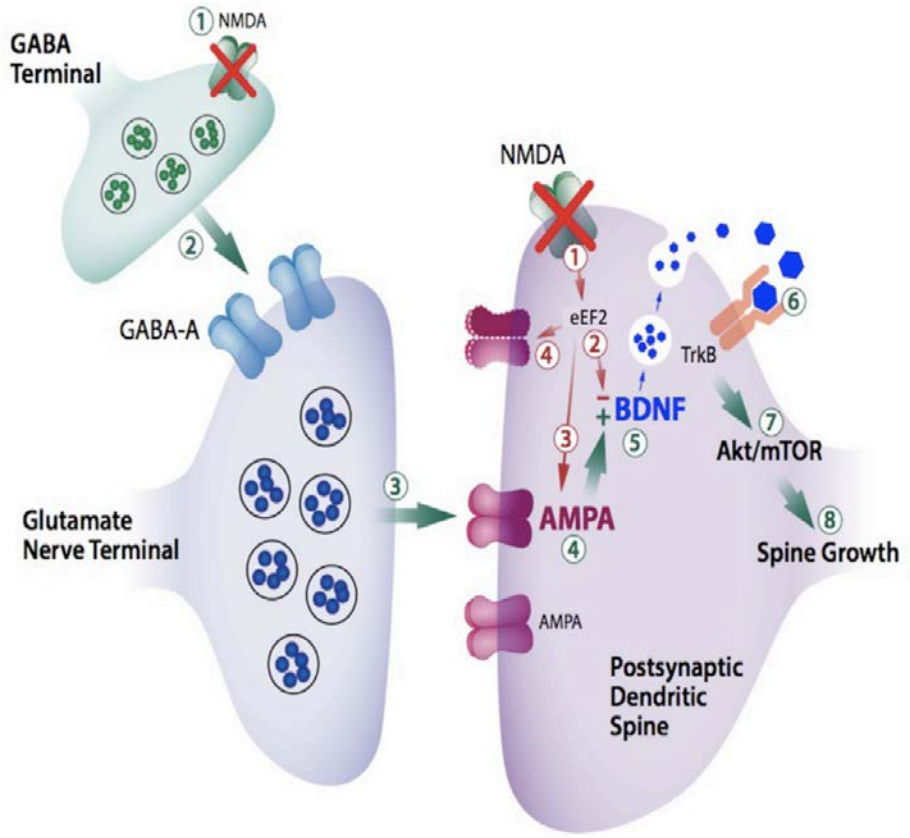

*Figure 1. Ketamine blocks the NMDA receptor localized to GABA neurons, thus disinhibiting pyramidal, glutamatergic neurons (*Krystal et al. 2019).

Ketamine as antidepressant

Ketamine has recently revolutionized the field of pharmacotherapy of depression because it relieves symptoms rapidly (in 2 hours) and has a sustained effect that lasts 7 days. Ketamine is a blocker of the NMDA receptor of glutamate localized to GABA interneurons, which is postulated to reduce GABAergic input to pyramidal neurons, thus disinhibiting glutamatergic transmission in the prefrontal cortex. Blockade of these NMDA receptors raises BDNF levels and boosts AMPA glutamate transmission, thus enhancing synaptic efficacy. AMPA receptor activation activates a signaling cascade that raises BDNF levels. Local release of BDNF is thought to stimulate TrkB receptors, engaging relevant signaling cascades and resulting in the activation of the molecular target of rapamycin complex 1 (mTORC1). This step, in turn, activates local protein synthesis necessary for increasing dendritic spine formation and restoring synaptic connectivity (Krystal et al. 2019).

In experiments with rodents, ketamine reduces immobility and increases swimming behaviors in the forced swim test (FST), a predictive animal model of antidepressant-like effects (Castagné et al. 2011; López-Gil et al. 2019). From a biochemical viewpoint, ketamine increases the release of 5-HT, (Amargós-Bosch et al. 2006; López-Gil et al. 2019), noradrenaline (Lorrain et al. 2003), as well as glutamate (Moghaddam et al. 1997; López-Gil et al. 2019) in the mPFC. Interestingly, the rapid antidepressant-like effects of ketamine at 30 min after its administration does not appear to be dependent on serotonergic transmission as it occurs 24 h after administration (Gigliucci et al. 2019). Unfortunately, ketamine has psychotomimetic adverse effects that compromise its therapeutic use. However, these adverse effects fade before the antidepressant effects emerge (Berman et al., 2000; Zarate et al., 2006).

Inhibition of GABA in the PFC as rapid antidepressant mechanism

GABA is the principal inhibitory neurotransmitter in the CNS and is opposed to the excitatory neurotransmitter glutamate. As an inhibitory neurotransmitter, GABA generates an inhibitory postsynaptic potential (IPSP) and causes hyperpolarization of the postsynaptic neuron while glutamate generates an excitatory postsynaptic potential (EPSP) and elicits depolarization of the postsynaptic neuron. A disruption in the balance between inhibition and excitation, or the glutamate-GABA equilibrium, could result in injury (e.g., strokes, Huntington's disease), overexcitation (e.g., epilepsy, spastic disorders), or excessive inhibition (e.g., hypersomnia, benzodiazepine overdose) (de Leon et al. 2021).

Therefore, the inhibition of cortical GABA transmission and the subsequent surge of glutamate transmission may be a relevant and novel strategy to achieve a rapid antidepressant response associated to the subsequent expression of the postsynaptic proteins involved in neuroplasticity and neurogenesis (Duman et al. 2016).

Serotonin or 5-hydroxytryptamine 5-HT_{1A} receptors

Given the unwanted effects of ketamine, novel mechanisms that also reduce GABA transmission in the prefrontal cortex have been under scrutiny.

Serotonin or 5-hydroxytryptamine 5-HT_{1A} receptors are attractive targets for pharmacotherapy

of different pathologies associated with dysfunctional serotonergic neurotransmission, including anxiety, depression, Parkinson's disease, pain and schizophrenia (Newman-Tancredi 2011).

5-HT_{1A} receptors are expressed both as presynaptic autoreceptors on serotonergic cell bodies in the dorsal raphe nucleus and as postsynaptic heteroreceptors in multiple brain regions including the cortex, hippocampus, septum and hypothalamus. Interestingly, activation of 5-HT_{1A} autoreceptors in the raphe nuclei have pro-depressive effects (Richardson-Jones et al. 2016; Bortolozzi et al. 2012), while stimulation of postsynaptic 5-HT_{1A} heteroreceptors is necessary for the antidepressant action (Blier et al. 1997; Fukumoto et al. 2018).

GABA interneurons of the prefrontal cortex bear a high density of 5-HT_{1A} receptors (Santana et al. 2004) and their activation also enhances pyramidal cell firing (Lladó-Pelfort et al. 2012). Thus, stimulation of 5-HT_{1A} receptors localized to prefrontal GABAergic interneurons are a promising alternative target to treat depressive states. Compounds that impact on these receptors, avoiding the stimulation of 5-HT_{1A} autoreceptors, are called 5-HT_{1A} biased (or functional) agonists. One of such compounds that is examined in the present work is NLX-101 (formerly F15599).

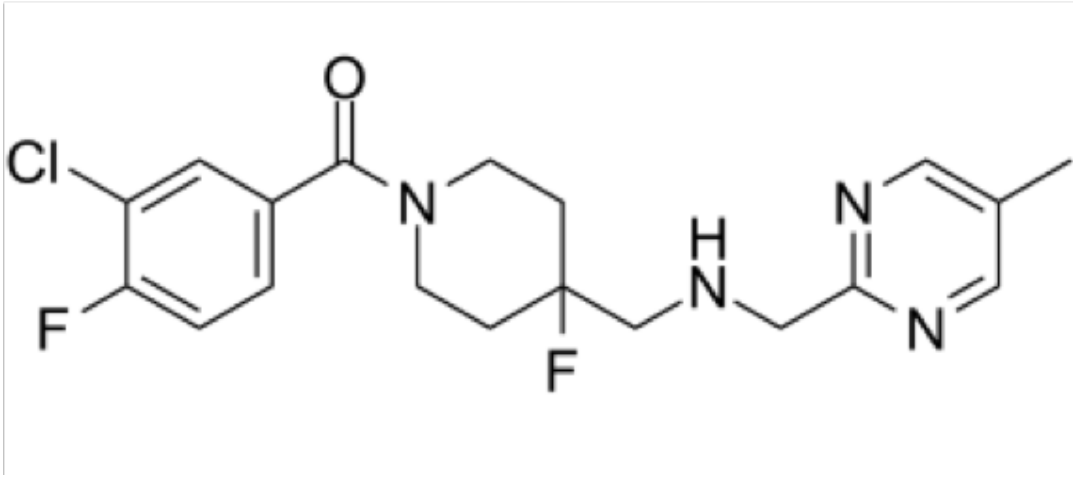

Figure 2. Molecule of NLX-101 also known as F15599.

NLX-101

NLX-101 (also known as F15599) is a highly selective and efficacious 'biased' agonist at cortical 5-hydroxytryptamine 1A (5-HT_{1A}) heteroreceptors preferentially activating Gαi versus Gαo G-protein subtypes. NLX-101 and also preferentially activates ERK1/2 phosphorylation. (Newman-Tancredi 2011). In rodents, NLX-101 possesses marked antidepressant-like activity, measured as suppression of immobility in the FST, albeit with a short duration of action. In addition, NLX-101 displays rapid-acting antidepressant-like effects in the rat chronic mild stress model (Depoortère et al. 2019). It is postulated that NLX-101 is able to inhibit GABA interneurons, thus increasing the release of glutamate in the synaptic cleft and stimulating the expression of proteins involved in neurogenesis and neuroplasticity.

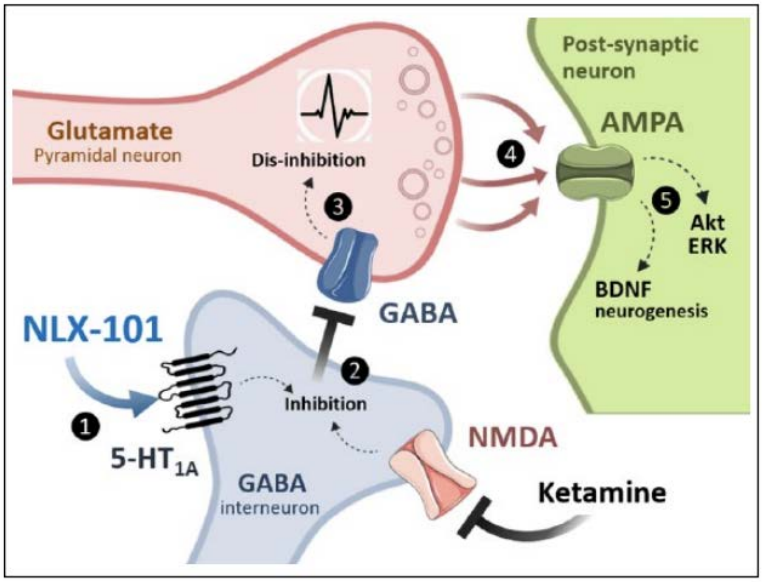

Figure 3. Postulated mechanism of action of NLX-101 (Depoortère et al. 2019).

Objectives

General Objectives: To examine the cellular and molecular mechanisms responsible for the antidepressant-like action of NLX-101.

Specific Objectives:

Objective 1. To assess the antidepressant-like effects of the intraperitoneal administration of 0.16 mg/kg of NLX-101 in the FST and the novelty-suppressed feeding test (NSFT).

Objective 2. To examine the release of noradrenaline, dopamine, serotonin, and glutamate in the mPFC after systemic administration of NLX-101. This will be done using in vivo intracerebral microdialysis coupled to ultra HPLC apparatus with electrochemical detection (Alexys®, Antec, The Netherlands).

Objective 3. To study the intracellular signaling pathways coupled to the administration of these compounds. This will be done using Western blotting analysis (CREB, ERK, mTOR, BDNF, p11, etc.).

Materials and methods

Animals

Male Sprague-Dawley albino rats of 240-260 g were used, maintained with light/dark cycles of 12/12 hours (lights on at 07:00), 22 ± 1 °C and with free access to water and food.

All experimental procedures were carried out in accordance with current Spanish legislation (RD 53/2013) and the Directive of the Council of the European Communities on "Protection of animals used for scientific purposes" of September 22, 2010 (2010/63/EEC) and were approved by the Institutional Committee for the Care and Use of Laboratory Animals of the University of Cantabria.

Pharmacological treatments

NLX-101 was generously donated by Neurolixis and dissolved in distilled water at the concentration of 0.16 mg/kg and injected in a volume of 5 ml/kg. Control rats were injected with distilled water in a volume of 5 ml/kg.

Forced swim test

The forced swim test (FST) evaluates the behavioral despair of the animal and has predictive validity to determine the antidepressant potential of a treatment. Rats were handled daily for 1 week before FST for habituation.

On day 1 (pretest), rats were placed in a clear plexiglas cylinder (46 cm height, 20 cm diameter) filled with 24 ± 1°C water to a height of 30 cm, for 15 min. After this pretest, animals were returned to their home cages and dried under a lamp for 30 min. Twenty-four hours after the pretest, rats received an i.p. injection of 0.16 mg/kg of NLX-101. Three tests were conducted 30 min, 24 h and 7 days after drug administration in the same cylinder for 5 min. The test

sessions were videotaped (ANY-maze, Stoelting Europe, Dublin, Ireland) and the duration of immobility in seconds (s) was annotated.

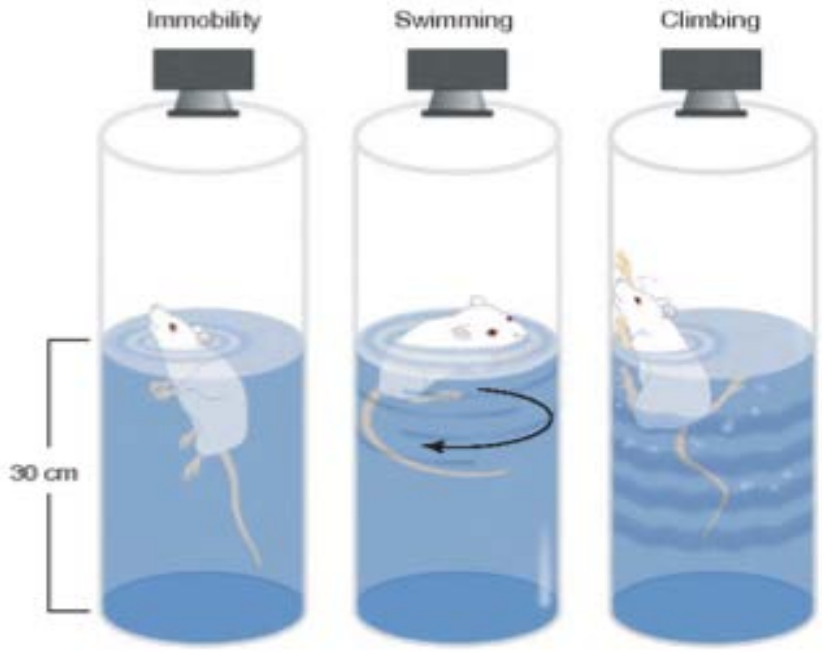

Figure 4. Forced swimming test. Outline of the three types of behavior that rats may exhibit during TNF (Cryan et al. 2002).

Open field test

To check for nonspecific changes in gross activity that would mask FST observations, locomotor activity was recorded for 15 min in an open field arena (100 cm × 100 cm × 40 cm) with gray plastic walls dimly lighted. Ambulatory behavior was video-tracked by a computerized system (Any-maze) and the total distance traveled was measured.

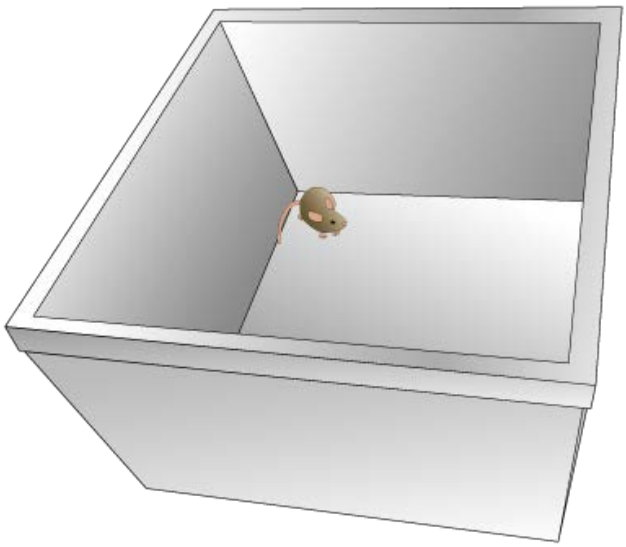

Figure 5. Open field box, From DataBase Center for Life Science (DBCLS) (https://brainstuff.org/blog/what-is-the-open-field-test).

Intracerebral Microdialysis

Concentric dialysis probes with a 4-mm membrane length were implanted under sodium pentobarbital anesthesia (60 mg/kg, i.p.) in the mPFC (AP +3.2 mm, L ±0.6 mm, DV -5.4 mm; from bregma), according to Paxinos and Watson (2007). In all cases, microdialysis experiments were conducted 24 h after surgery in freely moving rats by continuously perfusing probes with artificial cerebrospinal fluid (aCSF: 147 mM NaCl, 3 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$) at a rate of 1.5 µl/min. Dialysate samples of 30 µl were collected every 20 min, and monoamines and glutamate were determined using an Alexys® Analyzer (Antec Scientific, Leiden, The Netherlands) following manufacturer's methods. At the completion of dialysis experiments, rats were given an overdose of sodium pentobarbital and brains were processed for cresyl violet staining to assess the correct location of the probes.

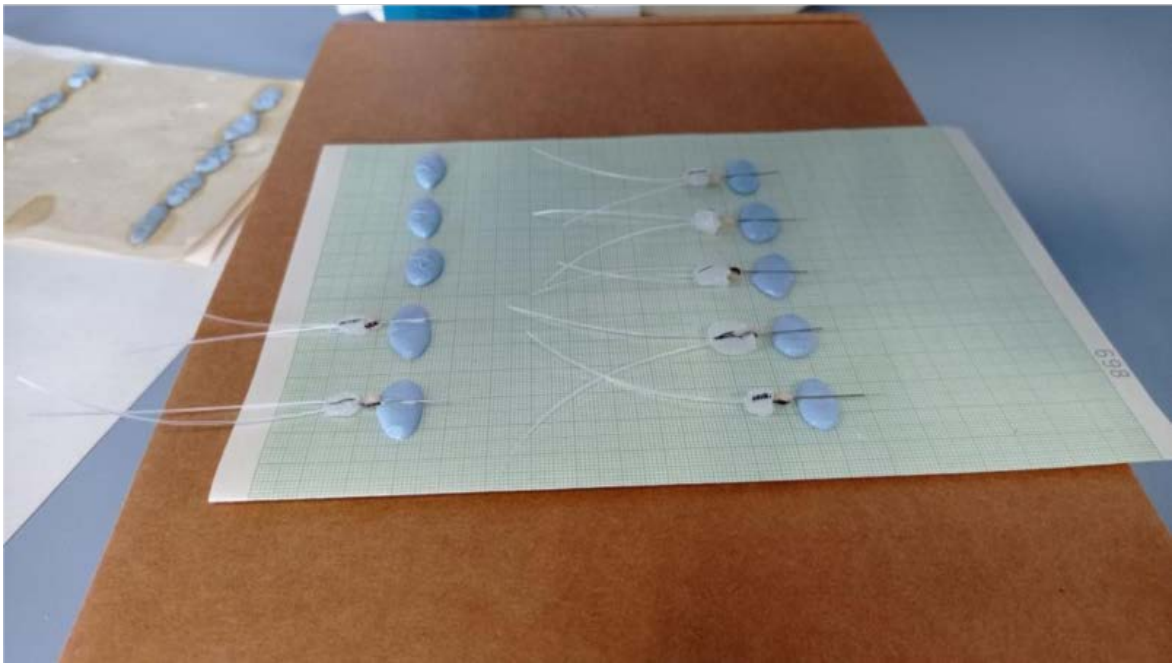

Figure 6. Concentric dialysis probes to perform microdialysis experiments.

Western Blot

To study protein expression after administration of NLX-101 i.p. (0.16 mg/kg), rats (n = 48) were sacrificed and divided in 4 groups: at 30 min, 1h, 2h after treatment. For each group, a control group was established to which the vehicle was injected (saline). Brains were then extracted, the mPFC was dissected on ice and stored at -80 °C.

For cell lysate, mPFC samples were homogenized (1:15) in a solution composed of 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 100 mM KCl, and the following protease and phosphatase inhibitors: 1 mM phenylmethylsulfonyl fluoride (PMSF), 0.2 mg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml pepstatin A, 10 µg/ml antipain, 10 µg/ml chemostatin, 1 mM Na_3VO_4 , 1 mM NaF. To this, lysis buffer (1% Igepal®, 0.5% sodium deoxycholate, 0.1% SDS and 2.5 mM CHAPS) was added and left 30 minutes in ice, then the solubilized proteins were collected in the supernatant after centrifuged for 10 min at 14000 rpm and 4 °C.

The quantification was performed by the Lowry method (Lowry et al. 1951) and aliquots were prepared, to which was added loading buffer (Laemmli Sample Buffer, BIO-RAD, California,

United States) with β-mercaptoethanol at 5% and heated 5 minutes to 100 °C to denature the proteins, then put it on ice for 3 minutes and centrifuged for 5 minutes at 3000 rpm. Afterwards, supernatants were collected and aliquots were stored at -20 °C until used. For each sample, 55 μg of protein (in duplicate) was loaded into discontinuous SDS-PAGE gels, consisting of a separation gel (10% or 15% acrylamide) depending on the molecular weight of the proteins to be analyzed and a packing gel (4% acrylamide). The gels were inserted into the electrophoresis cuvettes (Mini-PROTEAN® Tetra Cell, BIO-RAD, California, United States) with electrophoresis buffer and subjected to a current of 100 V for 15 minutes and 160 V for 50 minutes until the molecular weight marker (PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa, ThermoFisher Scientific, Waltham, Massachusetts, United States) was observed to reach the limit of the gel. The proteins were then transferred to nitrocellulose membranes, immersed in a 20% methanol transfer buffer. Transfer conditions were constant voltage (100 V) for 90 minutes at low temperature. The blockade of the nonspecific junctions was performed by incubating the membranes for an hour with 5% skimmed-milk powder and then incubated for one night at 4 °C with the primary antibodies diluted in 5% milk, to mark the following proteins: GluA1 subunit of glutamate AMPA receptor, postsynaptic density protein 95 (PSD-95), Brain-derived neurotrophic factor BDNF, protein p11, PKB (Protein kinase B) Akt, extracellular signal-regulated protein kinase ERK1/2, cAMP-response element binding protein CREB, β-arrestin 1, β-arrestin 2, mechanistic target of rapamycin mTOR, tubulin.

The blockade of the nonspecific junctions was performed by incubating the membranes for one hour with skimmed milk powder at 3% + NAV/NAF inhibitors and then incubated for one night at 4 °C with the primary antibodies diluted in skimmed milk powder at 3% + NAV/NAF inhibitors, to mark the phosphorylated proteins. For a special protein such as CREB and pCREB, the blockade was performed by incubating the membranes for one hour with bovine serum albumin (BSA) at 5% + NAV/NAF inhibitors and then incubated overnight at 4 °C with the primary antibodies CREB and pCREB, diluted in BSA at 5% + NAV/NAF inhibitors.

The next day the membranes were washed with Tween 20 at 0.05% in salt Tris buffer (TBS-T) and incubated for one hour with conjugated secondary antibodies for fluorescent detection against IgG of mouse, rabbit or goat, at a concentration of 1:15,000, provided by Li-Cor Biosciences (Lincoln, NE, USA). After washing the membranes again with TBS-T, the fluorescence detection was carried out in an Odyssey CLx scanner and for the quantification the Image Studio Lite program was used, both from LiCor Biosciences (Lincoln, NE, USA).

Densitometry values were normalized with respect to the tubulin band.

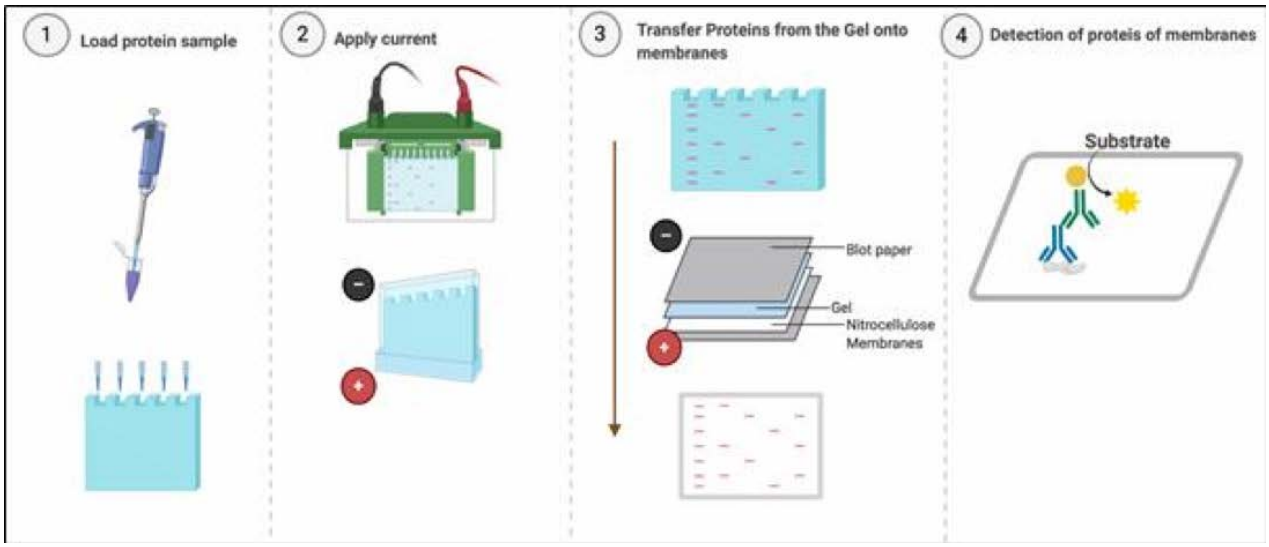

Figure 7 . Western blot 1) Load protein sample 2) Apply current (current of 100 V for 15 minutes and 160 V for 50 minutes) 3) Transfer proteins from the Gel onto membranes 4) Detection of proteins in the membranes (https://info.gbiosciences.com/blog/how-to-prepare-samples-for-Western-blot-analysis-1).

Antigen	*Molecular weight (kDa)*	*Commercial house*	*Reference code*	*Used concentration*	*Host animal*
AKT1	62	SANTA CRUZ	SC5298	1:1000	mouse
pAKT1	60	CELL SIGNALING	CS9271	1:500	rabbit
CREB	40	CELL SIGNALING	CS9104	1:500	mouse
pCREB	46	CELL SIGNALING	CS9198	1:500	rabbit
GLUA 1A	100	ABCAM	AB31232	1:1000	rabbit
pGLUA1 (ps831)	100	ABCAM	AB109464	1:250	rabbit
MTOR	289	CELL SIGNALING	CS4517	1:1000	rabbit
pMTOR	289	CELL SIGNALING	CS2971	1:250	rabbit
P11	11	ABCAM	AB187201	1:200	rabbit
BDNF	14	ABCAM	AB108319	1:250	rabbit
ERK	42-44	ABCAM	AB184699	1:1000	rabbit
pERK	42-44	SIGMA	M8159	1:200	mouse
B ARRESTIN 1	50	SANTA CRUZ	SC53780	1:100	mouse
B ARRESTIN 2	50	CELL SIGNALING	CS3857	1:500	rabbit
PSD95	95	SANTA CRUZ	SC8575	1:200	goat

Table 1. Primary antibodies used for protein marking in western blot. List of antibodies used throughout the study, indicating the molecular weight of the band marked, the commercial house, the concentration used for each of them and the animal from which they have been obtained.

Statistical analysis

Data are expressed as mean ± SEM. Differences between two groups were assessed by two-tailed Student's *t*-test. One or two-way analysis of variance (ANOVA) followed by post-hoc Tukey's multiple comparisons test was used to analyze differences among three or more independent groups.

For microdialysis experiments, changes in monoamines and glutamate concentrations were assessed by repeated measures ANOVA with drug and time as factors, followed by a post-hoc multiple comparisons test. In all cases the level of significance was set at $p < 0.05$.

Results

Forced swim test and open field test

Two-tailed Student's t-test of the forced swim test, show that IP administration of NLX-101 (0.16 mg/kg) reduced the immobility of the court of rats when measured 30 min (albeit not 24 h and 7 days) after drug administration (t=3,076, df=10; (p=0.0117)).

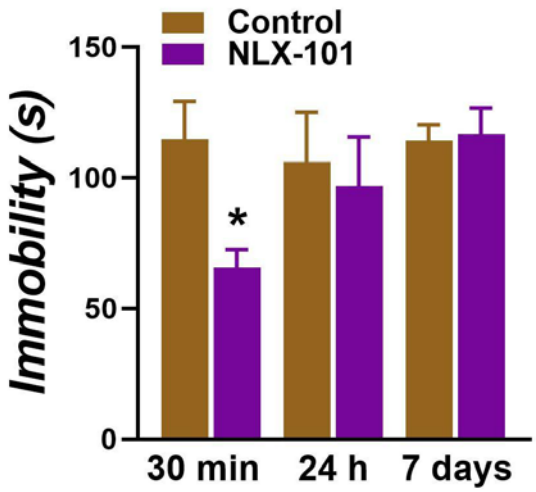

Figure 8. Immobility of a court of 6 rats (type) in the forced swim test when measured 30 minutes, 24 hours and 7 days later, after administration of NLX-101 (0.16 mg/kg). Reduced

immobility in the NLX-101 rats 30 minutes after IP administration.

The results of the open field test showed no difference in locomotion during the 10 minute period.

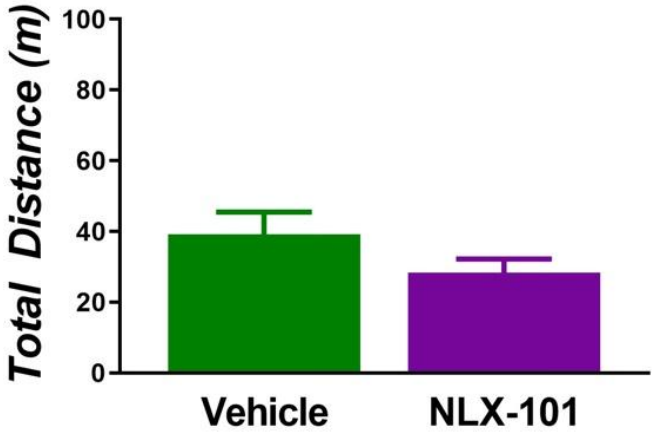

Figure 9. Open field test, total distance made by 5 rats, vehicle and NLX-101. No difference in change of mobility.

Microdialysis

Systemic administration of NLX-101 (0.16 mg/kg) increased the extracellular concentration of neurotransmitter dopamine and glutamate in the mPFC (Figure 10). In contrast, no changes were observed in the extracellular concentrations of noradrenaline and serotonin (5-HT).

For glutamate, the results showed a significant effects of treatment [F(1,12)= 7,351 (P=0,000005)], time [F(9,108)=4,311 (p=0,000083)] and the interaction treatment*time [F(9,108)=5,817 (p=0,000001)]. Post-hoc Tukey's test showed that glutamate concentration in NLX-101-treated rats was higher than that of vehicle treated rats (*p < 0.0005).

For dopamine, the results significant effects of treatment [F(1,11)= 7,1523 (P=0,021624), and the interaction treatment*time [F(9,99)=4,3049 (p=0,000096)]. As for glutamate, post-hoc Tukey's test showed that dopamine concentration in NLX-101-treated rats was higher than that of vehicle treated rats (*p < 0.05).

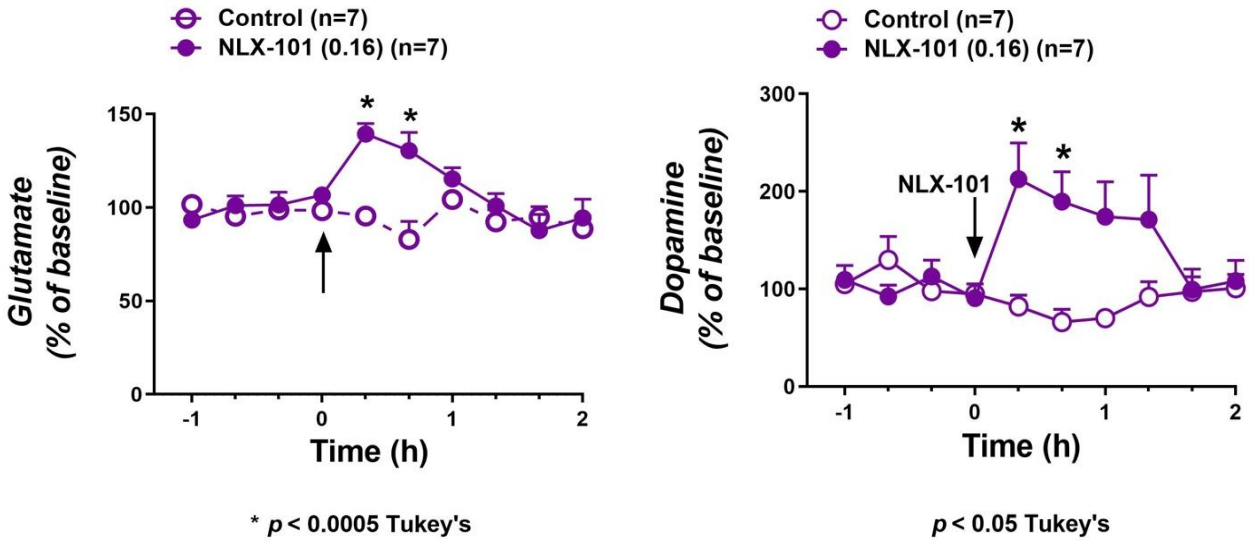

Figure 10. Systemic administration of NLX-101 increased the extracellular concentration of neurotransmitter glutamate and dopamine.

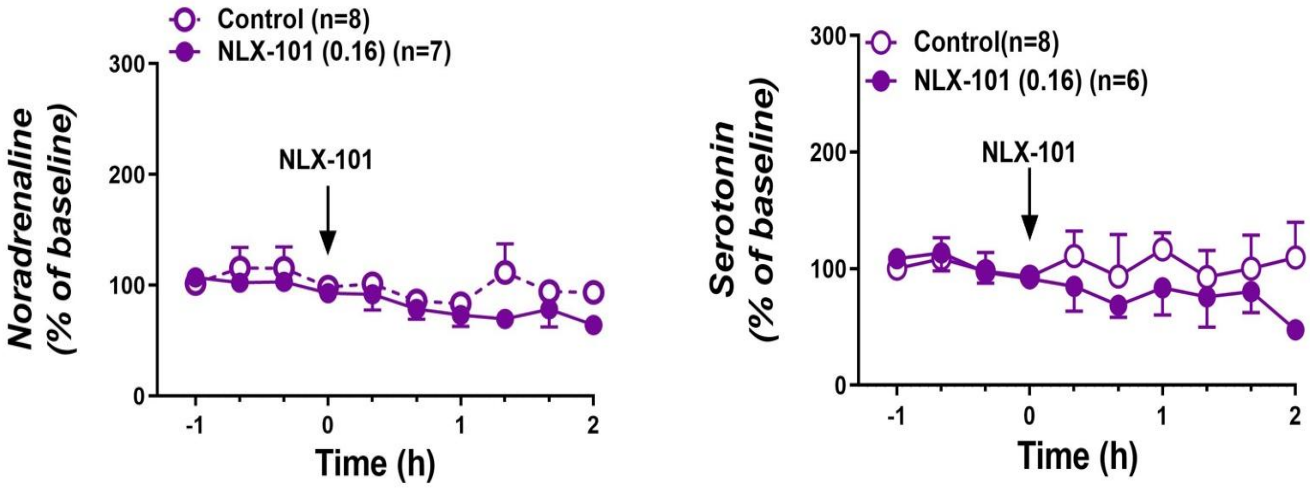

Figure 11. No change detected in the extracellular concentration of noradrenaline and serotonin.

Western blotting

Western blotting analyses were conducted 30 minutes, 1 hour, 2 hours and 6 hours after the administration of 0.16 mg/kg of NLX-101.

The proteins analysed were Akt, mTOR, BDNF, ERK ½, CREB, PSD95, p11, Glua1 with their corresponding phosphorilated form. No change was observed in the non-phosphorylated forms (data not shown) and the corresponding measurements were done with the active (phosphorylated) forms.

The systemic administration of NLX-101 (0.16 mg/kg) significantly increased the synthesis of pmTOR, pAkt, BDNF and pGluA1 although at different time points. The first proteins in elevating their levels were pmTOR and pGluA1 at 30 min and 1h, respectively (Fig. 12).

BDNF and pAkt displayed a more delayed increase (Fig. 13), whereas pCREB and the β-arrestins did not change up to a 6 h after NLX-101 administration (Fig. 14).

pERK1/2, pGluA1, PSD95 and p11 showed a nonsignificant trend to increase their levels 30 minutes after drug administration (Fig. 15).

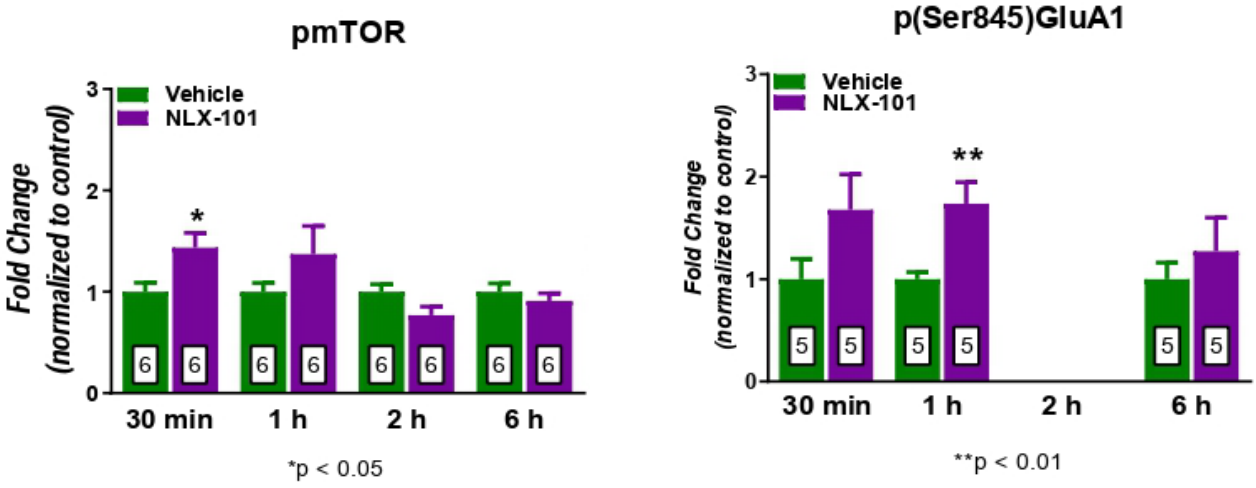

Figure 12. Systemic administration of NLX-101 increased the synthesis of pmTOR and

pGlua1.

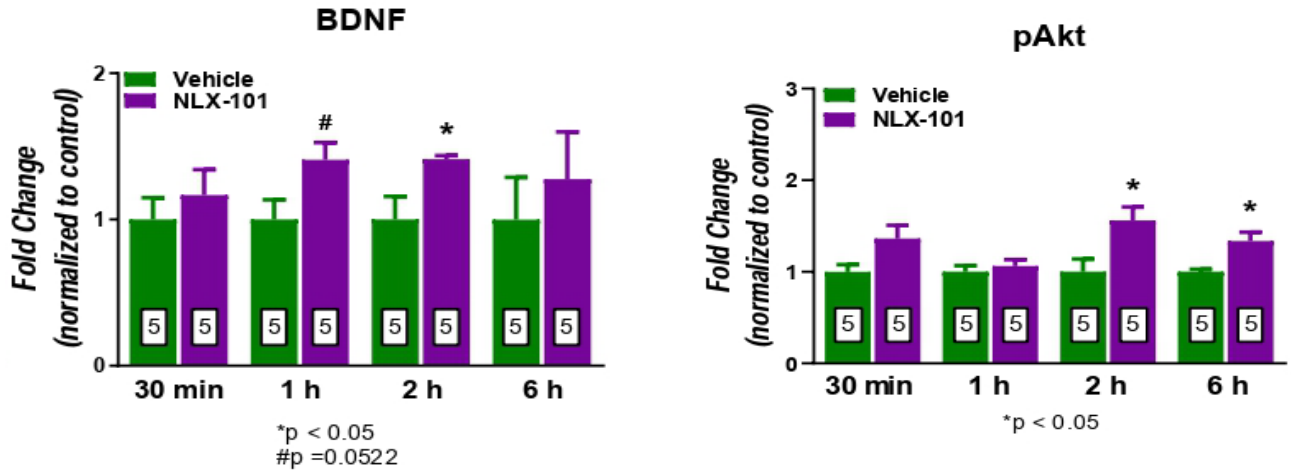

Figure 13. Systemic administration of NLX-101 increased the synthesis of BDNF and pAkt.

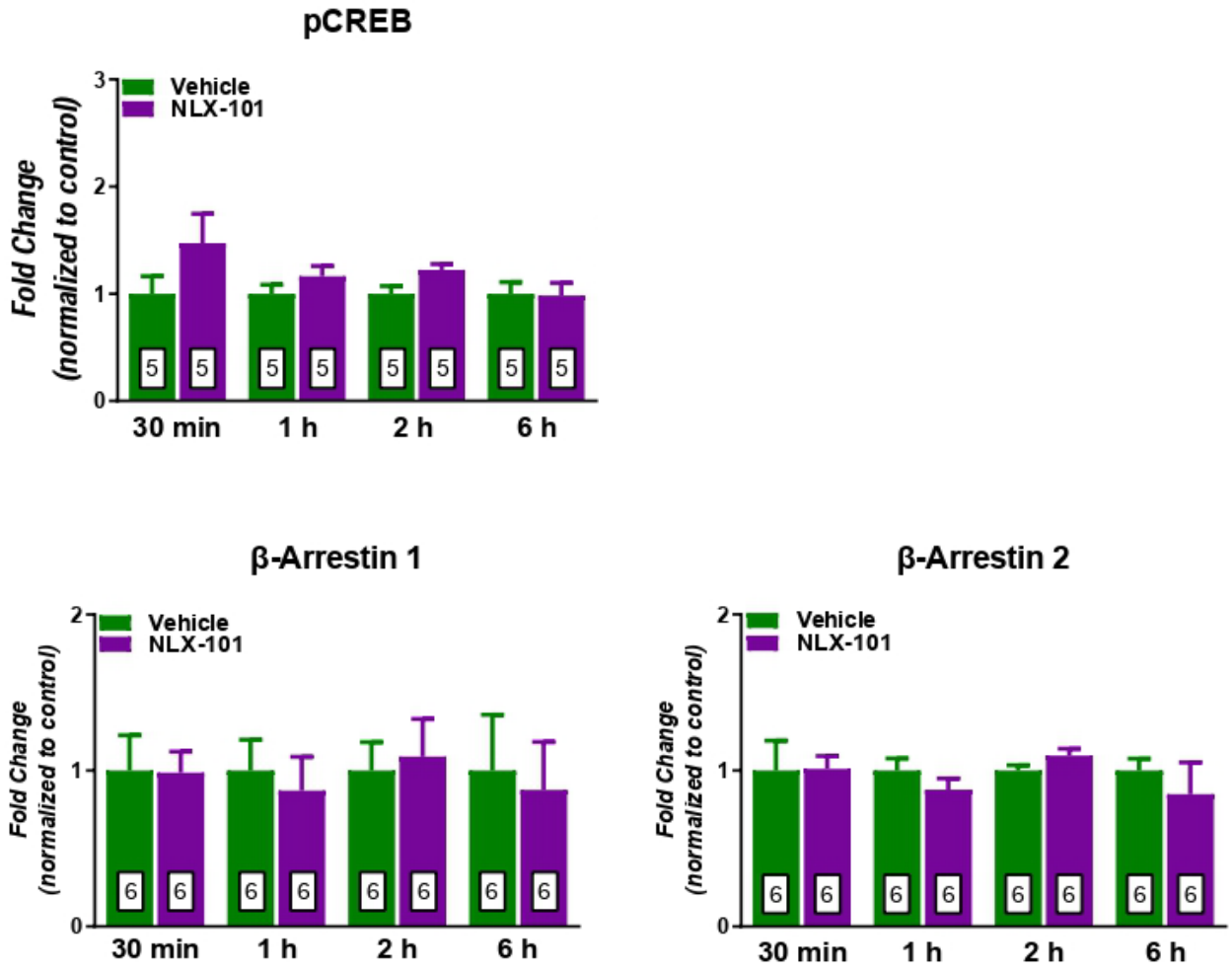

Figure 14. Systemic administration of NLX-101 did not change up the synthesis of pCREB and the β-arrestins.

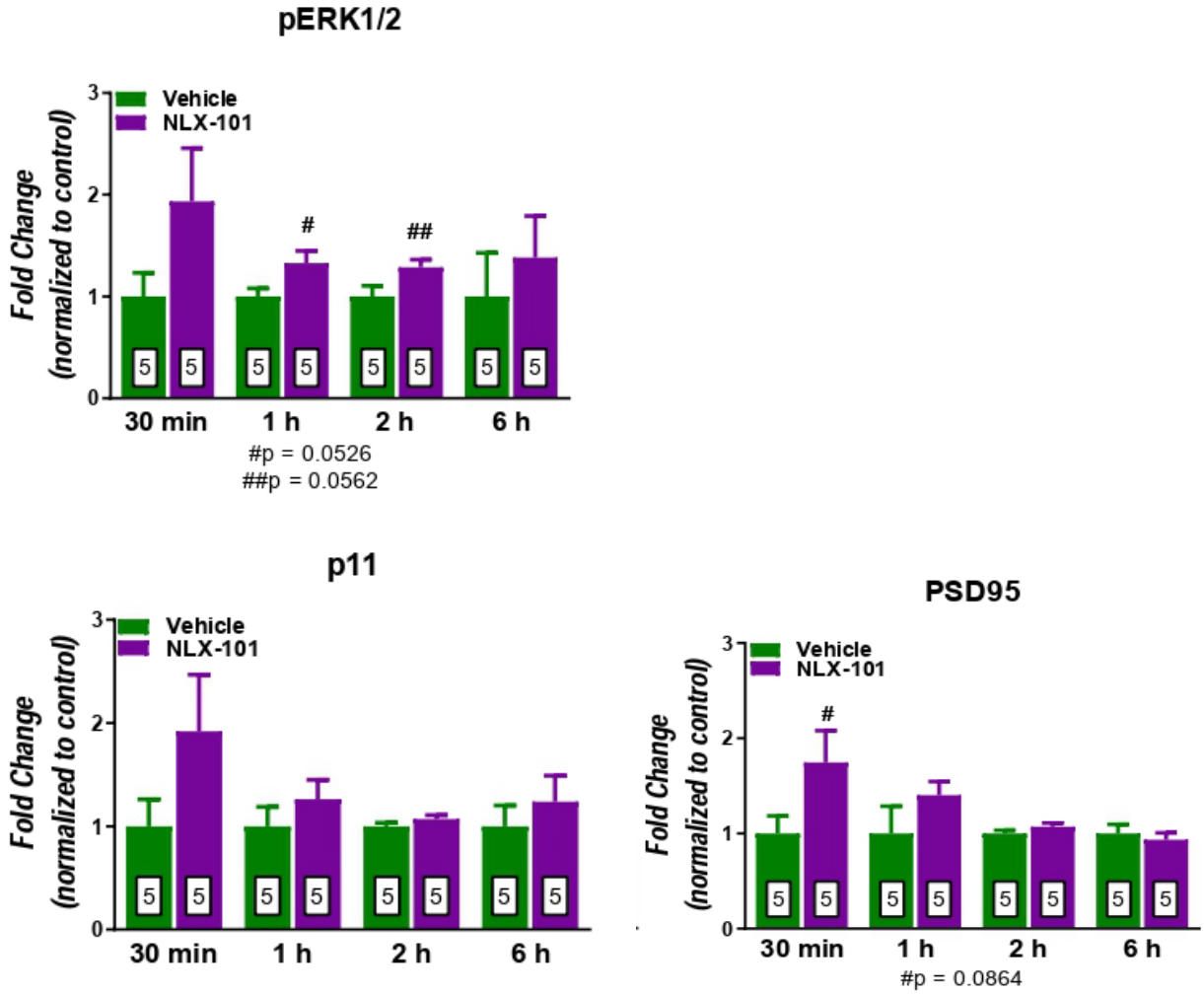

Figure 15. Systemic administration of NLX-101 showed a trend to increase the synthesis of pERK 1/2, p11 and PSD95.

NLX-101 showed an increased expression of synthesis of pmTOR after 30 minutes and an increased expression of p(Ser845)GLUA1 after 1 hour of drug administration.

Also, there is an increment in the BDNF and pERK ½ synthesis with a P of 0.0522 for BDNF and P=0.0526 for pERK ½ after 1 hour of drug administration, even if it is not significant.

Therefore, IP administration of NLX-101 (0.16 mg/kg) showed increased expression of synthesis of pAkt and BDNF after 2 hours of drug administration as shown in Figure 13. P= 0,0255 for pAkt and P=0,0623 and 0,0299 for BDNF.

There has been an increment also in the level of synthesis of pERK ½ 2 hours after drug

administration. P= 0.0562, even if it is not significant.

IP administration of NLX-101 (0.16 mg/kg) showed increased expression of synthesis of pAkt ALSO after 6 hours of drug administration.

PSD95 and p11 showed a nonsignificant trend to increase their levels only 30 minutes after drug administration, while no changes at all have been identified for pCREB, β-ARRESTIN 1 and 2 proteins.

Discussion

In the present work we have described that NLX-101 showed rapid –at 30 min but not 24 h or 7 days later- antidepressant-like effects in the FST associated with increased extracellular dopamine in the mPFC, as previously described (Lladó-Pelfort et al. 2010). The decreased immobility in the FST is not due to changes in locomotion as evidenced by a lack of effect of NLX-101 in the OFT.

We also show that NLX-101 increases prefrontal glutamate release. Taken together, our results suggest that blockade of GABAergic interneurons in the mPFC by NLX-101 (Fig. 3) disinhibited pyramidal neurons projecting to dopaminergic neurons in the ventral tegmental area (VTA) eventually leading to an increased release of dopamine in the mPFC, NLX-101 increased the release of dopamine in the mPFC. Interestingly, NLX-101 does not seem to disinhibit pyramidal neurons projecting to dorsal raphe nucleus and locus coeruleus as long as serotonergic and noradrenergic transmission in the mPFC was not altered. It is possible that the short-lasting increases in dopamine and glutamate (and the lack of effect on noradrenaline and serotonin) are responsible for the short-lived antidepressant-like effect of NLX-101 in the FST.

In the present work we also described, for the first time, the intracellular signaling mechanisms involved in the antidepressant-like effect of NLX-101. Our results show that systemic NLX-101 produced a rapid (in 30 min) increase in mPFC pmTOR. This is in line with the rapid increase of pmTOR observed after systemic ketamine (Li et al. 2010) and underscores the importance of mTOR signaling for obtaining a rapid antidepressant action. The most common upstream intermediaries of mTOR are Akt and ERK1/2.

However, the active form of Akt, pAkt, only increases 2 h after NLX-101 administration. On the other hand, pERK1/2 only exhibited a trend to be increased at 30 min after NLX-101 administration, which is in line with previous work showing that ERK1/2 is activated at earlier (< 30 min) stages (Newman-Tancredi 2011).

Furthermore, our results described a rapid increase in pGluA1, which together with the activation of downstream mTOR pathway, would suggest that GluA1 → mTOR pathway is the responsible for the rapid antidepressant response of NLX-101, as previously described for ketamine (Li et al. 2010). The apparent trend to an increased PSD95 would suggest the insertion of some proteins to the postsynaptic membrane, i.e. GluA1 and p11, and further support this view.

On the other hand, BDNF showed a delayed increase of its synthesis, which supports the view that it may be involved in neuroplasticity changes perhaps related to a more sustained antidepressant effect of NLX-101.

Altogether, our results suggest that NLX-101 has a rapid antidepressant-like response possibly by reducing the activity of GABA interneurons, thus disinhibiting the release of glutamate by the pyramidal neuron and increasing the production of pmTOR and pGluA1. The increase in other factors such as BDNF and Akt seemed to occur in a more delayed time course and may be involved in a long-term modulatory effect in regulation of neurogenesis.

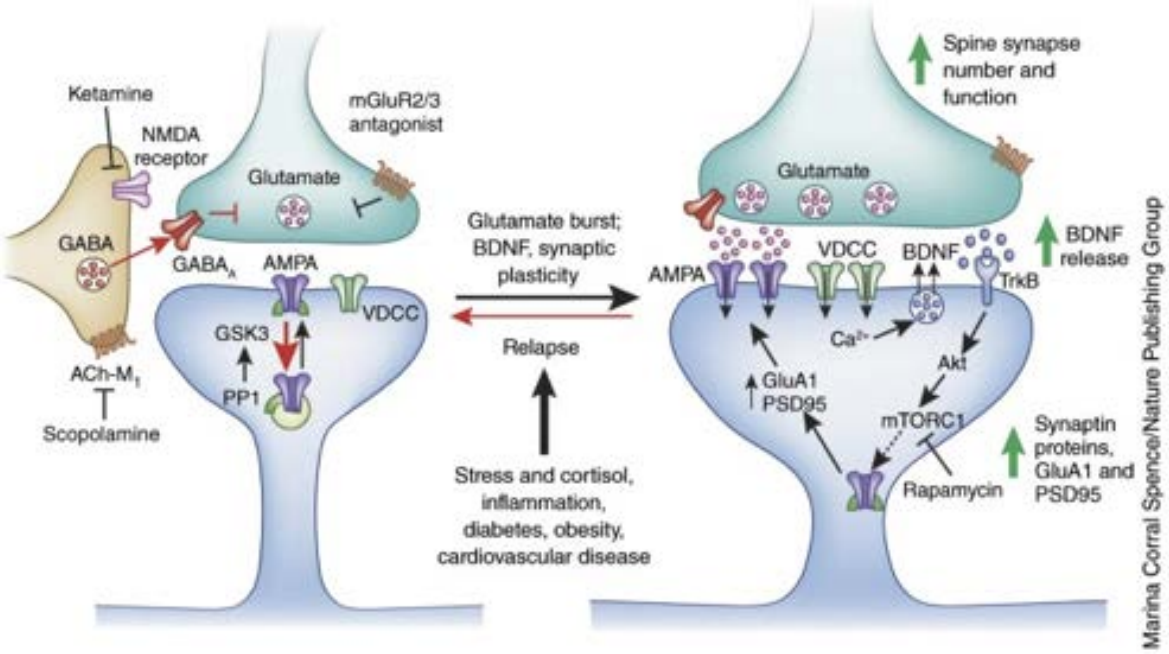

Figure 15. Molecular mechanisms of some protein studies such as BDNF, mTOR, PSD95, Glua1, Akt. (Duman et al. 2016)

Concluding remarks

Preferential targeting of cortical 5-HT$_{1A}$ receptors is a particularly attractive strategy because it would accelerate the onset of therapeutic efficacy in depression.

In addition, preferential targeting of cortical 5-HT$_{1A}$ receptors may increase the therapeutic margin with respect to side effects that arise from the activation of other 5-HT1A receptor subpopulations (Newman-Tancredi 2011).

The present results support this hypothesis. Unfortunately, the effects of NLX-101 are short-lived. Therefore, further research is needed to develop new 5-HT$_{1A}$ biased receptor agonists that evoke a more sustained response.

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Sharon Cabanu