Biomedicine & Pharmacotherapy 148 (2022) 112699

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Dissecting the contribution of 5-HT$_{1A}$ auto- and heteroreceptors in sucrose overconsumption in mice

Kate Beecher [a,1], Joshua Wang [a,1], Fatemeh Chehrehasa [b], Ronan Depoortere [c], Mark A. Varney [c], Adrian Newman-Tancredi [c], Selena E. Bartlett [a,*,2], Arnauld Belmer [a,*,2]

[a] Addiction and Obesity Laboratory, Faculty of Health, School of Clinical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
[b] Addiction and Obesity Laboratory, Faculty of Health, School of Biomedical Sciences, Queensland University of Technology, Brisbane, QLD, Australia
[c] Neurolixis SAS, 81100 Castres, France

ARTICLE INFO

Keywords:
Serotonin
Sugar consumption
5-HT$_{1A}$
Autoreceptors
Heteroreceptors
Pharmacology

ABSTRACT

The rise in obesity prevalence has been linked to overconsumption of high-sugar containing food and beverages. Recent evidence suggests that chronic sucrose consumption leads to changes in serotonergic neuroplasticity within the neural circuits involved in feeding control. Although there is a relationship between serotonin signalling in the brain and diet-induced obesity, the specific serotonin (5-HT) receptors or pathways involved remain unknown. The 5-HT$_{1A}$ receptor subtype plays a role in regulating mood, anxiety, and appetite, and has been associated with reversing addiction to substances of abuse. However, the respective role of 5-HT$_{1A}$ auto- *vs* heteroreceptors in sucrose consumption has not been examined. Mice were given controlled access to either 5%, 10% or 25% w/v sucrose, or water as a control, for 12 weeks using the well-established "drinking in the dark" protocol (n = 6–8 mice per group). Ligands selectively targeting 5-HT$_{1A}$ auto- and/or heteroreceptors (NLX-112, unbiased 5-HT$_{1A}$ receptor agonist; NLX-101, preferential heteroreceptor agonist; F13714, preferential autoreceptor agonist) were administered i.p. acutely after 6 and 12 weeks of sucrose consumption. The specific involvement of 5-HT$_{1A}$ receptors in these effects was verified by blockade with the selective 5-HT$_{1A}$ receptors antagonist WAY-100,635. The specific subpopulation of 5-HT$_{1A}$ receptors involved in sucrose consumption was dependent on the concentration of sucrose solution and the duration of exposure to sucrose (6 weeks *vs* 12 weeks). Long-term sucrose consumption leads to accentuated 5-HT$_{1A}$ autoreceptor function. Thus, targeting 5-HT$_{1A}$ autoreceptors might represent an effective therapeutic strategy to combat the rise in obesity resulting from the overconsumption of high-sugar diet.

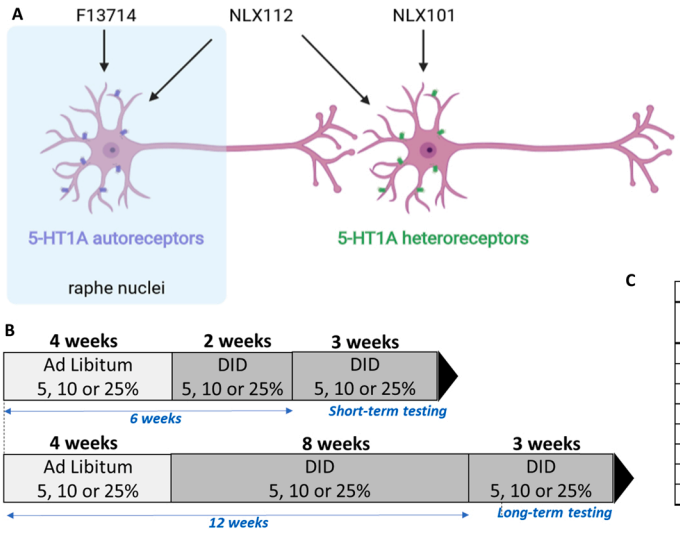
1. Introduction

The neural substrates of the reward produced by sugar consumption appear to be more potent than those of certain substances of abuse, such as alcohol or cocaine, possibly reflecting past selective evolutionary pressures for seeking and consuming foods high in sugar and calories [25,32]. The link between sugar consumption and the serotonergic system has been shown in the regulation of hedonic feeding [20,31]. Previously we and other laboratories have identified that alcohol and sugar dependence share similar signalling pathways and circuitry [4,5,41,46,48]. Therefore, we hypothesised that the effect of long-term sucrose consumption on serotonin receptor function may share similar mechanisms to alcohol-induced alterations on serotonergic neuroplasticity (for review see Belmer et al. [8]).

Serotonin exerts its function by stimulating 7 different families of receptors (5-HT1–7) totalling 14 receptor subtypes, most of which are G-protein coupled receptors (GPCR), except the 5-HT3 receptor, which is a ligand-gated ion channel [21]. The 5-HT$_{1A}$ receptors regulate mood, anxiety and appetite and are implicated in ethanol binge-like drinking behaviour [7,43]. The 5-HT$_{1A}$ receptor is a subtype of serotonin receptor located in presynaptic and postsynaptic regions (Fig. 1). 5-HT$_{1A}$ receptors expressed on 5-HT raphe neurons are autoreceptors that inhibit 5-HT neuron activity and limit the release of 5-HT at the nerve terminals [6], whereas the postsynaptic response of 5-HT release is mediated by

* Corresponding authors.
E-mail addresses: Selena.bartlett@qut.edu.au (S.E. Bartlett), Arnauld.belmer@qut.edu.au (A. Belmer).
[1] Equal co-first authors.
[2] Equal co-last authors.

https://doi.org/10.1016/j.biopha.2022.112699
Received 22 September 2021; Received in revised form 20 January 2022; Accepted 2 February 2022



C

Latin square design n=8			
Animal #	dose (mg/kg)		
	week1	week 2	week3
1	0	0.16	0.64
2			
3			
4	0.64	0	0.16
5			
6			
7	0.16	0.64	0
8			

Fig. 1. A. The 5-HT$_{1A}$ receptor is expressed somatodendritically on 5-HT producing neurons as an autoreceptor in the raphe nuclei which inhibits 5-HT neuron activity and limits the release of 5-HT at the nerve terminals. 5-HT$_{1A}$ receptors are also found in target regions as a heteroreceptors that mediate the post-synaptic response of 5-HT release. 5-HT$_{1A}$ receptor agonist selective for both auto- (located in the raphe nuclei) and heteroreceptors (located in the other brain areas). **B.** Experimental design of short- and long-term Drinking-in-the-Dark with 5%, 10% or 25% sucrose and drug testing in Latin-square design. **C.** Example of Latin-square design and pseudo-random testing of various doses of NLX compounds (0, 0.16 or 0.64 mg/kg), with n = 8 animals.

5-HT$_{1A}$ heteroreceptors at regions efferent to the dorsal (DR) or median raphe (MR), such as the prefrontal cortex, amygdala, dorsal striatum or hippocampus [1]. 5-HT$_{1A}$ receptors have been shown to be hypersensitized following chronic ethanol exposure increasing the net excitation of DR neurons [30] and 5-HT$_{1A}$ receptor signalling and expression [27,33].

Our laboratory has demonstrated the role of 5-HT$_{1A}$ receptors in alcohol withdrawal-induced anxiety and neurogenic deficits following long-term chronic ethanol binge-like consumption (12 weeks) in mice [7,41]. Pindolol, a dual beta-adrenergic antagonist and weak 5-HT$_{1A}$ partial agonist with preference for autoreceptors [44], significantly reduces ethanol consumption in mice (but not sucrose consumption) following long-term but not short-term exposure [41], suggesting changes in 5-HT$_{1A}$ receptor signalling depending on duration of exposure. Buspirone, a non-selective full agonist at 5-HT$_{1A}$ autoreceptors and partial agonist at 5-HT$_{1A}$ heteroreceptors, significantly reduced both ethanol and sucrose consumption following long-term exposure [41]. Tandospirone, a more potent and selective partial agonist of 5-HT$_{1A}$ receptor than buspirone, with also full agonist activity at 5-HT$_{1A}$ autoreceptors and a partial agonist at 5-HT$_{1A}$ heteroreceptors Ki = 27 ± 5 nM) [47,49], reduced ethanol consumption and, at higher dose, sucrose intake [7]. Interestingly, long-term sucrose consumption decreases the hyperphagic response following activation of 5-HT$_{1A}$ autoreceptors and reduces the hyperlocomotor effect following activation of 5-HT$_{1A}$ heteroreceptors, elicited by the agonist 8-OH-DPAT [18,23,24,38], suggesting that 5-HT$_{1A}$ receptor signalling is affected by sucrose consumption and that 5-HT$_{1A}$ auto- and heteroreceptors might differentially contribute to short and/or long-term sucrose consumption.

Recently, new highly selective and potent biased 5-HT$_{1A}$ receptor agonists have become available: NLX-101 (a.k.a. F15599) and F13714 (Fig. 1; [2,3,11,13,28,29,36]). For recent review of the pharmacology, physiology and behavioral effects of NLX agonists, see [35]. NLX-101 mediates its effects through Gαi protein activation and pERK1/2 downstream signalling [2,28,29]. F13714 preferentially activates somatodendritic 5-HT$_{1A}$ autoreceptors [3]. The unbiased selective agonist NLX-112, preferentially activates Gα$_o$ proteins and elicits pronounced phosphorylation of ERK1/2 [11,35,37]. NLX-101, F13714 and NLX-112 all elicit anxiolytic and antidepressant activity rodents [17,19,26,42].

Therefore, we used these three 5-HT$_{1A}$ biased agonists to dissect the respective contribution of 5-HT$_{1A}$ auto- and heteroreceptors in short- (6 weeks) and long- (12 weeks) term consumption of 5%, 10% or 25% sucrose solution. We have shown for the first time that 5-HT$_{1A}$ autoreceptors *vs* heteroreceptors distinctly control the consumption of sucrose. Our results demonstrate that short- and long-term exposure to high sugar diet differentially alter 5-HT$_{1A}$ auto- and heteroreceptor signalling.

2. Methods

2.1. Animals and housing

Four-week-old C57BL/6 male mice (ARC, WA, Australia) were individually housed (GM500 Greenline cage for Mice, Tecniplast) at the Pharmacy Australia Centre of Excellence (PACE) Research Animal Facility under reverse-light cycle conditions (lights off from 9:00 a.m. to 9:00 p.m.) in a climate-controlled room (~ 18–23 °C and 30–40% humidity) with *ad libitum* access to food (Meat Free Mouse Diet, pellets, Specialty Feeds) and filtered water. Following one week of habituation to the housing conditions, mice were offered sucrose solution or water during the drinking sessions. All procedures were approved by The University of Queensland and The Queensland University of Technology Animal Ethics Committees under approval QUT/053/18 and complied with the policies and regulations regarding animal experimentation and other ethical matters, in accordance with the Queensland Government Animal Research Act 2001, associated Animal Care and Protection Regulations (2002 and 2008), as well as the Australian Code for the Care and Use of Animals for Scientific Purposes, 8th Edition.

2.2. Sucrose consumption

Mice (n = 6–8 per group) had *ad libitum* (24/7) access to 5%, 10% or 25% sucrose (w/v) solution for the first 4 weeks before being switched to the "Drinking-in-the-Dark" paradigm (DID) for the remaining 8 weeks (12 weeks of sucrose consumption total). During the 4 week *ad libitum* access to sucrose, mice were given access to one bottle of sucrose and one bottle of water available at all times. Mice and sucrose and water containing bottles were weighed daily to calculate the adjusted g/kg intake. During the DID period, they had access to one bottle of 5%, 10% or 25% (w/v) sucrose for a 2 h period (3 h into the dark cycle) Monday to Thursday and a 4 h period on Friday as previously published [4,5,7,41]. Sucrose containing bottles were weighed prior to, 30 min and 2 h (Monday–Thursday) or 4 h (Friday) after presentation. All mice had *ad libitum* access to food and water. The sucrose solution and filtered water were presented in 50 ml plastic falcon tubes fitted with rubber stoppers and a 6.35 cm stainless-steel sipper tube with double ball bearings.

Table 1
Pharmacology of the NLX compounds. Affinity and selectivity over other 5-HT (5-HTR), dopamine (DAR) and noradrenaline (NAR) are indicated. ?: not known.

Drug	Target	Affinity (pKi)							Metabolism – Cytochrome p450 activation/inhibition
		5-HTR			DAR		NAR		Fold selectivity	
		1A	1B	2A, B, C	D1, 2, 3	D4	α1	α2		
NLX112	AutoR + HeteroR	8.96	< 5	< 5	< 5	5.7	5.35	5.43	> 1000	no change
NLX101	HeteroR	8.65	*< 5*	*< 5*	*< 5*	*< 5*	*< 5*	*< 5*	> 1000	no change
F13714	AutoR	10.1	*< 6*	*< 6*	*< 6*	*< 6*	7.1	< 6	1000	?

2.3. Drug treatments

Drug administration occurred at 6 and 12 weeks. NLX-112 (befiradol or F13640; 3-chloro-4-fluorophenyl-[4-fluoro-4-([(5-methylpyridin-2-yl)methylamino]methyl)piperidin-1-yl] methanone, fumarate salt), NLX-101 (F15599; 3-Chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-pyrimidin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl)-methanone, fumarate salt) and F13714 (3-chloro-4-fluorophenyl-(4-fluoro-4-{[(5-methyl-6-methylaminopyridin-2-ylmethyl)-amino]-methyl}-piperidin-1-yl-methanone, fumarate salt)) and WAY-100,635 maleate (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]- N-(2-pyridyl) cyclohexanecarboxamide, maleate salt). NLX-101, F13714 and NLX-112 were provided by Neurolixis (See summary of pharmacological data in Table 1). WAY-100,635 was commercially obtained from Abcam (ab120550). NLX compounds and WAY-100,635 were dissolved in 0.9% (w/v) sterile sodium chloride to 0.64 mg/kg; NLX drugs were then serially diluted to 0.16 mg/kg. All doses refer to the weight of the free base. The drugs were tested on sucrose consumption by intraperitoneal (i.p.) injections (10 ml/kg, Fig. 1B) in a Latin-square design, where each mouse received each of the three doses of the drug over three testings, with each mouse serving as its own control (Fig. 1C). Drugs were tested after 6 weeks (short term) and or 12 weeks (long-term) of sucrose consumption as we previously showed that alterations in 5-HT_{1A} function happen after long-term but not short-term alcohol consumption. The doses were chosen according to previous published work with these compounds, with respective potencies ranging from 0.1 to 1 mg/kg [10,16,42].

2.4. Locomotor activity

Locomotor activity was measured using an open-field apparatus (open arena of 30 × 30 × 40 cm) and video-tracked using the ANY-maze software (Version 6.18, Stoelting Co., USA). A separate group of mice was used to access locomotor activity. Mice were habituated for three consecutive days, from 9 a.m. to 5 p.m., before locomotor testing on the fourth day. On the first day mice were placed in the arena for 30 min, scruff handled then placed back into the arena for 30 min. On the second day, after 30 min in the box, mice were scruff handled then abdominally pricked with a syringe needle and placed back into the arena for 1 h. On the third day, mice were given an intraperitoneal saline

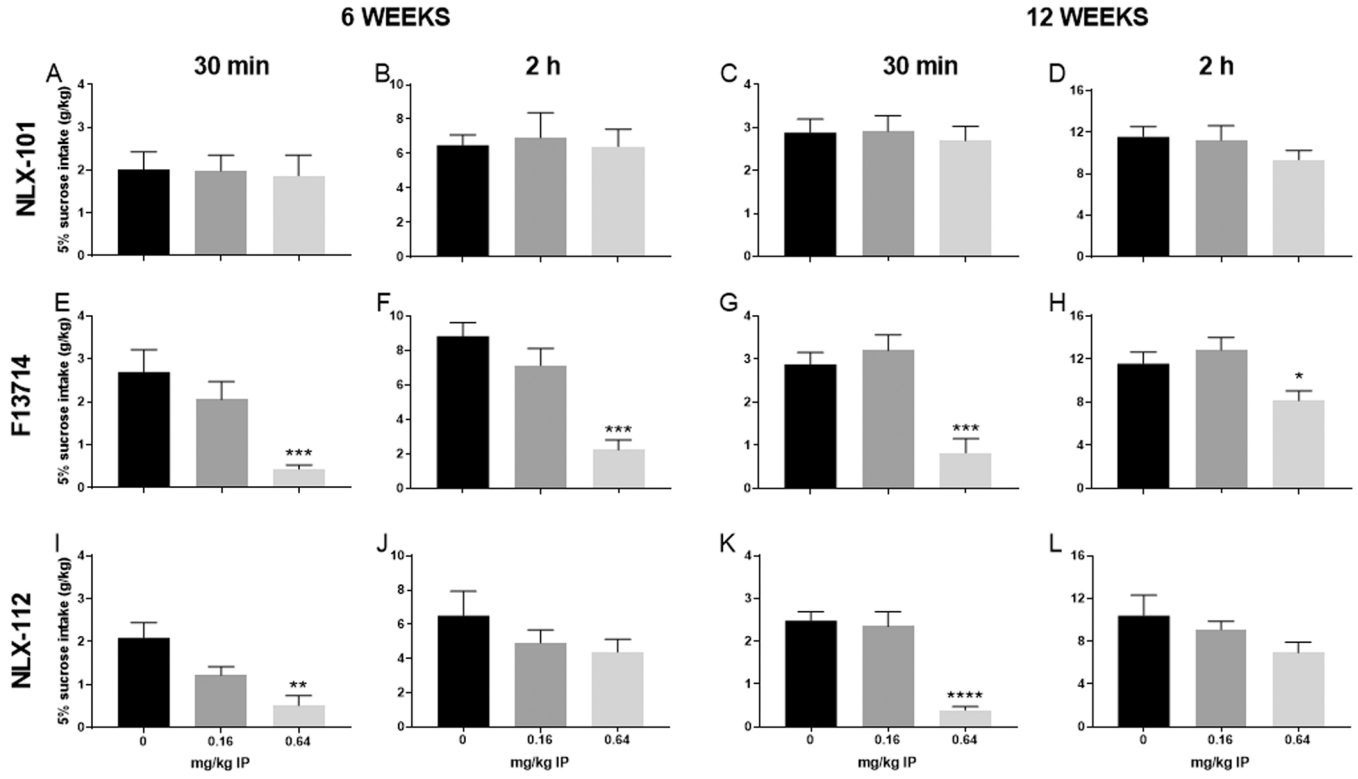


Fig. 2. Effect of NLX-101, F13714 and NLX-112 on 5% sucrose intake following short-term (6 weeks) and long-term (12 weeks) exposure at 30 and 2 h post-start of session. Agonists (0.16 and 0.64 mg/kg, i.p.) were administered 30 min before the start of the drinking session. **(A–D)** NLX-101 had no effect on sucrose consumption following short-term or long-term exposure. F13714 significantly reduced sucrose consumption at the highest dose (0.64 mg/kg) at 30 min and 2 h following short-term **(E–F)** and long-term **(G–H)**. **(I–J)** NLX-112 only reduced sucrose intake at 30 min at the highest dose (0.64 mg/kg) after short-term **(I)** and long-term **(K)** exposure but had no effect on 2 h drinking **(J–L)**. Values are expressed as mean sucrose consumed (g/kg) ± SEM (one-way ANOVA followed by Bonferroni's *post hoc* test). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with vehicle (n = 6).

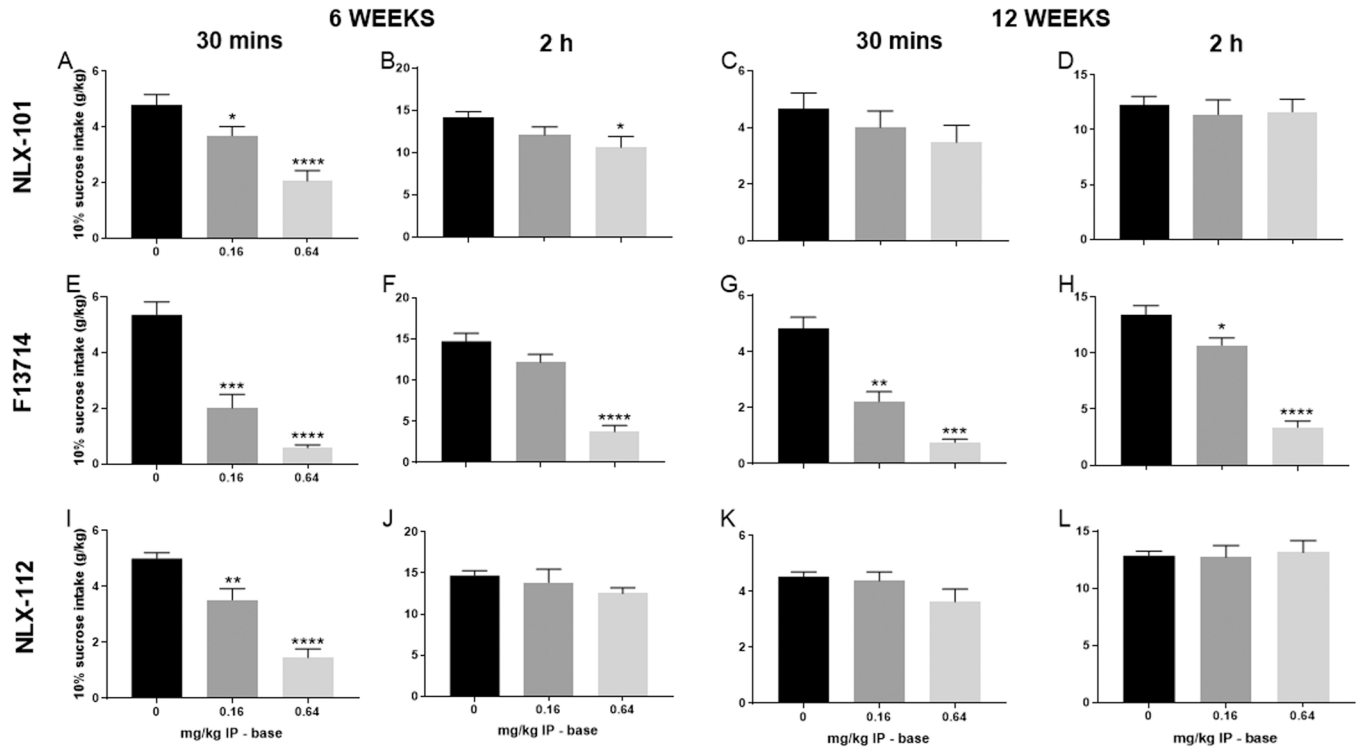


Fig. 3. Effect of NLX-101, F13714, NLX-112 on 10% sucrose intake following short-term (6 weeks) and long-term (12 weeks) exposure at 30 and 2 h post-start of session. Agonists (0.16 and 0.64 mg/kg, i.p.) were administered 30 min before the start of the drinking session. NLX-101 significantly reduced the 30 min and 2 h sucrose consumption following short-term **(A–B)** but not long-term exposure **(C–D)**. F13714 significantly reduced sucrose consumption at the at 30 min and 2 h following short-term **(E–F)** and long-term **(G–H)**. NLX-112 reduced sucrose intake at 30 min but not 2 h following short-term **(I–J)** but not long-term **(K–L)** exposure. Values are expressed as mean sucrose consumed (g/kg) ± SEM (one-way ANOVA followed by Bonferroni's *post hoc* test). *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ compared with vehicle ($n = 8$).

injection after 30 min of habituation in the arena, then placed back into it for 1 h. On the testing day, mice received an i.p. injection of either saline, NLX-101, F13714 or NLX-112 (0.64 mg/kg each) and placed immediately in the locomotor activity apparatus for 1 h.

2.5. Data analysis

Data are expressed as the mean ± SEM. Sucrose consumption was analysed with a one-way ANOVA for repeated measures (summarised in Supplementary Table 1), followed, if appropriate, by a Bonferroni multiple comparisons *post-hoc* test (summarised in Supplementary Tables 2 and 3). For the locomotor activity, Area Under the Curve (AUC) values were analysed with an unpaired student's t-test to compare the saline-treated. All statistical analyses were done with GraphPad Prism 8 (GraphPad Software Co., CA, USA). P values < 0.05 were considered significant.

3. Results

5-HT_{1A} partial agonists reduce ethanol and sucrose intake following long-term exposure [7,39–41]. Given that the agonists tandospirone, pindolol, and buspirone cannot specifically discriminate between 5-HT_{1A} auto- and heteroreceptors in reducing sugar consumption, we used the 5-HT_{1A} receptor biased agonists NLX-101, F13714 and NLX-112 on sucrose intake. We tested the effect of NLX-101, F13714 and NLX-112 drugs on sucrose intake at 6 weeks and 12 weeks of binge consumption of 5%, 10% and 25% sucrose solutions.

3.1. Activation of 5-HT_{1A} receptors reduces binge-like consumption of 5% sucrose following short-term and long-term exposure

Preferential stimulation of the 5-HT_{1A} heteroreceptor by NLX-101 had no effect on 5% sucrose intake at 6 or 12 weeks (Fig. 2A–D). However, F13714 (autoreceptor) had an overall main effect at 30 min and 2 h at both 6 and 12 weeks (Fig. 2E–H). Bonferroni's *post-hoc* analysis revealed that F13714 significantly reduced 5% sucrose intake at 30 min and 2 h at the highest dose at 6 weeks (Fig. 2E–F). NLX-112 (unbiased) had an overall main effect on 5% sucrose at 30 min at both 6 and 12 weeks at 30 min (Fig. 2I and K) but not at 2 h (Fig. 2J and L). Bonferroni's *post hoc* analysis revealed that NLX-112 significantly reduced sucrose intake only at 30 min and at the highest dose at 6 and 12 weeks (Fig. 2I and K). There was no effect of any dose of NLX-112 on 2 h sucrose intake following short- or long-term consumption (Fig. 2J and L).

3.2. Activation of 5-HT_{1A} receptors reduces binge-like consumption of 10% sucrose following short-term and long-term exposure

NLX-101(which activates heteroreceptors) had an overall main effect on 10% sucrose at 30 min and 2 h following short-term exposure (Fig. 3A–B) with no effect seen after long-term consumption (Fig. 3C–D). Bonferroni's *post hoc* analysis revealed that NLX-101 reduced the 30 min consumption of 10% sucrose at both doses following short-term (Fig. 3A), and the 2 h consumption only at the highest dose for the 2 h period (Fig. 3B) but not long-term consumption (Fig. 3C–D). F13714 (autoreceptor biased) had an overall main effect on 10% sucrose at 30 min and 2 h at both 6 and 12 weeks (Fig. 3E–H). Bonferroni's *post hoc* analysis revealed that F13714 significantly reduced sucrose intake at 30 min and 2 h at both doses at 6 and 12 weeks (Fig. 3E–H). NLX-112

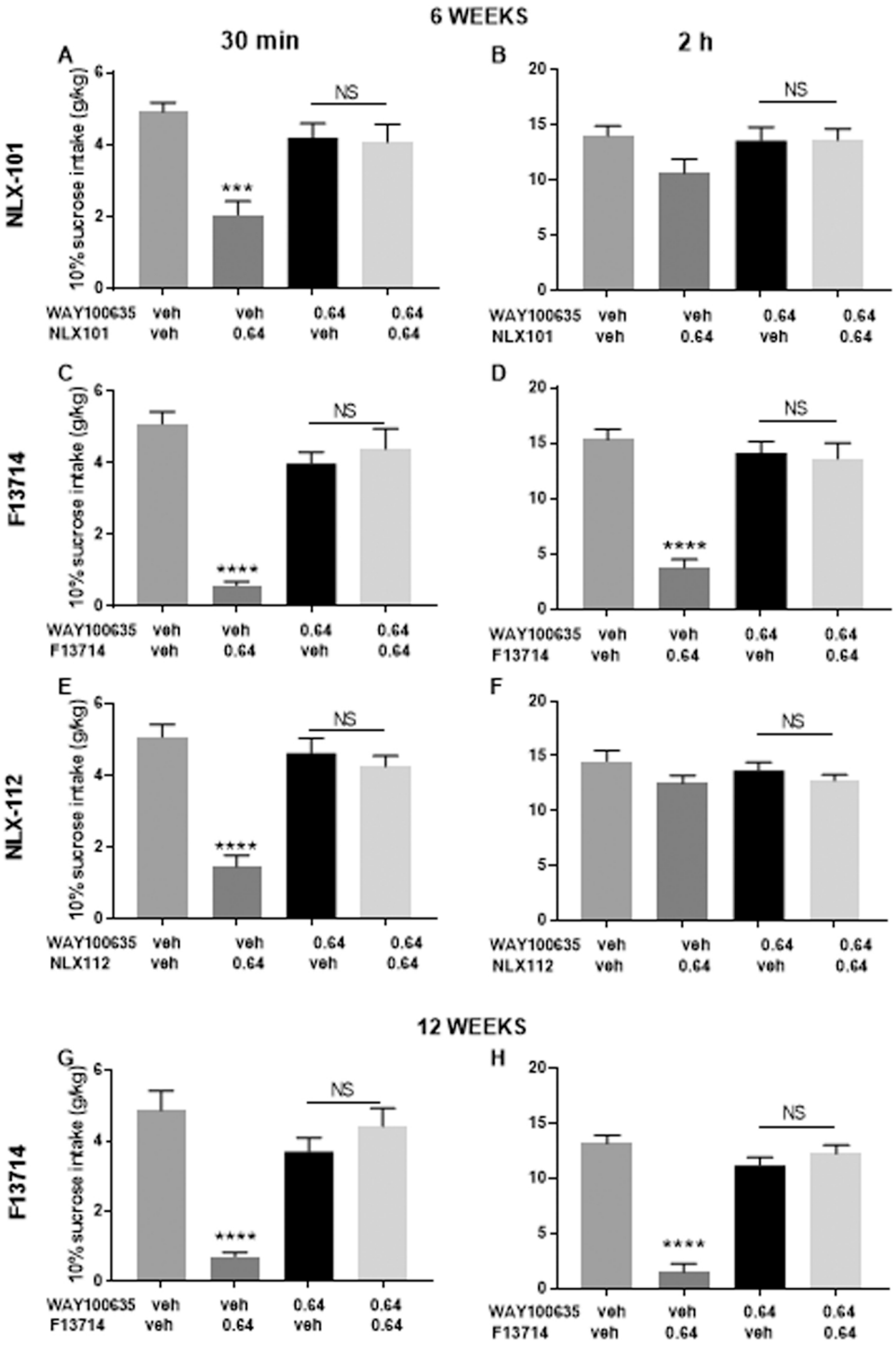


Fig. 4. Blockade of NLX-101, F13714, NLX-112 effect on short-term (6 weeks) and long-term (12 weeks) consumption of 10% sucrose intake by WAY-100,635. WAY-100,635 pre-treatment was administered 1 h before the drinking session. Agonists (0.64 mg/kg, i.p.) were administered 30 min before the start of the drinking session. **(A–F)** The effect induced by NLX-101, F13714, NLX-112 was blocked by pre-treatment with WAY-100,635 following short-term consumption at 30 min and 2 h. **(G–H)** After long term consumption of 10% sucrose, pre-treatment with WAY-100,635 blocked the effect of NLX-112. Values are expressed as mean sucrose consumed (g/kg) ± SEM (one-way ANOVA followed by Bonferroni's *post hoc* test). ***$p < 0.001$, ****$p < 0.0001$ compared with vehicle ($n = 8$).

(unbiased) had an overall main effect on 10% sucrose at 30 min at 6 weeks (Fig. 3I) and 12 weeks (Fig. 3K). No effect of NLX-112 was observed at 2 h at 6 weeks (Fig. 3J) and 12 weeks (Fig. 3L). Bonferroni's *post hoc* analysis revealed that NLX-112 significantly reduced 10% sucrose at both doses at 30 min at 6 weeks (Fig. 3I) and only at the highest dose at 12 weeks (Fig. 3K).

We confirmed the specific contribution of 5-HT_{1A} receptors in the effects of the three NLX compounds on 10% sucrose by blocking their effect on sucrose consumption with the selective 5-HT_{1A} receptor antagonist, WAY-100,635. Indeed, WAY-100,635 (0.64 mg/kg) did not have any significant effect on sucrose intake by itself, but totally blocked the effect of the highest dose (0.64 mg/kg) of NLX-101, F13714 and NLX-112 on short-term consumption at 30 min and 2 h (Fig. 4A–F).

Pre-treatment with WAY-100,635 also completely blocked the effect of F13714 following long-term sucrose exposure (Fig. 4G–H). The blocking by WAY-100,635 of the reducing effects of the NLX drugs confirmed the specific involvement of 5-HT_{1A} receptors in sucrose consumption.

3.3. Activation of 5-HT_{1A} receptors reduces binge-like consumption of 25% sucrose following short-term and long-term exposure

NLX-101 (heteroreceptors) had an overall main effect on 25% sucrose at 30 min and 2 h following short- and long-term exposure

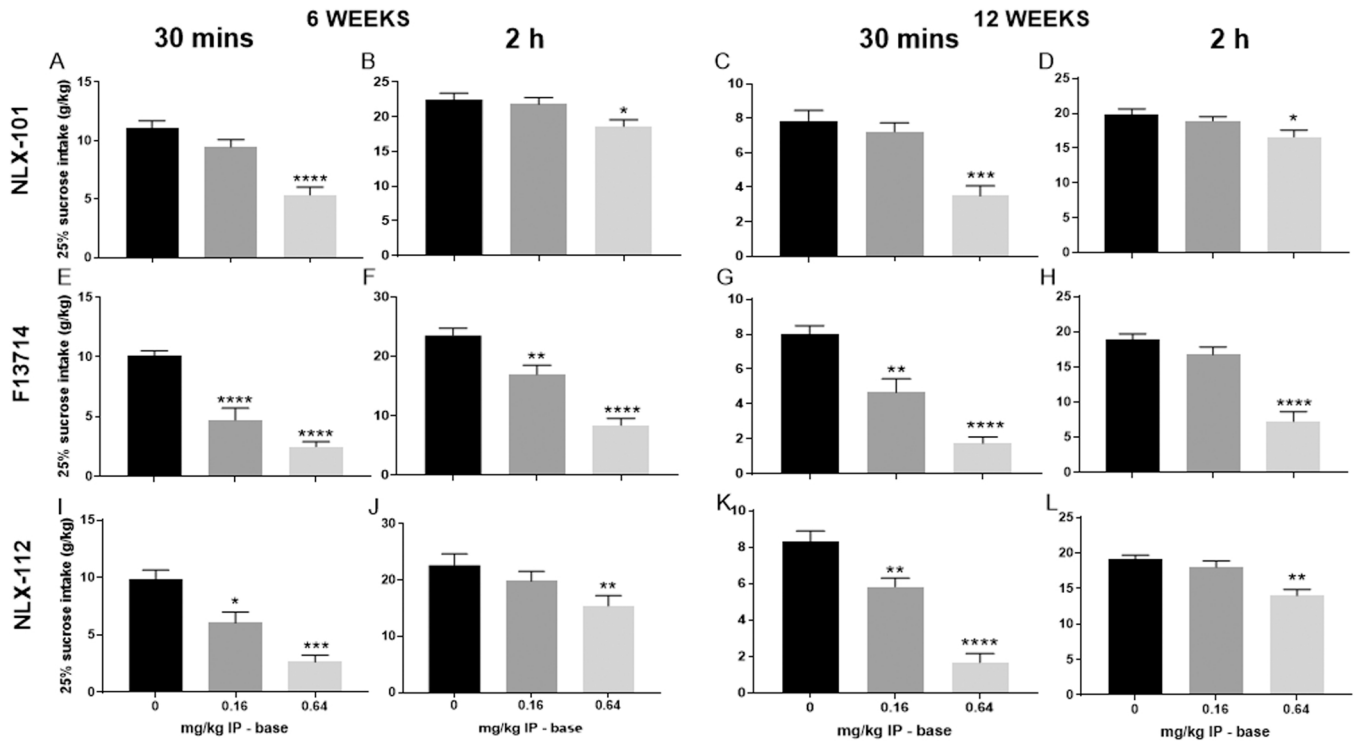


Fig. 5. Effect of NLX-101, F13714, NLX-112 on 25% sucrose intake following short-term (6 weeks) and long-term (12 weeks) exposure at 30 and 2 h post-start of session. Agonists (0.16 and 0.64 mg/kg, i.p.) were administered 30 min before the start of the drinking session. **(A–D)** NLX-101 significantly reduced sucrose consumption at the highest dose (0.64 mg/kg) at 30 min and 2 h following short-term **(A–B)** and long-term **(C–D)** exposure. F13714 significantly reduced sucrose consumption at 30 min and at 2 h following short-term **(E–F)** and long-term consumption **(G–H)**. **(I–L)** NLX-112 reduced sucrose intake at 0.16 mg/kg and 0.64 mg/kg at 30 min and the highest dose (0.64 mg/kg) at 2 h after short- **(I–J)** and long-term **(K–L)** consumption. Values are expressed as mean sucrose consumed (g/kg) ± SEM (one-way ANOVA followed by Bonferroni's *post hoc* test). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ compared with vehicle ($n = 8$).
To verify that the reduction of 25% sucrose consumption by NLX drugs was specifically mediated by 5-HT_{1A} receptors, we also used the selective 5-HT_{1A} receptor antagonist, WAY-100,635. The effect induced by NLX-101, F13714, NLX-112 (0.64 mg/kg) was totally blocked by pre-treatment with WAY-100,635 (0.64 mg/kg) following short- and long-term sucrose consumption at both 30 min and 2 h (Fig. 6A–L).

(Fig. 5A–D). Bonferroni's *post hoc* test revealed that NLX-101 reduced 25% sucrose intake only at the highest dose (0.64 mg/kg) at both 30 min and 2 h following short-term consumption (Fig. 5A–B), and long-term consumption (Fig. 5C–D). One-way ANOVA showed that F13714 (autoreceptors) had an overall main effect on 25% sucrose at both 30 min and 2 h following short- and long-term exposure (Fig. 5E–H). Bonferroni's *post hoc* analysis revealed that F13714 significantly reduced sucrose intake at 30 min, at both 0.16 mg/kg and 0.64 mg/kg at 6 and 12 weeks (Fig. 5E and G). While both F13714 doses reduced sucrose intake at 2 h after short-term consumption (Fig. 5 F) only the highest dose reduced the 2 h intake after long-term consumption (Fig. 4H). NLX-112 (unbiased) had an overall main effect on 25% sucrose at 30 min and 2 h following 6 and 12 weeks of exposure (Fig. 5I–L). Bonferroni's *post hoc* analysis revealed that NLX-112 significantly reduced 25% sucrose at both doses at 30 min at 6 and 12 weeks (Fig. 5I and K) and only at the highest dose at 2 h following 6 and 12 weeks of exposure (Fig. 5J and L).

3.4. Reduction of sugar intake by NLX agonists is not mediated by reduced locomotor activity

To evaluate the contribution of any non-specific locomotor effects in the efficacy of NLX-101, F13714 and NLX-112 in reducing sucrose consumption, we tested their effects on sucrose-naive, water consuming mice and assessed general locomotor activity. NLX-101 did not change general locomotor activity at 30 min after NLX-101 injection with the analysis of area under the curve (AUC) values for 30–60 min period (which corresponds to the period of 30 min sucrose intake) revealing no significant difference between vehicle (saline) controls and NLX-101 0.64 mg/kg (Fig. 7A; $p = 0.08$, t-test). F13714 increased general locomotor behaviour 30 min post- injection with the AUC values for 30–60 min period revealed a significant difference between vehicle-saline controls and vehicle-F13714 0.64 mg/kg (Fig. 7B; $p = 0.0005$, t-test). NLX-112 also significantly increased general locomotor activity at 30 min with the AUC values for 30–60 min period revealing a significant difference between vehicle (saline) controls and NLX-112 0.64 mg/kg (Fig. 7C; $p = 0.0001$, t-test).

4. Discussion

The present study suggests that short- and long-term consumption of increasing concentrations of sucrose differently affect 5-HT_{1A} auto- and heteroreceptor function and that targeting these receptor populations may represent a potential treatment strategy for sugar dependence. Previous work within our laboratory has demonstrated the role of 5-HT_{1A} receptors in chronic ethanol or sucrose binge-like consumption and how the pharmacological response to agonists changes between short- and long-term exposure [7,39,40]. Pindolol, a weak partial agonist of 5-$HT_{1A/1B}$ receptors and antagonist at β-adrenergic receptors [34] reduced ethanol consumption but not sucrose consumption in long-term, binge ethanol-consuming mice but not after short-term consumption [41]. This has suggested that changes in receptor function occur following prolonged exposure. On the other hand, buspirone, a full agonist at 5-HT_{1A} autoreceptors and partial agonist at 5-HT_{1A} heteroreceptors [12], reduced the binge consumption of both ethanol and sucrose following long-term exposure, hence suggesting the contribution of 5-HT_{1A} receptors in binge-sucrose consumption [41]. The 5-HT_{1A} receptor partial agonist, tandospirone is approximately two to three

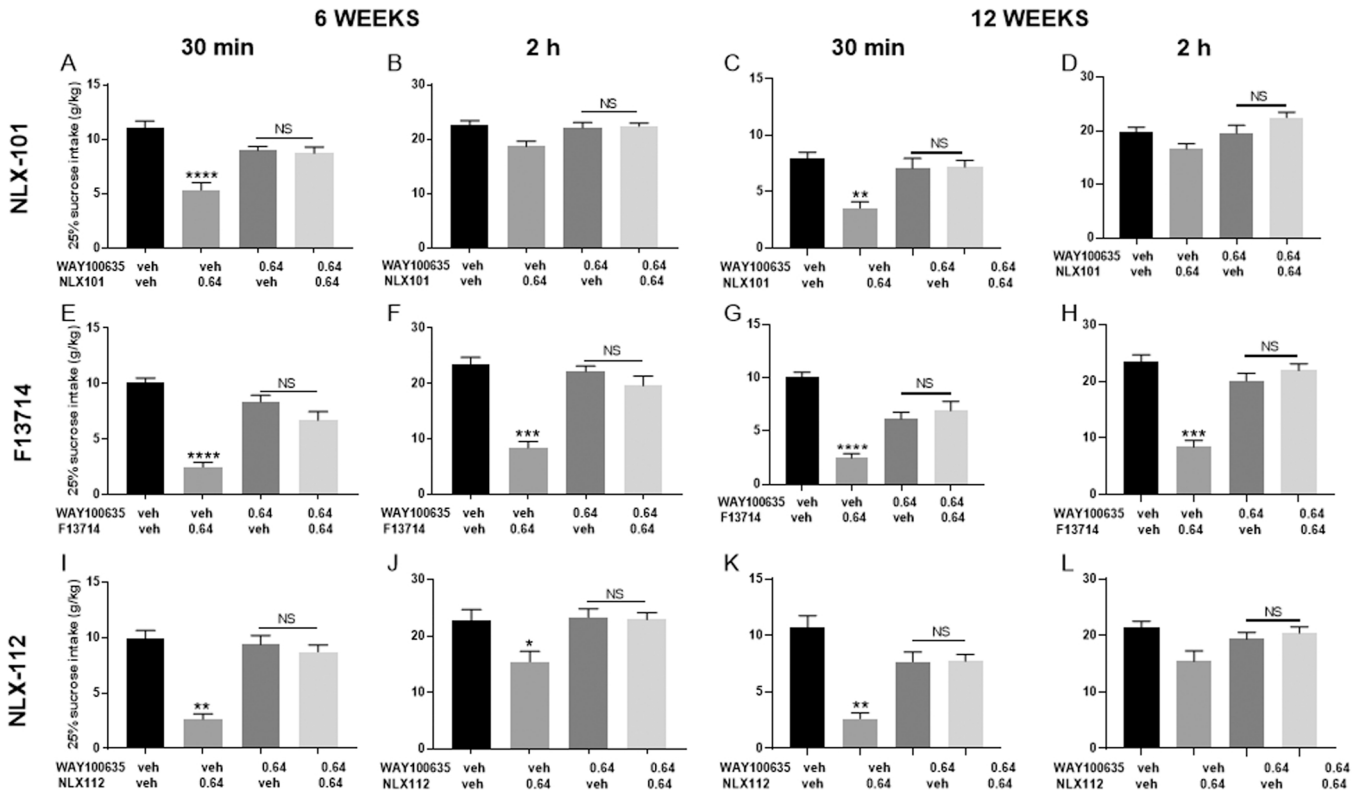


Fig. 6. Blockade of NLX-101, F13714, NLX-112 effect on short-term (6 weeks) and long-term (12 weeks) consumption of 25% sucrose intake by WAY-100,635. WAY-100,635 pre-treatment was administered 1 h before the drinking session. Agonists (0.64 mg/kg, i.p.) were administered 30 min before the start of the drinking session. **(A–L)** The effect induced by NLX-101, F13714, NLX-112 was blocked by pre-treatment with WAY-100,635 following short- **(A–B & E–F)** and long-term **(C–D & G–H & K–L)** consumption at 30 min and 2 h. Values are expressed as mean sucrose consumed (g/kg) ± SEM (one-way ANOVA followed by Bonferroni's *post hoc* test). $^{**}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ compared with vehicle (n = 8).

orders of magnitude less potent at 5-HT_2, 5-HT_{1C}, α1-adrenergic, α2-adrenergic and dopamine D1 and D2 receptors [22], with full agonist activity at 5-HT_{1A} autoreceptors in the raphe nuclei and partial agonist activity at postsynaptic 5-HT_{1A} receptors [47,49], Although low-doses of tandospirone were shown to reduce ethanol consumption, it only reduced long-term sucrose intake at higher doses (> 3 mg/kg, unpublished data [7]). Since sucrose consumption was shown to reduce the responsiveness of both presynaptic (hyperphagia) and postsynaptic (hyperactivity) 5-HT_{1A} receptors [24], it is likely that 5-HT_{1A} receptor signalling is influenced by sucrose consumption. However, the nonspecific pharmacology of classical 5-HT_{1A} agonists has made it previously impossible to discriminate the respective roles of 5-HT_{1A} auto- and heteroreceptors on sucrose consumption, until the recent development of novel 5-HT_{1A} biased agonists preferentially targeting either autoreceptors or heteroreceptors.

We therefore used these novel 5-HT_{1A} biased agonists to dissect the role of 5-HT_{1A} auto- and heteroreceptor synaptic 5-HT_{1A} receptors on sucrose consumption. Due to differences in serotonergic control over ethanol consumption following both short-term and long-term chronic ethanol intake [41], we evaluated the efficacy of these agonists in reducing sucrose intake after short-term (6 weeks) and long-term (12 weeks) sucrose consumption, see summary of results in Table 2. Targeting of 5-HT_{1A} heteroreceptors with NLX-101 did not affect 5% sucrose intake, but NLX-101 became more effective at reducing the consumption of higher sucrose concentrations. Indeed, the highest dose of NLX-101 (0.64 mg/kg) reduced the 30 min consumption of both 10% and 25% sucrose solutions, at 6 and 12 weeks, suggesting that 5-HT_{1A} heteroreceptors are recruited more during the binge-like consumption of higher concentrations of sucrose. On the other hand, targeting 5-HT_{1A} autoreceptors with F13714 was effective at reducing sucrose intake during both the binge-like period (30 min) and 2 h period of consumption, across all three sucrose concentrations. Interestingly, only the highest dose (0.64 mg/kg) was effective at reducing 5% sucrose consumption, with the lower dose (0.16 mg/kg) becoming effective at reducing the consumption of only higher concentration of sucrose (10% and 25%). This suggests that chronic consumption of higher concentrations of sucrose sensitises 5-HT_{1A} autoreceptors. As a result, the unbiased stimulation of 5-HT_{1A} receptors by NLX-112 agonist produced an effect that closely mimicked the combined effects of NLX-101 and F13714 on sucrose intake at both short- and long-term exposure, for all sucrose concentrations. This reduction of sugar intake by 5-HT_{1A} agonists was unlikely mediated by reduced locomotor activity. Instead, 5-HT_{1A} agonists caused no change (NLX-101) or an increase in locomotor activity (F13714 and NLX-112), which could potentially be attributed to their anxiolytic properties [15], which could result in an augmentation of exploratory behaviour in a novel environment [35].

A limitation of this study is that, while the results point out to the behavioural of sensitisation 5-HT_{1A} autoreceptors after sucrose consumption, further validations are needed to determine whether 5-HT_{1A} autoreceptor sensitisation occurs at physiological or molecular levels. Electrophysiological, biochemical or autoradiography studies are therefore needed to identify if the observed sensitisation is mediated by upregulation of 5-HT_{1A} autoreceptor function/G protein coupling or expression as previously reported [27]. A second limitation of this study is that we cannot rule out a change in pharmacokinetic parameters of the NLX compounds following chronic sucrose consumption, since high sucrose intake has been shown to affect hepatic function. However it seems that chronic sucrose mostly reduces the activity of cytochrome P450 [50], the enzyme that metabolises most drugs, hence likely resulting in increased systemic availability of the NLX compounds. However, we observed both an increase and a decrease in the efficacy of the NLX compounds as a function of sugar concentration or time of

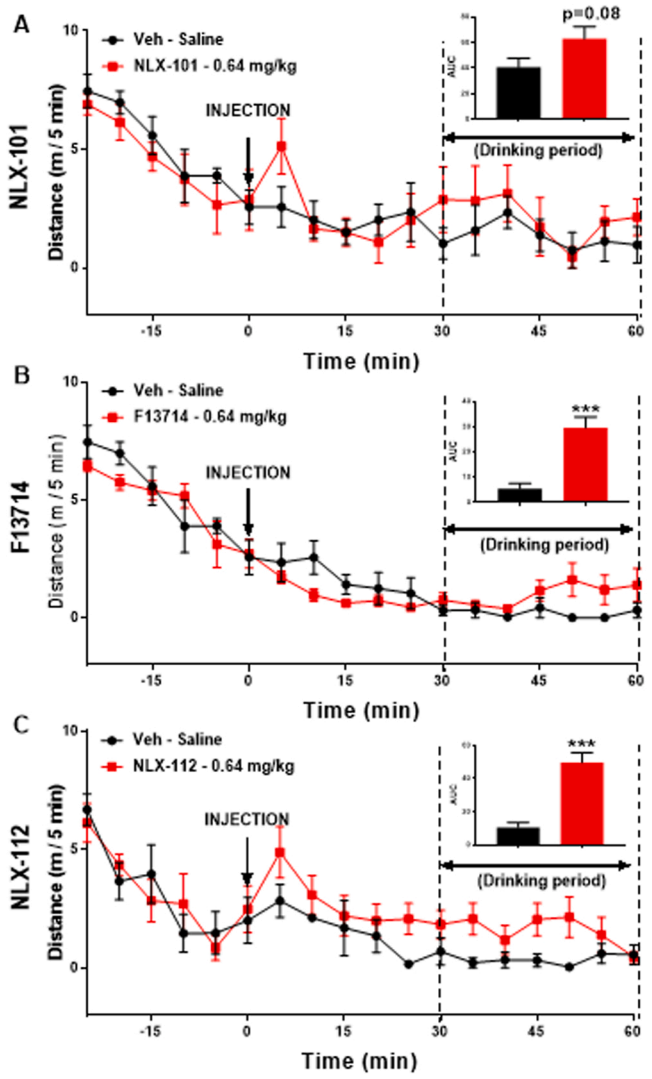


Fig. 7. Effects of NLX-101, F13714 and NLX-112 on locomotor activity as measured by distance travelled (m/5 min) over 60 min activity sessions. Water consuming animals were administered with agonists 30 min after baseline recording as indicated by the arrow. Area under curve (AUC) analysis values were recorded 30–60 min after injection of the 5-HT_{1A} agonists in order to observe the behaviour under the 30 min drinking period. **(A)** Analysis of AUC values for 30–60 min period revealed no significant difference in general locomotor behaviour between vehicle-saline controls and vehicle-NLX-101 0.64 mg/kg. **(B)** Analysis of AUC values for 30–60 min period revealed a significant increase in general locomotor behaviour between vehicle (saline) controls and F13714 0.64 mg/kg. **(C)** Analysis of AUC values for 30–60 min period revealed a significant increase in general locomotor behaviour between vehicle controls and NLX-112 0.64 mg/kg.

testing (week 6 *vs* week 12 of 10% sucrose: decrease in NLX-101 & NLX-112 efficacy *vs* increase in NLX-101 & NLX-112 efficacy for 10% *vs* 25% sucrose at week 12). Thus, it is likely that these differences are mediated by different pharmacological profiles rather than sucrose-induced changes in pharmacokinetics of the NLX compounds. Preclinical development data suggests that NLX-112 and NLX-101 do not induce or inhibit cytochrome p450 enzymes in naïve rats at these doses (Neurolixis data on file), suggesting an absence of hepatic drug-drug interaction, between NLX compounds and sucrose. Nonetheless, further work is needed to clarify this point.

The current study confirmed that 5-HT_{1A} receptor signalling is influenced by sucrose intake with 5-HT_{1A} autoreceptors particularly involved in sucrose drinking behaviour. Autoreceptors are located on

Table 2
Summary of results from NLX-101, F13714 and NLX-112 at 6 and 12 weeks of sucrose consumption. Sucrose consumption increases the recruitment of 5-HT_{1A} receptors in a dose-dependent manner. There is a shift in 5-HT1A receptor subpopulations' effects between 6 and 12 weeks of sucrose consumption.

	6 weeks				12 weeks			
Time after onset of drinking	30 min		2 h		30 min		2 h	
NLX-101 treatment (mg/kg)	0.16	0.64	0.16	0.64	0.16	0.64	0.16	0.64
NLX-101								
5%	–	–	–	–	–	–	–	–
10%	↓	↓	–	↓	–	–	–	–
25%	–	↓	–	↓	–	↓	–	↓
F13714								
5%	–	↓	–	↓	–	↓	–	↓
10%	↓	↓	–	↓	↓	↓	↓	↓
25%	↓	↓	↓	↓	↓	↓		↓
NLX-112								
5%	–	↓	–	–	–	↓	–	–
10%	↓	↓	–	–	–	–	–	–
25%	↓	↓	–	↓	↓	↓	–	↓

serotonin neurons from the raphe nuclei, however, another limitation of the current study is that we cannot determine which raphe nuclei (dorsal, median, caudal) mediates the effect of 5-HT_{1A} autoreceptors on sugar intake. Further studies using specific brain microinjection techniques in particular raphe nuclei are needed to address this question. The 5-HT_{1A} autoreceptors expressed on 5-HT raphe neurons exert a negative control on 5-HT neuron activity [9], which inhibits the release of 5-HT at the nerve terminals [6]. Previous work in our laboratory suggests the overconsumption of sucrose leads to increased locomotor activity, reduced impulse control and neurogenic deficits [4]. Potential serotonergic pathways/circuits affected by sucrose consumption could include: hyperactivity (DR to caudate-putamen and nucleus accumbens [51]), impulsivity (DR to prefrontal cortex/orbitofrontal cortex [14]) and neurogenesis (DR or MR to hippocampus [45]). Further work using optogenetic or chemogenetic techniques is therefore required to identify which serotonin neuronal subpopulations and brain circuits are involved in the control of sugar consumption.

CRediT authorship contribution statement

Kate Beecher: Conceptualization, Investigation, Visualization, Data curation, Formal analysis, Writing – original draft. **Joshua Wang:** Investigation, Formal analysis, Data curation, Writing – original draft. **Fatemeh Chehrehasa:** Supervision, Writing – original draft, Writing – review & editing. **Ronan Depoortere:** Resources, Writing – review & editing, Supervision. **Mark A. Varney:** Resources, Writing – review & editing, Supervision. **Adrian Newman-Tancredi:** Resources, Conceptualization, Writing – review & editing, Supervision, Project administration. **Selena E. Bartlett:** Conceptualization, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Arnauld Belmer:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

Conflict of Interests

ANT, RD and MV are shareholders and/or employees of Neurolixis and/or have a proprietary interest in NLX-101 and NLX-112.

Acknowledgements

This study was supported by the National Health and Medical Research Council (NHMRC) (GNT1146417) to Selena E Bartlett. We

would like to thank Syed Aoun Ali, Ignatius Alvarez Cooper, and Fatema Nasrin for their support in animal welfare. We are thankful to PACE animal facility manager Lisa Foster and her staff Rachel Smith, Miranda Sleath, and Corey Peterson for the exquisite care of our animals.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.112699.

References

[1] P.R. Albert, F. Vahid-Ansari, C. Luckhart, Serotonin-prefrontal cortical circuitry in anxiety and depression phenotypes: pivotal role of pre- and post-synaptic 5-HT1A receptor expression, Front. Behav. Neurosci. 8 (2014) 199, https://doi.org/10.3389/fnbeh.2014.00199.

[2] M.-B. Assié, L. Bardin, A.L. Auclair, E. Carilla-Durand, R. Depoortère, W. Koek, M. S. Kleven, F. Colpaert, B. Vacher, A. Newman-Tancredi, 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 (10) (2010) 1285–1298, https://doi.org/10.1017/S1461145709991222.

[3] M.-B. Assié, H. Lomenech, V. Ravailhe, V. Faucillon, A. Newman-Tancredi, 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 (2) (2006) 170–178, https://doi.org/10.1038/sj.bjp.0706859.

[4] K. Beecher, I. Alvarez Cooper, J. Wang, S.B. Walters, F. Chehrehasa, S.E. Bartlett, A. Belmer, Long-term overconsumption of sugar starting at adolescence produces persistent hyperactivity and neurocognitive deficits in adulthood, Front. Neurosci. 15 (2021), 670430, https://doi.org/10.3389/fnins.2021.670430.

[5] K. Beecher, J. Wang, A. Jacques, N. Chaaya, F. Chehrehasa, A. Belmer, S.E. Bartlett, Sucrose consumption alters serotonin/glutamate co-localisation within the prefrontal cortex and hippocampus of mice, Front. Mol. Neurosci. 14 (2021), 678267, https://doi.org/10.3389/fnmol.2021.678267.

[6] A. Belmer, L. Maroteaux, Regulation of raphe serotonin neurons by serotonin 1A and 2B receptors, Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. 44 (1) (2019) 218–219, https://doi.org/10.1038/s41386-018-0214-6.

[7] A. Belmer, O.L. Patkar, V. Lanoue, S.E. Bartlett, 5-HT1A receptor-dependent modulation of emotional and neurogenic deficits elicited by prolonged consumption of alcohol, Sci. Rep. 8 (1) (2018) 2099, https://doi.org/10.1038/s41598-018-20504-z.

[8] A. Belmer, O.L. Patkar, K.M. Pitman, S.E. Bartlett, Serotonergic neuroplasticity in alcohol addiction, Brain Plast. 1 (2) (2016) 177–206, https://doi.org/10.3233/BPL-150022.

[9] P. Blier, C. de Montigny, Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain, Synapse 1 (5) (1987) 470–480, https://doi.org/10.1002/syn.890010511.

[10] J.H. Broadbear, R.Y. Depoortere, K. Vacy, D. Ralph, B.J. Tunstall, A. Newman-Tancredi, Discriminative stimulus properties of the 5-HT1A receptor biased agonists NLX-101 and F13714, in rats trained to discriminate 8-OH-DPAT from saline, Behav. Pharmacol. 32 (8) (2021) 652–659, https://doi.org/10.1097/FBP.0000000000000659.

[11] J. Buritova, G. Berrichon, C. Cathala, F. Colpaert, D. Cussac, Region-specific changes in 5-HT1A agonist-induced extracellular signal-regulated kinases 1/2 phosphorylation in rat brain: a quantitative ELISA study, Neuropharmacology 56 (2) (2009) 350–361, https://doi.org/10.1016/j.neuropharm.2008.09.004.

[12] Z. Chilmonczyk, A.J. Bojarski, A. Pilc, I. Sylte, Functional selectivity and antidepressant activity of serotonin 1A receptor ligands, Int. J. Mol. Sci. 16 (8) (2015) 18474–18506, https://doi.org/10.3390/ijms160818474.

[13] F.C. Colpaert, J.P. Tarayre, W. Koek, P.J. Pauwels, L. Bardin, X.-J. Xu, Z. Wiesenfeld-Hallin, C. Cosi, E. Carilla-Durand, M.B. Assié, B. Vacher, Large-amplitude 5-HT1A receptor activation: a new mechanism of profound, central analgesia, Neuropharmacology 43 (6) (2002) 945–958, https://doi.org/10.1016/S0028-3908(02)00119-3.

[14] M. Darna, J.J. Chow, J.R. Yates, R.J. Charnigo, J.S. Beckmann, M.T. Bardo, L. P. Dwoskin, Role of serotonin transporter function in rat orbitofrontal cortex in impulsive choice, Behav. Brain Res. 293 (2015) 134–142, https://doi.org/10.1016/j.bbr.2015.07.025.

[15] J. De Vry, 5-HT1A receptor agonists: recent developments and controversial issues, Psychopharmacology 121 (1) (1995) 1–26, https://doi.org/10.1007/BF02245588.

[16] R. Depoortère, A.L. Auclair, A. Newman-Tancredi, NLX-101, a highly selective 5-HT1A receptor biased agonist, mediates antidepressant-like activity in rats via prefrontal cortex 5-HT1A receptors, Behav. Brain Res. 401 (2021), 113082, https://doi.org/10.1016/j.bbr.2020.113082.

[17] R. Depoortère, L. Bardin, M.A. Varney, A. Newman-Tancredi, Serotonin 5-HT1A receptor biased agonists display differential anxiolytic activity in a rat social interaction model, ACS Chem. Neurosci. 10 (7) (2019) 3101–3107, https://doi.org/10.1021/acschemneuro.8b00661.

[18] C.T. Dourish, P.H. Hutson, G.A. Kennett, G. Curzon, 8-OH-DPAT-induced hyperphagia: its neural basis and possible therapeutic relevance, Appetite (7 Suppl.) (1986) S127–S140, https://doi.org/10.1016/s0195-6663(86)80058-7.

[19] M. Głuch-Lutwin, K. Sałaciak, A. Gawalska, M. Jamrozik, J. Sniecikowska, A. Newman-Tancredi, M. Kołaczkowski, K. Pytka, The selective 5-HT1A receptor biased agonists, F15599 and F13714, show antidepressant-like properties after a single administration in the mouse model of unpredictable chronic mild stress, Psychopharmacology 238 (8) (2021) 2249–2260, https://doi.org/10.1007/s00213-021-05849-0.

[20] J.C.G. Halford, E.J. Boyland, J.E. Blundell, T.C. Kirkham, J.A. Harrold, Pharmacological management of appetite expression in obesity, Nat. Rev. Endocrinol. 6 (5) (2010) 255–269, https://doi.org/10.1038/nrendo.2010.19.

[21] D. Hoyer, J.P. Hannon, G.R. Martin, Molecular, pharmacological and functional diversity of 5-HT receptors, Pharmacol. Biochem. Behav. 71 (4) (2002) 533–554, https://doi.org/10.1016/s0091-3057(01)00746-8.

[22] X. Huang, J. Yang, S. Yang, S. Cao, D. Qin, Y. Zhou, X. Li, Y. Ye, J. Wu, Role of tandospirone, a 5-HT1A receptor partial agonist, in the treatment of central nervous system disorders and the underlying mechanisms, Oncotarget 8 (60) (2017) 102705–102720, https://doi.org/10.18632/oncotarget.22170.

[23] P.H. Hutson, C.T. Dourish, G. Curzon, Neurochemical and behavioural evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors, Eur. J. Pharmacol. 129 (3) (1986) 347–352, https://doi.org/10.1016/0014-2999(86)90445-0.

[24] Q.-U.-A. Inam, M.A. Haleem, D.J. Haleem, Effects of long term consumption of sugar as part of meal on serotonin 1-a receptor dependent responses, Pak. J. Pharmaceut. Sci. 19 (2) (2006) 94–98.

[25] A. Jacques, N. Chaaya, K. Beecher, S.A. Ali, A. Belmer, S. Bartlett, The impact of sugar consumption on stress driven, emotional and addictive behaviors, Neurosci. Biobehav. Rev. 103 (2019) 178–199, https://doi.org/10.1016/j.neubiorev.2019.05.021.

[26] M. Jastrzębska-Więsek, A. Partyka, J. Rychtyk, J. Śniecikowska, M. Kołaczkowski, A. Wesołowska, M.A. Varney, A. Newman-Tancredi, Activity of serotonin 5-HT1A receptor biased agonists in rat: anxiolytic and antidepressant-like properties, ACS Chem. Neurosci. 9 (5) (2018) 1040–1050, https://doi.org/10.1021/acschemneuro.7b00443.

[27] Sabah Kelaï, Thibault Renoir, Laurent Chouchana, Françoise Saurini, Naïma Hanoun, Michel Hamon, Laurence Lanfumey, Chronic voluntary ethanol intake hypersensitizes 5–HT1A autoreceptors in C57BL/6J mice, J. Neurochem. 107 (6) (2008) 1660–1670, https://doi.org/10.1111/j.1471-4159.2008.05733.x.

[28] L. Lemoine, M. Verdurand, B. Vacher, E. Blanc, D. Le Bars, A. Newman-Tancredi, L. Zimmer, [18F]F15599, a novel 5-HT1A receptor agonist, as a radioligand for PET neuroimaging, Eur. J. Nucl. Med. Mol. Imaging 37 (3) (2010) 594–605, https://doi.org/10.1007/s00259-009-1274-y.

[29] L. Lladó-Pelfort, M.-B. Assié, A. Newman-Tancredi, F. Artigas, P. Celada, Preferential in vivo action of F15599, a novel 5-HT(1A) receptor agonist, at postsynaptic 5-HT(1A) receptors, Br. J. Pharmacol. 160 (8) (2010) 1929–1940, https://doi.org/10.1111/j.1476-5381.2010.00738.x.

[30] E.G. Lowery-Gionta, C.A. Marcinkiewcz, T.L. Kash, Functional alterations in the dorsal raphe nucleus following acute and chronic ethanol exposure, Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. 40 (3) (2015) 590–600, https://doi.org/10.1038/npp.2014.205.

[31] C.M. Mathes, J.R. Gregson, A.C. Spector, The selective serotonin reuptake inhibitor paroxetine decreases breakpoint of rats engaging in a progressive ratio licking task for sucrose and quinine solutions, Chem. Senses 38 (3) (2013) 211–220, https://doi.org/10.1093/chemse/bjs096.

[32] S. Murray, A. Tulloch, K. Criscitelli, N.M. Avena, Recent studies of the effects of sugars on brain systems involved in energy balance and reward: relevance to low calorie sweeteners, Physiol. Behav. 164 (Pt B) (2016) 504–508, https://doi.org/10.1016/j.physbeh.2016.04.004.

[33] I. Nevo, X. Langlois, A.-M. Laporte, M. Kleven, W. Koek, L. Lima, C. Maudhuit, M.-P. Martres, M. Hamon, Chronic alcoholization alters the expression of 5-HT1A and 5-HT1B receptor subtypes in rat brain, Eur. J. Pharmacol. 281 (3) (1995) 229–239, https://doi.org/10.1016/0014-2999(95)00238-G.

[34] A. Newman-Tancredi, C. Chaput, S. Gavaudan, L. Verrièle, M.J. Millan, Agonist and antagonist actions of (-)pindolol at recombinant, human serotonin$_{1A}$ (5-HT$_{1A}$) receptors, Neuropsychopharmacology 18 (5) (1998) 395–398, https://doi.org/10.1016/S0893-133X(97)00169-3.

[35] A. Newman-Tancredi, R.Y. Depoortère, M.S. Kleven, M. Kołaczkowski, L. Zimmer, Translating biased agonists from molecules to medications: serotonin 5-HT1A receptor functional selectivity for CNS disorders, Pharmacol. Ther. 229 (2021), 107937, https://doi.org/10.1016/j.pharmthera.2021.107937.

[36] A. Newman-Tancredi, J.-C. Martel, M.-B. Assié, J. Buritova, E. Lauressergues, C. Cosi, P. Heusler, L. Bruins Slot, F.C. Colpaert, B. Vacher, D. Cussac, Signal transduction and functional selectivity of F15599, a preferential post-synaptic 5-HT1A receptor agonist, Br. J. Pharmacol. 156 (2) (2009) 338–353, https://doi.org/10.1111/j.1476-5381.2008.00001.x.

[37] A. Newman-Tancredi, J.-C. Martel, C. Cosi, P. Heusler, F. Lestienne, M.A. Varney, D. Cussac, Distinctive in vitro signal transduction profile of NLX-112, a potent and efficacious serotonin 5-HT1A receptor agonist, J. Pharm. Pharmacol. 69 (9) (2017) 1178–1190, https://doi.org/10.1111/jphp.12762.

[38] M.T. O'Connell, G. Curzon, A comparison of the effects of 8-OH-DPAT pretreatment of different behavioural responses to 8-OH-DPAT, Eur. J. Pharmacol. 312 (2) (1996) 137–143, https://doi.org/10.1016/0014-2999(96)00496-7.

[39] O.L. Patkar, A. Belmer, K. Beecher, A. Jacques, S.E. Bartlett, Pindolol rescues anxiety-like behavior and neurogenic maladaptations of long-term binge alcohol intake in mice, Front. Behav. Neurosci. 13 (2019) 264, https://doi.org/10.3389/fnbeh.2019.00264.

[40] O.L. Patkar, A. Belmer, J.Y. Holgate, P.M. Klenowski, S.E. Bartlett, Modulation of serotonin and noradrenaline in the BLA by pindolol reduces long-term ethanol intake, Addict. Biol. (2018), https://doi.org/10.1111/adb.12630.
[41] O.L. Patkar, Arnauld Belmer, Joan Y. Holgate, Tarren, R. Josephine, R. Shariff Masroor, Michael Morgan, Matthew J. Fogarty, Bellingham, C. Mark, Bartlett, E. Selena, Klenowski, M. Paul, The antihypertensive drug pindolol attenuates long-term but not short-term binge-like ethanol consumption in mice, Addict. Biol. 22 (3) (2017) 679–691, https://doi.org/10.1111/adb.12359.
[42] W.H. Powell, L.E. Annett, R. Depoortere, A. Newman-Tancredi, M.M. Iravani, The selective 5-HT1A receptor agonist NLX-112 displays anxiolytic-like activity in mice, Naunyn-Schmiedeberg's Arch. Pharmacol. 395 (2021) 149–157, https://doi.org/10.1007/s00210-021-02183-2.
[43] J.W. Richardson-Jones, C.P. Craige, T.H. Nguyen, H.F. Kung, A.M. Gardier, A. Dranovsky, D.J. David, B.P. Guiard, S.G. Beck, R. Hen, E.D. Leonardo, Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety, J. Neurosci. 31 (16) (2011) 6008–6018, https://doi.org/10.1523/JNEUROSCI.5836-10.2011.
[44] L. Romero, N. Bel, F. Artigas, C. de Montigny, P. Blier, Effect of pindolol on the function of pre- and postsynaptic 5-HT1A receptors: in vivo microdialysis and electrophysiological studies in the rat brain, Neuropsychopharmacology 15 (4) (1996) 349–360, https://doi.org/10.1016/0893-133X(95)00240-E.
[45] E. Segi-Nishida, The effect of serotonin-targeting antidepressants on neurogenesis and neuronal maturation of the hippocampus mediated via 5-HT1A and 5-HT4 receptors, Front. Cell. Neurosci. 0 (2017), https://doi.org/10.3389/fncel.2017.00142.
[46] M. Shariff, M. Quik, J. Holgate, M. Morgan, O.L. Patkar, V. Tam, A. Belmer, S. E. Bartlett, Neuronal nicotinic acetylcholine receptor modulators reduce sugar intake, PLoS One 11 (3) (2016), e0150270, https://doi.org/10.1371/journal.pone.0150270.
[47] H. Shimizu, T. Tatsuno, H. Tanaka, A. Hirose, Y. Araki, M. Nakamura, Serotonergic mechanisms in anxiolytic effect of tandospirone in the Vogel conflict test, Jpn. J. Pharmacol. 59 (1) (1992) 105–112, https://doi.org/10.1254/jjp.59.105.
[48] P. Steensland, J.A. Simms, J. Holgate, J.K. Richards, S.E. Bartlett, Varenicline, an α4β2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking, Proc. Natl. Acad. Sci. USA 104 (30) (2007) 12518–12523, https://doi.org/10.1073/pnas.0705368104.
[49] H. Tanaka, H. Shimizu, Y. Kumasaka, A. Hirose, T. Tatsuno, M. Nakamura, Autoradiographic localization and pharmacological characterization of [3H] tandospirone binding sites in the rat brain, Brain Res. 546 (2) (1991) 181–189, https://doi.org/10.1016/0006-8993(91)91479-k.
[50] U.M. Zanger, M. Schwab, Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation, Pharmacol. Ther. 138 (1) (2013) 103–141, https://doi.org/10.1016/j.pharmthera.2012.12.007.
[51] K. Zhang, E. Davids, F.I. Tarazi, R.J. Baldessarini, Serotonin transporter binding increases in caudate-putamen and nucleus accumbens after neonatal 6-hydroxydopamine lesions in rats: implications for motor hyperactivity, Dev. Brain Res. 137 (2) (2002) 135–138, https://doi.org/10.1016/S0165-3806(02)00436-4.