The selective 5-HT$_{1A}$ receptor agonist, NLX-112, exerts anti-dyskinetic and anti-parkinsonian-like effects in MPTP-treated marmosets

Ria Fisher[a], Atsuko Hikima[a], Rebecca Morris[a], Michael J. Jackson[a], Sarah Rose[a],
Mark A. Varney[b], Ronan Depoortere[b], Adrian Newman-Tancredi[b],*

[a] Faculty of Life Sciences and Medicine, Hodgkin Building, King's College London, Guy's Campus, London, SE1 1UL, UK
[b] Neurolixis SAS, 2 Rue Georges Charpak, 81100, Castres, France

HIGHLIGHTS

- L-DOPA-induced dyskinesia (LID) develops in many Parkinson's disease patients.
- NLX-112 is a highly selective and high efficacy serotonin 5-HT$_{1A}$ receptor agonist.
- In MPTP-treated marmosets NLX112 reduced LID without impairing therapeutic activity.
- NLX-112 by itself exhibited antiparkinsonian activity (decreased motor disability).
- NLX-112 is a promising drug candidate for treatment of Parkinson's disease.

ARTICLE INFO

ABSTRACT

L-DOPA is the gold-standard pharmacotherapy for treatment of Parkinson's disease (PD) but can lead to the appearance of troubling dyskinesia which are attributable to 'false neurotransmitter' release of dopamine by serotonergic neurons. Reducing the activity of these neurons diminishes L-DOPA-induced dyskinesia (LID), but there are currently no clinically approved selective, high efficacy 5-HT$_{1A}$ receptor agonists. Here we describe the effects of NLX-112, a highly selective and efficacious 5-HT$_{1A}$ receptor agonist, on LID in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets, a non-human primate model of PD.

NLX-112 exhibited modest plasma half-life (~2h) and marked plasma protein binding (96%). When administered to parkinsonian marmosets with L-DOPA (7 mg/kg p.o.), NLX-112 (0.025, 0.1 and 0.4 mg/kg p.o.) reduced LID scores at early time-points after administration, whilst only minimally interfering with the L-DOPA-induced reversal of motor disability. In contrast, the prototypical 5-HT$_{1A}$ receptor agonist, (+)8-OH-DPAT (0.6 and 2 mg/kg p. o.), reduced LID but also abolished L-DOPA's anti-disability activity.

Administered by itself, NLX-112 (0.1, 0.2 mg/kg p.o.) produced very little dyskinesia or locomotor activity, but reduced motor disability scores by about half the extent elicited by L-DOPA, suggesting that it may have motor facilitation effects of its own. Both NLX-112 and (+)8-OH-DPAT induced unusual and dose-limiting behaviors in marmoset that resembled 'serotonin behavioral syndrome' observed previously in rat.

Overall, the present study showed that NLX-112 has anti-LID activity at the doses tested as well as reducing motor disability. The data suggest that additional investigation of NLX-112 is desirable to explore its potential as a treatment for PD and PD-LID.

1. Introduction

Parkinson's disease (PD) is characterized by a loss of nigrostriatal dopaminergic neurons resulting in the cardinal motor symptoms of tremor, rigidity, slowness of movement, and difficulty with walking (Schapira et al., 2006). Treatment of these motor symptoms relies primarily on the gold-standard medication, L-3,4-dihydroxyphenylalanine (L-DOPA) but, over time, as the disease progresses, more and more neurons die, and the buffering capacity of dopamine is reduced. As a result, a sizeable proportion of patients develop dose-limiting dyskinesia, characterized by hyperkinetic movements, including chorea, dystonia, and athetosis (Jackson et al., 2004; Jenner

---

et al., 2011; Sellnow et al., 2019). Such L-DOPA-induced dyskinesia (LID) can become increasingly troublesome but its treatment has been hampered by a lack of sufficiently efficacious and well-tolerated medications, although some success has been achieved by use of the glutamate receptor antagonist amantadine. However, its use, like that of other glutamate NMDA receptor antagonists is limited by tolerance issues and suboptimal efficacy in some patients (Pilleri and Antonini, 2015) and a clear need therefore exists for novel pharmacological approaches (Cenci et al., 2019). Over recent years, the 5-hydroxytryptamine (5-HT) system has emerged as a key element in the etiology of dyskinesia. 5-HT neurons possess the enzyme necessary to convert exogenous L-DOPA to dopamine (DA), which can be stored in vesicles and released as a 'false neurotransmitter' (Carta et al., 2008; Politis et al., 2014). However, 5-HT neurons lack appropriate control mechanisms to regulate synaptic DA levels, resulting in excessive and physiologically inappropriate DA release and pulsatile stimulation of post-synaptic DA receptors that generate dyskinesia. Several lines of evidence support this model for induction of LID, as well as the desirability of a potent, selective and high efficacy 5-HT$_{1A}$ agonist to treat LID: (i) destruction of serotonergic neurons with the toxin, 5,7-dihydroxytryptamine, leads to a near-complete suppression of LID in hemi-parkinsonian rats (Carta et al., 2007); (ii) transplanting serotoninergic neurons into the striatum of hemi-parkinsonian rats worsens LID (Carlsson et al., 2007, 2009); (iii) silencing of 5-HT neurons via activation of pre-synaptic 5-HT$_{1B}$ or 5-HT$_{1A}$ autoreceptors reduces or eliminates LID in rat and non-human primate models of LID (Carta et al., 2007; Eskow et al., 2009; Munoz et al., 2008); (iv) the 5-HT precursor, 5-hydroxytryptophan reduces dyskinesia in hemi-parkinsonian rats through displacement of DA from serotonergic vesicles and via activation of 5-HT auto-receptors (Tronci et al., 2013).

Several attempts have been made to use serotonergic agonists, such as buspirone, sarizotan or eltoprazine, as treatments for LID. However, only moderate efficacy was observed, and the beneficial anti-parkinsonian effects of L-DOPA was reduced. The causes of these disappointing results are likely related to several factors, including metabolic instability (for buspirone) (Caccia et al., 1983; Politis et al., 2014), interaction with off-target sites (notably DA D$_2$ receptor antagonism by sarizotan and buspirone) (Peroutka, 1985; Rabiner et al., 2002) and lack of sufficient agonist efficacy at 5-HT$_{1A}$ receptors (partial agonism by buspirone and eltoprazine). The question still remains, therefore, as to whether a selective and high efficacy 5-HT$_{1A}$ receptor agonist can show efficacious anti-LID properties without interfering with the beneficial anti-parkinsonian effects of L-DOPA.

The novel 5-HT$_{1A}$ receptor agonist, NLX-112, fits the criteria for a potentially superior anti-LID therapy. Unlike previous 5-HT$_{1A}$ receptor agonists, NLX-112 (a.k.a. befiradol or F13640) is a potent (nanomolar affinity), 5-HT$_{1A}$ receptor 'full' agonist and highly selective (> 1000-fold selectivity over a wide palette of off-target sites (Colpaert et al., 2002). NLX-112 exhibits a distinctive profile for in vitro 5-HT$_{1A}$ receptor activation, preferentially activating specific intracellular G-proteins and signal transduction cascades. Thus, unlike 8-OH-DPAT or buspirone, NLX-112 preferentially activates Gαo proteins and elicits pronounced phosphorylation of ERK1/2, both in vitro and ex vivo (Buritova et al., 2009; Newman-Tancredi et al., 2017). NLX-112 rapidly penetrates the brain following systemic dosing (Bardin et al., 2005). Radiolabeled ^{18}F-NLX-112 binds specifically to rat, cat, non-human primate (macaque) as well as human brain regions known to express 5-HT$_{1A}$ receptors (Colom et al., 2020; Vidal et al., 2016, 2018). Notably, NLX-112 efficaciously and completely inhibits the electrical activity of dorsal raphe 5-HT neurons via activation of pre-synaptic 5-HT$_{1A}$ autoreceptors (Llado-Pelfort et al., 2012). Accordingly, striatal serotonin release is profoundly reduced by NLX-112 in 6-OH-DA-lesioned hemi-parkinsonian rats treated with L-DOPA (Iderberg et al., 2015b). This is accompanied by a blunting of L-DOPA-induced dopamine release, confirming the ability of NLX-112 to inhibit serotonergic neurons responsible for the "false neurotransmitter" surge in DA release (Iderberg

et al., 2015a).

The same doses of NLX-112 that produce these neurochemical effects also abolish the electrophysiological signature (cortical slow-wave oscillations) of LID in 6-OH-DA-lesioned rats (Brys et al., 2018) and reverse haloperidol-induced catalepsy, a read-out of D$_2$ receptor blockade-induced motor impairment (Iderberg et al., 2015b). Abnormal Involuntary Movements (AIMs) in hemi-parkinsonian rats chronically treated with L-DOPA were dose-dependently and completely eliminated by NLX-112 and this effect was entirely reversed by a selective 5-HT$_{1A}$ receptor antagonist (Iderberg et al., 2015b). In addition, the anti-AIMs activity of NLX-112 was maintained upon chronic treatment (14 days) (McCreary et al., 2016a). Autoradiography studies showed that NLX-112 dose-dependently occupies 5-HT$_{1A}$ receptors at doses that correspond to those that are active in rat models of LID (Bardin et al., 2005). Moreover, NLX-112 elicited marked ipsilateral rotations in hemi-parkinsonian rats, suggestive of motor facilitation effects (Iderberg et al., 2015b).

Taken together, these studies show that NLX-112 has strong anti-LID activity in rodents but the question of whether its anti-LID activity can translate from rodent to other species remains to be investigated. A previous study on a related compound, F15599 (a.k.a. NLX-101), showed that it decreased LID in MPTP-treated cynomolgus macaques, without interfering with L-DOPA's therapeutic effects (Huot et al., 2015), suggesting that the anti-LID activity of NLX-112 would similarly translate to primate species. The purpose of the present study was, therefore, to test NLX-112 in a non-human primate model of LID, i.e. MPTP-treated marmosets, which have shown high translational value to human (Veyres et al., 2018). Prior to carrying out behavioral observations, the pharmacokinetics of NLX-112 in marmoset were investigated to determine plasma exposure at the doses tested.

2. Methods

2.1. Animals

Common marmosets (*Callithrix jacchus*, 5 females and 3 males, aged 8–14 years, Harlan UK Ltd. Loughborough, UK and Manchester University, Manchester, UK), previously (6–8 years) treated with MPTP were used for this study. They were housed alone or in pairs, in a room maintained at constant temperature (24 ± 1 °C), 50% relative humidity and with a 12-h light/dark cycle. All animals were given 1 meal of mashed cereal and 1 meal of fresh fruit daily and had *ad libitum* access to food pellets and fresh water. On behavioral test days, water was supplied *ad libitum* and food was given after completion of testing.

2.2. Administration of MPTP and L-DOPA priming

Locomotor and behavioral deficits were induced by subcutaneous administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride; Sigma Chemical, UK) 2 mg/kg daily for up to 5 consecutive days dissolved in sterile 0.9% saline solution (Baxter Healthcare Ltd.). Following recovery from acute effects of MPTP-treatment, dyskinesia was induced by chronic treatment with L-DOPA (10–12.5 mg/kg p.o., plus benserazide (10 mg/kg po) administered up to twice daily for up to 30 days (Pearce et al., 1995). At the start of the study, all MPTP-treated marmosets exhibited stable motor deficits including a marked reduction of basal locomotor activity, poor coordination of movement, abnormal and/or rigid posture, reduced alertness and head checking movements. Procedures were carried out in accordance with the UK Animals (Scientific Procedures Act) 1986 and with approval of the King's College London Ethical Review Panel under project license PPL 70/8541 and were compliant with the minimal standards as defined by the European Communities Council Directive (85/609/EEC).

Following priming for dyskinesia expression, the animals had been previously used for the assessment of the therapeutic and dyskinetic

effects of other dopaminergic and non-dopaminergic agents alone and/ or in combination with L-DOPA or dopamine agonists. One animal showed little or no dyskinesia in response to L-DOPA treatment and was excluded from subsequent analysis. All animals received a washout of at least 2 months prior to the start of this study. They were declared fit for study by the named veterinary surgeon and were certified for re-use by the UK Home Office.

2.3. Drugs treatments

NLX-112 (3-chloro-4-fluorophenyl-[4-fluoro-4-([(5-methylpyridin-2-yl)methylamino]methyl)piperidin-1-yl]methanone, fumarate) was provided by Neurolixis and dissolved in distilled water to prepare a stock solution and then diluted in a 10% w/v sucrose solution to provide the required dose and administered orally at a volume of 2 ml/kg. (+)8-OH-DPAT (Tocris Cookson, Inc., Bristol, UK) was dissolved in sterile 0.9% saline and injected s.c. at a volume of 1 ml/kg. Benserazide HCl (Sigma, UK) was dissolved in a 10% w/v sucrose solution and administered p.o. at a volume of 2 ml/kg. L-DOPA methyl ester HCl (Sigma, UK) was dissolved in a 10% w/v sucrose solution. In all cases, drug doses are expressed as the weight of the free base.

For NLX-112 plasma exposure determination, four marmosets (2 M, 2 F) received a single dose of 0.4 mg/kg p.o. For behavioral observations, the following combinations were tested: (i) L-DOPA (7 mg/kg p.o.) in combination with NLX-112 (0.025, 0.1 and 0.4 mg/kg p.o.) or vehicle (10% sucrose). (ii) NLX-112 (0.1 and 0.2 mg/kg p.o.) or vehicle (10% sucrose) administered alone (i.e. in the absence of L-DOPA). (iii) L-DOPA (7 mg/kg p.o.) given in combination with (+)8-OH-DPAT (0.6 and 2 mg/kg s.c.) or vehicle (0.9% saline). Treatments were administered twice a week according to a modified latin square design, with a minimum of 2 days drug free washout period between treatment days. Benserazide was administered at t-60 min before L-DOPA, and test drug treatment (L-DOPA, NLX-112 and (+)8-OH-DPAT) or vehicles was then administered at time = 0 min (p.o. for L-DOPA, NLX-112; s.c. for (+)8-OH-DPAT). Locomotor activity, motor disability and dyskinesia were monitored before and for up to 5 h after test drug administration as described below.

The doses of NLX-112 were based on allometric scaling, following FDA guidelines that predict that marmoset doses should be similar to those in rat, based on body weight and surface (FDA, 2005; Nair and Jacob, 2016). The doses of (+)8-OH-DPAT were based on the previous marmoset study on this compound (Iravani et al., 2006). The dose of L-DOPA used herein (7 mg/kg p.o.) consistently triggers substantial, but not severe, levels of LID, mimicking the clinical situation where mild-moderate levels of LID are commonly seen. In addition, this dose of L-DOPA produces robust (over 80% decrease) in motor disability score, confirming its strong anti-parkinsonian-like pharmacological activity under these conditions.

2.4. Plasma pharmacokinetics and protein binding of NLX-112 in MPTP-treated marmosets

Following administration of NLX-112, blood was collected over a 24-h period (0, 1, 2, 4, 6 and 24 h), and plasma separated, frozen and stored at −80 °C until analyzed. Total levels of NLX-112 were quantified in plasma by LC-MS/MS based on the method described previously (Bardin et al., 2005). For protein binding experiments to marmoset plasma, three concentrations of NLX-112 (100, 500 and 2500 nM) were selected and tested in vitro using the method of equilibrium dialysis. Briefly, the proportion of the drug able to cross a dialysis membrane provided a measure of the 'free' drug concentration, detected by microdialysis.

2.5. Assessment of locomotor activity

Locomotor activity was assessed with automated activity units

(50 × 60 × 90 cm) fitted with a clear Perspex observation door and 8 horizontal photoelectric emitters/detectors (light beams). Two horizontal wooden perches were fitted and drinking water was available. Interruption of a light beam was recorded as a single locomotor count. Animals were acclimatized individually to the activity unit for 90 min (t −90 to t0 min), during which baseline locomotor activity was recorded. Subsequently, locomotor activity was recorded during serial 30 min intervals.

2.6. Assessment of motor disability and dyskinesia

Motor disability and dyskinesia were assessed simultaneously in the automated activity units at 20 or 30 min intervals (see figures for details). For each scoring period the animals were observed for the last 10 min of the period (e.g. for the first 30 min period, the animals were observed from 20 to 30 min). The lowest motor disability and the greatest dyskinesia scores were recorded for each period. The assessment of motor disability was performed with an established rating scale: alertness (normal = 0, reduced = 1, sleepy = 2); checking (present = 0, reduced = 1, absent = 2); posture (normal = 0, abnormal trunk +1, abnormal tail + 1, abnormal limbs + 1, flexed = 4); balance (normal = 0, impaired = 1, unstable = 2, spontaneous falls = 3); reaction to stimuli (normal = 0, reduced = 1, slow = 2, absent = 3); vocalization (normal = 0, reduced = 1, absent = 2); motility (normal = 0, bradykinesia = 1, akinesia = 2). These values were summed, a maximum score of 18 indicating severe motor disability, a minimum score of 0 indicating normal motor behavior.

Assessment of dyskinesia was performed with an established rating scale: 0 = absent; 1 = mild, fleeting and rare dyskinetic postures and movements; 2 = moderate: more prominent abnormal movements, but not significantly affecting normal behavior; 3 = marked, frequent and at times continuous dyskinesia affecting the normal pattern of activity; 4 = severe, virtually continuous dyskinetic activity, disabling to the animal and replacing normal behavior.

The assessment of motor disability and dyskinesia was performed by an observer blinded to the treatment.
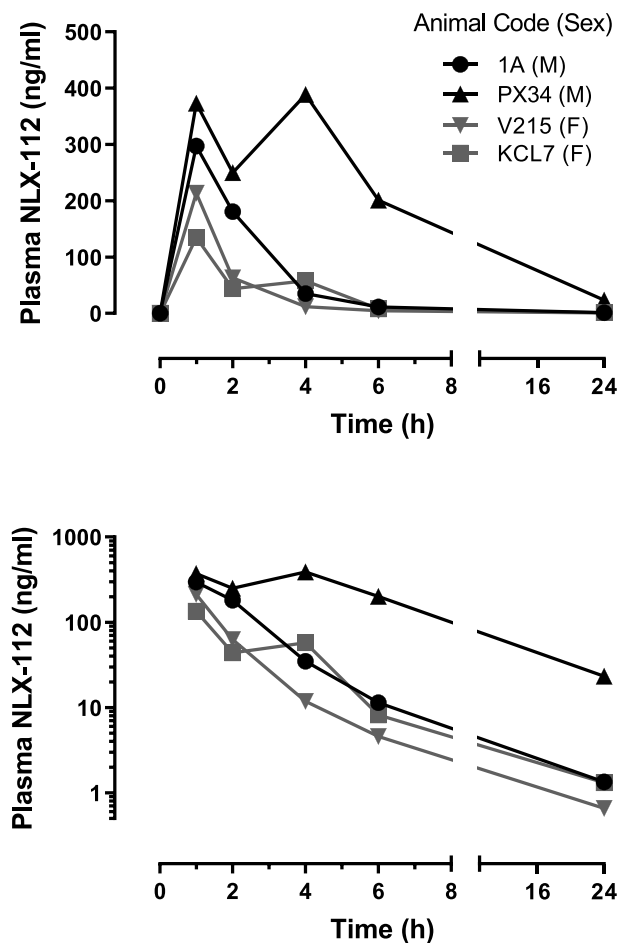
2.7. Statistical and data analysis

Data for the plasma level determination experiment are presented as the mean of duplicate measures. Data for dyskinesia and motor disability being ordinal in nature, are presented graphically as median values at each time point. They were then subjected to a square root transformation [y = sqrt(y)] to make them amenable to parametric one or two-way ANOVA (see below). Data for locomotor activity are presented graphically as mean values at each time point. Data for the effects of NLX-112 (when administered alone) were analyzed with a one-way ANOVA for repeated measures with time as the within-subjects factor. Data for the combination of NLX-112 and L-DOPA were analyzed with a two-way ANOVA, with treatment and time as the within-subjects factors. Where applicable, ANOVAs were followed by Dunnett's post-hoc tests for comparison with the −60 min time point (one-way ANOVA) or with the L-DOPA alone group as the control, for each time point (two-way ANOVA).

3. Results

3.1. Plasma levels and protein binding of NLX-112 in MPTP-treated marmosets

In 3 out of 4 animals tested (2 M, 2 F), peak plasma exposure of NLX-112 occurred at 1-h post dosing with levels between 130 and 370 ng/ml (Fig. 1). For animal PX34, exposure was higher and more sustained over 4 h post-dosing. NLX-112 was fairly rapidly eliminated such that, in 3 out of 4 animals, plasma levels at 6 h were around 10 ng/ml. Although the numbers of animals in this study is too small to draw

Fig. 1. Plasma Levels of NLX-112 in Marmosets. Symbols represent the mean of duplicate measures taken from the same plasma sample following administration of 0.4 mg/kg p.o. NLX-112 was quantified by LC-MS/MS. The same data are plotted on a linear scale y-axis (upper panel) and on a $\log_{10}$ scale y-axis (lower panel). M: male, F: female.

firm conclusions, it is noted that the two male marmosets exhibited higher exposure levels than the two females. The data suggests that plasma half-life in marmoset is about 2 h.
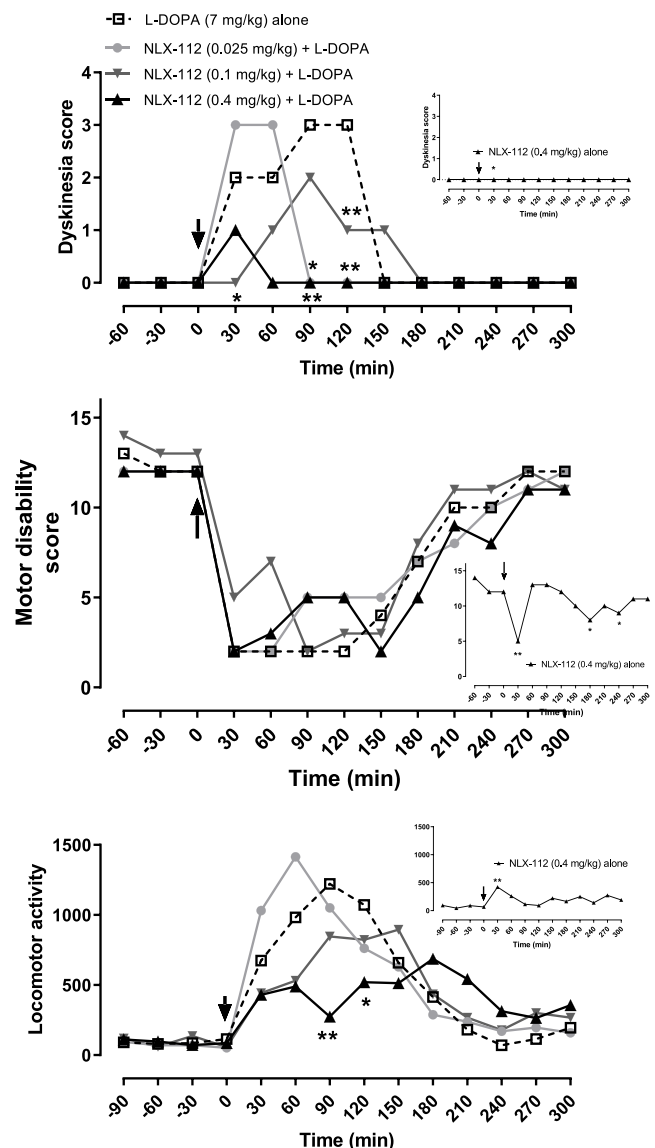
As concerns plasma protein binding, the percentage of unbound NLX-112 was independent of initial concentration in all cases. The unbound fraction of NLX-112 was 3.8–4.5%, giving a plasma protein binding (PPB) value of 96%.

3.2. Effects of NLX-112 on L-DOPA-induced effects

3.2.1. Dyskinesia score

L-DOPA, by itself, increased dyskinesia scores, with peak median values of 3 at 90 and 120 min post treatment (open squares, Fig. 2, top panel). NLX-112 attenuated dyskinesia scores, reducing median values to 2 or less at doses of 0.1 and 0.4 mg/kg. There was a significant time effect [$F_{(12,72)} = 14.22$; $P < 0.0001$] and treatment × time interaction [$F_{(36,216)} = 1.77$; $P < 0.01$, but not treatment effect [$F_{(3,18)} = 0.68$; ns]]. Subsequent post-hoc analysis showed that NLX-112 significantly reduced dyskinesia scores by around 50% at various time x dose combinations between 30 and 120 min (see statistical symbols in top panel Fig. 2).

Compared to the score observed before drug administration (t −60 min), NLX-112 alone (0.4 mg/kg) significantly [$F_{(12,72)} = 3.20$; $P < 0.01$] modified the dyskinesia score at 30 min with 3 out of 7 animals showing fleeting bouts of dyskinesia. Note: this is not represented graphically as the figure (inset, Fig. 2) shows median values,

Fig. 2. Time course of effects of NLX-112 in combination with L-DOPA (main panels) or alone (insets). Main panels: NLX-112 or vehicle and L-DOPA (7 mg/kg) were administered p.o. at t = 0 min (arrows). Benserazide was administered at t = −60 min. For dyskinesia and motor disability, data are presented as median values and were analyzed by two-way ANOVA on transformed data [y = sqrt(y)]. For locomotor activity, data are presented as mean values, and were analyzed by two-way ANOVA on raw data. *$p < 0.05$, **$p < 0.01$, versus L-DOPA alone at the considered time point, Dunnett's post-hoc tests. N = 7 per group. Insets: NLX-112 was administered alone p.o. at t = 0 min (arrows). For dyskinesia and motor disability, data are presented as median values and were analyzed by one-way ANOVA on transformed data [y = sqrt (y)]. For locomotor activity, data are presented as mean values, and were analyzed by one-way ANOVA on raw data. *$p < 0.05$, **$p < 0.01$ versus value at t = −60 min (dyskinesia and motor disability) or t = −90 min (locomotor activity), Dunnett's post-hoc tests.

whereas the statistical analysis was done on square-root transformed raw score.

3.2.2. Motor disability score

Administered alone, L-DOPA decreased motor disability score in the period from 30 to 180 min, with a slow return to pre L-DOPA levels from 180 min onward (open squares, Fig. 2, middle panel). NLX-112 did not significantly modify the beneficial effects of L-DOPA, i.e. did not interfere with the decrease in disability score (treatment factor: [F

$(3,18) = 2.70$, ns], treatment × time interaction [F (36,216) = 1.19; ns], but significant time effect [F (12,72) = 52.17; P < 0.0001]). On its own, NLX-112 significantly decreased the motor disability score [F (12, 72) = 2.92, P < 0.01, inset], most prominently at 30 min, but also at 180 and 210 min post-treatment.

3.2.3. Locomotor activity

Administration of L-DOPA stimulated locomotor activity, with peak effect recorded between 180 and 240 min (open squares, Fig. 2, bottom panel). NLX-112 significantly attenuated this hyperlocomotor activity, most prominently from 60 to 120 min post-administration. There was a significant treatment × time interaction effect [F (39,234) = 1.85; P < 0.01] and time effect [F (13,78) = 7.31; P < 0.0001, but not treatment effect [F (3, 18) = 0.64; ns]. By itself, NLX-112 very modestly increased locomotor activity [F (13, 78) = 4.57; P < 0.0001], with a significant increase observed only at 30 min post-treatment (Fig. 2, bottom panel, inset).

3.2.4. Induction of unusual behaviors by NLX-112

At 0.1 and 0.4 mg/kg, NLX-112 in combination with L-DOPA produced mainly dystonia and scratching, most prominently at the 0.4 mg/kg dose. Sedation-like, dystonia and scratching behaviours were also observed with NLX-112 alone at 0.4 mg/kg (Supplementary Table).

3.3. Effects of NLX-112 alone

3.3.1. Dyskinesia score

Vehicle-treated marmosets presented no observable dyskinesia during the observation period (open diamonds, Fig. 3, top panel). NLX-112 slightly increased dyskinesia scores, with peak effect (median value of 1) observed for each dose at some time points. Statistical analysis revealed that there was a significant time effect [F (15, 105) = 4.56; P < 0.0001], treatment effect [F (2, 14) = 5.33; P < 0.05] and treatment × time interaction [F (30, 210) = 2.19; P < 0.001]. The peak dyskinesia scores reflect occasional mild and fleeting dyskinetic postures and movements.

3.3.2. Motor disability score

Following administration of vehicle, marmosets presented a stable and high level of motor disability (median scores fluctuating between 9 and 12 over the whole observation period: Fig. 3, middle panel). NLX-112 significantly lowered the disability score (treatment effect: [F (2, 14) = 6.64; p < 0.01]; time effect: [F (15, 105) = 8.73; P < 0.0001] and treatment × time interaction [F (30, 210) = 1.88; P < 0.01]), with median scores being reduced to 4.5–5 at most time points from 20 to 160 min post-treatment.

3.3.3. Locomotor activity

Administration of vehicle had no effect on locomotor activity (Fig. 3, bottom panel). NLX-112 produced a small but significant augmentation of locomotor activity, most notably at the lower dose tested, at various time points, with peak effects around 200% above those of vehicle values [treatment effect: F (2, 14) = 8.08; P < 0.01, time effect: F (13, 91) = 3.80, P < 0.0001 and treatment × time interaction: F (26, 182) = 2.21, P < 0.01.

3.4. Effects of (+)8-OH-DPAT on L-DOPA-induced effects

3.4.1. Dyskinesia score

L-DOPA by itself produced a time-dependent increase in the dyskinesia score, with median peak values of 2 from 30 to 90 min post-treatment (open symbols, Fig. 4, top panel). In combination with L-DOPA, (+)8-OH-DPAT at both doses reduced dyskinesia (non significant treatment effect [F (2,12) = 0.15; ns], but significant time effect [F (12, 72) = 5.64; P < 0.0001] and treatment × time interaction [F (24,144) = 1.79; P < 0.05]. Subsequent post-hoc analysis
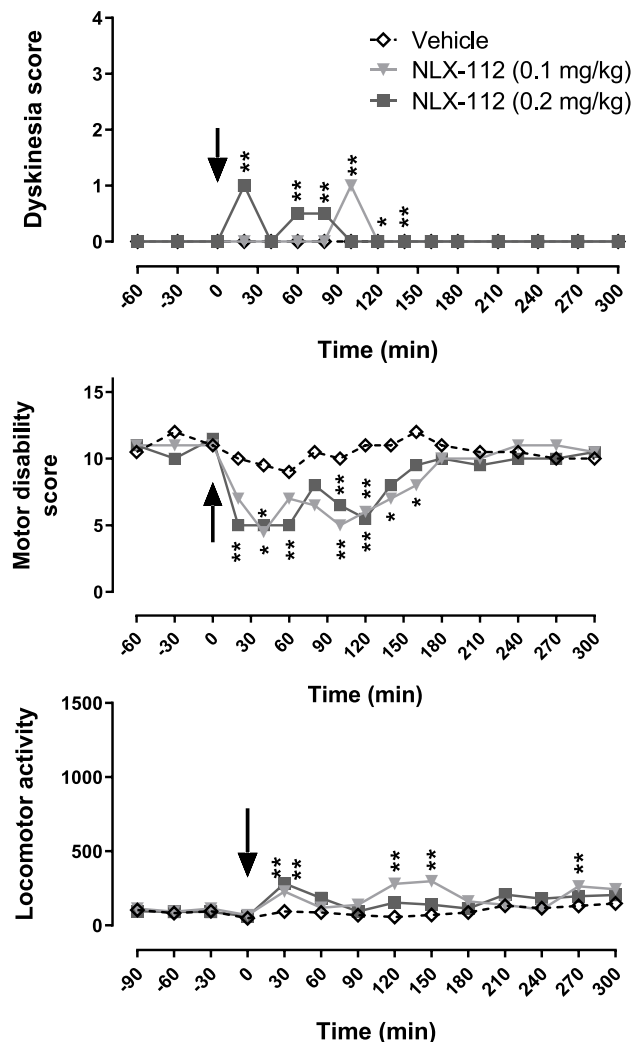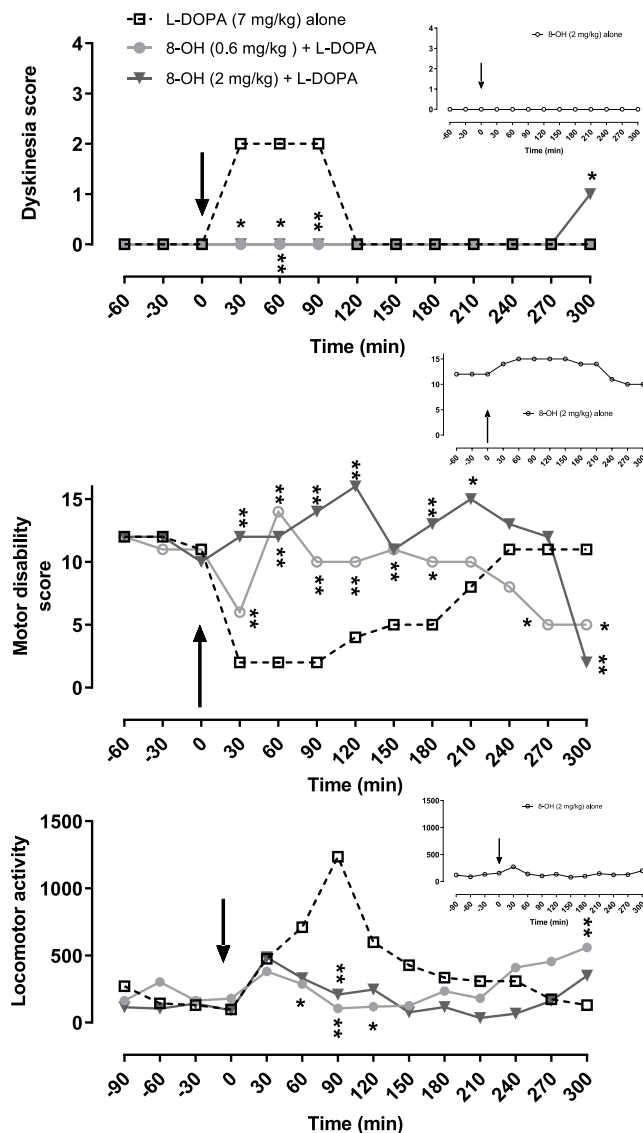

Fig. 3. Time course of the effects of NLX-112 alone. NLX-112 or vehicle were administered at t = 0 min; benserazide was administered at t = −60 min. For dyskinesia and motor disability, data are presented as median values and were analyzed by two-way ANOVA on transformed data [y = sqrt(y)]. For locomotor activity, data are presented as mean values, and were analyzed by two-way ANOVA on raw data. *p < 0.05, **p < 0.01, versus vehicle at the considered time point, Dunnett's post-hoc tests. N = 7 per group.

showed that (+)8-OH-DPAT significantly reduced dyskinesia scores at both doses from 30 to 90 min post-treatment. By itself, (+)8-OH-DPAT, at 2 mg/kg s.c., produced no dyskinesia (inset; treatment effect [F (12,72) = 1.47; ns]).

3.4.2. Motor disability score

Administered alone, L-DOPA decreased motor disability from 30 to 210 min, with a return to pre-treatment levels from 210 min onward (open squares, Fig. 4, middle panel). When co-administered with L-DOPA, (+)8-OH-DPAT attenuated the anti-disability effect of L-DOPA (treatment factor: [F (2, 12) = 10.17; p < 0.01], with significant time effect [F (12, 72) = 5.46; P < 0.0001] and treatment × time interaction [F (24, 144) = 5.73; P < 0.0001]. Post-hoc analysis detected increased motor disability scores at most time points from 30 to 210 min post-treatment, compared to L-DOPA alone. (+)8-OH-DPAT, administered alone, significantly increased the disability score (treatment factor: [F (12, 72) = 1.94; p < 0.05]; however, post-hoc analysis detected no time at which it was significantly different to pretreatment levels (inset).

Fig. 4. Time course of effects of (+)8-OH-DPAT alone or in combination with L-DOPA. Main panels: (+)8-OH-DPAT (8-OH) and its vehicle were administered i. p. and L-DOPA (7 mg/kg) was administered p. o. at t = 0 min (arrows). Benserazide was administered at t = −60 min. For dyskinesia and motor disability, data are presented as median values and were analyzed by two-way ANOVA on transformed data [y = sqrt(y)]. For locomotor activity, data are presented as mean values, and were analyzed by two-way ANOVA on raw data. *p < 0.05, **p < 0.01, versus L-DOPA alone at the considered time point, Dunnett's post-hoc tests. N = 7 per group. Insets: (+)8-OH-DPAT was administered alone i. p. at t = 0 min (arrows). For dyskinesia and motor disability, data are presented as median values and were analyzed by one-way ANOVA on transformed data [y = sqrt(y)]. For locomotor activity, data are presented as mean values and were analyzed by one-way ANOVA on raw data.

3.4.3. Locomotor activity

Administration of L-DOPA stimulated locomotor activity, with peak effect recorded at 90 min (open squares, Fig. 4, bottom panel). In combination with L-DOPA, (+)8-OH-DPAT attenuated this locomotor enhancement (non significant treatment effect [F (2, 12) = 1.37; ns], but significant time [F (13, 78) = 2.80, P < 0.01] and treatment × time interaction [F (26, 156) = 2.73, P < 0.0001]. Post-hoc analysis showed that (+)8-OH-DPAT decreased locomotion as compared to L-DOPA alone mostly at 0.06 mg/kg from 60 to 120 min post-treatment. (+)8-OH-DPAT had no effect on locomotor activity as compared to pre-treatment values (inset, Fig. 4 bottom panel; treatment effect [F (13, 78) = 0.86; ns]).

3.4.4. Induction of unusual behaviors by (+)8-OH-DPAT

At 0.6 and 2 mg/kg s.c., (+)8-OH-DPAT in combination with L-DOPA produced various behavioral effects, including dystonia and sedation-like behavior, most prominently at the 2 mg/kg dose. Severe dystonia and flat body posture were also observed when (+)8-OH-DPAT was administered alone at 2 mg/kg (Supplementary Table).

4. Discussion

NLX-112 (a.k.a. befiradol or F13640), is a centrally acting, highly efficacious and highly selective 5-HT$_{1A}$ receptor agonist being developed for the treatment of LID in PD. Although it has been extensively tested in multiple models in rodent species, including electrophysiology, microdialysis, behavior and brain imaging (Brys et al., 2018; Iderberg et al., 2015b; McCreary et al., 2016a; Newman-Tancredi et al., 2018a,b; Vidal et al., 2018), this is the first report of its effects in the MPTP-treated marmoset model of PD. The principal findings are as follows: (i) The duration of plasma exposure of NLX-112 in marmosets was relatively modest, which may limit its pharmacological effects in this species. (ii) NLX-112 exhibited moderate but potent dose-dependent anti-dyskinetic activity in L-DOPA-treated parkinsonian marmosets (effects notably at 0.4 mg/kg p.o.). (iii) NLX-112 only marginally interfered with the anti-parkinsonian effects of L-DOPA, unlike reference 5-HT$_{1A}$ receptor agonist, (+)8-OH-DPAT, which reduced LID but also abolished the anti-parkinsonian effects of L-DOPA. (iv) NLX-112 alone exhibited anti-parkinsonian-like activity in parkinsonian marmosets. (v) Both NLX-112 and (+)8-OH-DPAT produced serotonergic behavioral effects in marmosets, which were dose-limiting and interfered with some behavioral observations. Such motor effects were not previously observed in another NHP species, cynomolgus macaques (*Macaca fascicularis*) at doses equivalent to or even higher than those used here, or in previous clinical trials with NLX-112, suggesting that marmosets are particularly sensitive to serotonergic activation. These findings are discussed in more detail below.

4.1. Contrasting effects of NLX-112 and (+)8-OH-DPAT on L-DOPA-induced behaviors

NLX-112 elicited anti-dyskinetic activity in parkinsonian marmosets at specific time-points following drug administration. The lower doses (0.025 and 0.1 mg/kg p.o.) reduced dyskinesia scores at a couple of time points, but the higher dose (0.4 mg/kg p.o.) elicited significant effects at most time points between 30 and 120 min post-treatment (Fig. 2). The median dyskinesia scores for the 0.4 mg/kg p.o. dose were reduced from 2 or 3 down to 1 or zero at all time-points, indicating a reduction from troublesome to non-troublesome dyskinesia. One factor that may influence the extent and duration of the anti-LID activity of NLX-112 is the relatively short half-life of NLX-112 plasma exposure in marmoset (Fig. 1). Indeed, NLX-112 exposure in 3 out of 4 animals was strongly reduced within 2 h of administration, a profile which more closely resembles that of rats (around 1–2 h (Bardin et al., 2005)) than that of human subjects (> 20 h) (Paillard et al., 2016).

A marked inter-individual variability in LID scores was observed, suggesting that a larger cohort of marmosets might have increased statistical resolution, particularly at early time-points when NLX-112 exposure was highest. NLX-112 also elicited unusual behaviors which resembled serotonergic behavioral syndrome. This might have confounded scoring of LID, in addition to preventing higher (and potentially more active) doses from being tested (see additional discussion on this point below). Nevertheless, despite these limitations, the present data are consistent with significant oral anti-LID activity of NLX-112. They also extend to marmoset the observations previously reported in hemi-parkinsonian rat where NLX-112 abolished L-DOPA-induced AIMs (Iderberg et al., 2015b), and retained this activity upon 14-day repeated administration (McCreary et al., 2016a).

Notably, NLX-112 did not markedly interfere with the anti-disability

effects of L-DOPA. The latter strongly reduced parkinsonism scores of MPTP-treated marmosets (median values decreased from about 12 to 2–3) and NLX-112 only slightly opposed this effect at a single time-point at the highest dose (0.4 mg/kg p.o., Fig. 2). This sharply distinguishes NLX-112 from the prototypical 5-HT$_{1A}$ receptor agonist (+)8-OH-DPAT (Huot, 2018). The latter was tested herein at the same doses as previously reported (i.e. 0.6 and 2.0 mg/kg p.o.) (Iravani et al., 2006), and reduced dyskinesia elicited by L-DOPA (Fig. 4, top). However, whereas L-DOPA produced a strong decrease in mean motor disability scores, from 12 to about 2–5 (Fig. 4 middle), (+)8-OH-DPAT completely abolished this therapeutic-like effect (Fig. 4 middle). This observation is in accordance with a previous studies of the effects of (+)8-OH-DPAT (Iravani et al., 2006) and of other 5-HT$_{1A}$ receptor agonists which also found that reduction of dyskinesia was accompanied by loss of anti-disability effects (Bezard et al., 2013; Gregoire et al., 2009; Iravani et al., 2006). NLX-112 therefore exhibits a distinctive pattern of activity, attenuating LID in parkinsonian marmosets, but with minimal effects on L-DOPA-induced therapeutic-like properties on motor disability. In addition, in a recent study, NLX-112 also elicited robust anti-LID properties (without impacting L-DOPA anti-parkinsonian activity) in a second non-human primate species, MPTP-treated cynomolgus macaques, over a dose-range similar to the one used herein (0.03–0.3 mg/kg p.o.) (Johnston et al.; manuscript in preparation).

4.2. Anti-parkinsonian-like effects of NLX-112

In addition to attenuating LID, NLX-112, by itself, also reduced parkinsonism behavior. Vehicle-treated marmosets had motor disability scores of 10–12 and these were reduced to about 5–6 by low doses of NLX-112 (0.1 and 0.2 mg/kg p.o.; Fig. 3, middle panels). Interestingly, the higher dose of NLX-112 (0.4 mg/kg p.o.) appeared less active than the lower doses, only reducing disability at a single early time-point (compare Fig. 3 middle panel with inset of Fig. 2 middle panel), suggesting an inverted dose-response relationship. To our knowledge, this is the first time that such anti-parkinsonian effects have been observed in NHP for a 5-HT$_{1A}$ receptor agonist. An indication that NLX-112 may possess a motor facilitation activity was seen in hemi-parkinsonian rats, in which NLX-112 stimulated rotation behavior, a response associated with anti-parkinsonian effects (Iderberg et al., 2015b). The underlying mechanism for that activity was not established, but may involve direct activation of 5-HT$_{1A}$ receptor subpopulations in basal ganglia. Indeed, brain imaging studies using ^{18}F-labelled NLX-112 in rat, cat and NHP have identified binding sites in caudate/striatum which may be functionally active (Vidal et al., 2018). NLX-112 also elicits a specific glucose uptake (^{18}F-fluorodeoxyglucose PET labelling) pattern in rat cortical and subcortical areas (Levigoureux et al., 2019b) and ongoing human brain imaging will determine if these brain regions are labelled in human brain (Colom et al., 2020). In any case, the effects of NLX-112 contrast strongly with those of (+)8-OH-DPAT (Fig. 4) (Iravani et al., 2006). The latter showed no anti-parkinsonian activity by itself but, on the contrary, *increased* motor disability at a low dose of 0.6 mg/kg (Iravani et al., 2006) (see discussion below). This is consistent with the diversity in the effects of 5-HT$_{1A}$ receptor agonists that has been also observed in rat: (+)8-OH-DPAT and another agonist, tandospirone, both exhibit anti-AIMs effects in rats (Bishop et al., 2009; Iderberg et al., 2015a) but elicited contralateral rotation in 6-OH-DA-lesioned hemi-parkinsonian rats (Gerber et al., 1988; Iderberg et al., 2015a; Matsubara et al., 2006) whereas NLX-112 produces ipsilateral rotations (Iderberg et al., 2015b). Overall, these observations indicate that different 5-HT$_{1A}$ receptor agonists can elicit divergent effects on motor control in both rodent and NHP models of PD. In particular, the data suggest that NLX-112 may have therapeutic activity against the primary motor symptoms of PD as well as reducing LID. Nevertheless, caution is warranted as to the translatability of anti-parkinsonian effects from marmoset to human, and further investigation is necessary to understand the exact mechanisms underlying such differences – see discussion below.

4.3. Mechanisms underlying the distinctive profile of NLX-112 in MPTP-treated marmosets

The neurological mechanisms underlying the properties of NLX-112 remain to be fully elucidated. At a molecular level, NLX-112 preferentially activates Gαo proteins and ERK1/2 phosphorylation via 5-HT$_{1A}$ receptors, a characteristic that differentiates it from other 5-HT$_{1A}$ receptor agonists, including (+)8-OH-DPAT (Buritova et al., 2009; Newman-Tancredi et al., 2017). It may, therefore, be speculated that NLX-112 more specifically activates 5-HT$_{1A}$ receptor signal transduction pathways associated with brain regions controlling motor responses. At a neurochemical level, anti-dyskinetic activity is most likely related to inhibition of serotonergic projections from the raphe nuclei to the basal ganglia, which would blunt 'false neurotransmitter' DA release. Strong support for this mechanism has been generated in rodent models (see Introduction), and imaging data in human also supports this interpretation (Politis et al., 2014; Roussakis et al., 2016). NLX-112 has some 'biased agonism' for activation of 5-HT$_{1A}$ autoreceptors (Buritova et al., 2009) but other mechanisms may be involved, such as a direct effect of NLX-112 in striatal regions. This is suggested by the fact that local administration of 5-HT$_{1A}$ receptor agonists (including ($\pm$)8-OH-DPAT and F13714, a close chemical analogue of NLX-112) in rat striatum elicited efficacious anti-dyskinetic activity in rats, pointing to the presence of active populations of 5-HT$_{1A}$ receptors in that brain region (Bishop et al., 2009; Meadows et al., 2017). Moreover, PET imaging of 5-HT$_{1A}$ receptors in marmoset brain using ^{18}F–F13714 showed labelling in subcortical areas and raphe nuclei (Yokoyama et al., 2016), as expected, but also in caudate nucleus and putamen (Yokoyama et al., 2016). Subsequent PET imaging using ^{18}F-NLX-112 in rat, cat and Rhesus macaques (*Macaca mulatta*) found pronounced labeling in thalamus and dorsal raphe (Vidal et al., 2018). An ongoing clinical PET imaging investigation with ^{18}F-NLX-112 is expected to shed light on whether such patterns of labelling are also observed in healthy human subjects (Colom et al., 2020). Ultimately, it will be interesting to determine whether NLX-112 differentially activates 5-HT$_{1A}$ receptors in patients with Parkinson's disease compared with healthy controls. Initial preclinical investigation of this issue using hemi-parkinsonian (6-OH-DA-lesioned) rats suggests that induction of dyskinesia is associated with decreased 5-HT$_{1A}$ receptor expression in sub-cortical regions and increased sensitivity to agonist stimulation (Levigoureux et al., 2019a).

4.4. Serotonergic effects of NLX-112 and (+)8-OH-DPAT

NLX-112 (0.1–0.4, but not 0.025 mg/kg p.o.), administered acutely, produced dose-dependent unusual behaviors in the marmosets, including sedation, scratching, wet dog shakes and dystonia in the tail. Similar effects were observed upon administration of the reference 5-HT$_{1A}$ receptor agonist, (+)8-OH-DPAT. Although a formal scoring system for such behaviors in marmoset has not been described, these signs are reminiscent of the 'serotonergic behavioral syndrome' observed in rat, including forepaw treading, flat body posture, Straub tail and slowed behavior (Aubert et al., 2012; Iderberg et al., 2015b; Smith and Peroutka, 1986). This suggests that the behaviors result from common neurobiological mechanisms i.e. activation of 5-HT$_{1A}$ receptors, thus providing evidence that the compounds do engage the intended receptor target. However, these behaviors became dose-limiting: higher doses of NLX-112 could, potentially, have been more efficacious in reducing LID. It should be noted that, in rats, repeated or continuous treatment with NLX-112, or other 5-HT$_{1A}$ receptor agonists elicits tachyphylaxis to this serotonergic behavioral syndrome (Iderberg et al., 2015b; Prinssen et al., 2000). This is in contrast to the anti-AIMs effects of NLX-112, where tachyphylaxis is not seen and effects are maintained over chronic administration (McCreary et al., 2016b). Serotonergic behavioral syndrome in rat has been suggested to imply

potential side effects at a clinical level (Lindenbach et al., 2015). However, in the study of NLX-112 in MPTP-treated cynomolgus macaques mentioned above, NLX-112 did not elicit movements or behaviours that could be classified as "serotonergic behavioral syndrome" up to 0.3 mg/kg p.o. (Johnston et al.; manuscript in preparation). It is also interesting that when F15599, a close chemical analogue of NLX-112, was tested in MPTP-treated parkinsonian cynomolgus macaques, no "serotonergic behavioral syndrome" effects were observed in that species (Huot et al., 2015). Moreover, NLX-112 previously underwent regulatory 3-month toxicology studies in cynomolgus macaques and did not induce "serotonergic behavioral syndrome" (with a dose titration from 0.025 to 2 mg/kg bid p.o. over 14 days: Neurolixis data on file). Finally, when NLX-112 was tested previously in clinical trials (administered to over 500 subjects for non-PD indications in clinical trials lasting up to 2 months), side-effects consisted principally of nausea and dizziness, but motor side-effects were not observed (unpublished observations and (Paillard et al., 2016)). Taken together, these observations suggest that marmosets may be a species that is particularly sensitive to "serotonergic behavioral syndrome" and that the latter, unlike the anti-dyskinetic effects of 5-HT$_{1A}$ receptor activation, does not translate to other NHP species or to human. It should be noted that 5-HT$_{1A}$ receptor activation is also associated with other, potentially beneficial, properties. Indeed, NLX-112 exhibits potent antidepressant-like activity (Iderberg et al., 2015b) in rodent behavioral tests and increases extracellular DA concentrations in the medial prefrontal cortex (Llado-Pelfort et al., 2012), consistent with activity against mood deficits. Moreover, NLX-112 is potently active in a range of rat pain models (Colpaert, 2006), suggesting possible utility against the chronic pain symptoms experienced by many PD patients (Rana et al., 2013, 2016). However, the extent to which such beneficial serotonergic properties are expressed in a clinical setting remains to be determined.

5. Conclusions

The present observations indicate that NLX-112 has anti-dyskinetic activity in MPTP-treated marmosets at the doses tested. This is consistent with prior observations in hemi-parkinsonian rats and, importantly, NLX-112 exerted only very slight interference with the antiparkinsonian effects of L-DOPA, unlike reference 5-HT$_{1A}$ receptor agonist, (+)8-OH-DPAT, which completely suppressed the effects of L-DOPA. The present data therefore further highlight the distinctive profile of NLX-112 in comparison with other serotonergic compounds. Nevertheless, the magnitude of the anti-LID effects in marmoset was likely limited by the rather short plasma half-life of the compound in this species and by the dose-limiting induction of the "serotonergic behavioral syndrome" – such effects were not seen with NLX-112 in other non-human primate species (Cynomolgus or Rhesus macaques) or in human. In addition to anti-LID activity, NLX-112 also exhibited anti-motor disability activity on its own in the MPTP-treated marmosets. To our knowledge, this is the first observation of such activity for a 5-HT$_{1A}$ receptor agonist in an NHP species. Such activity is intriguing and suggests that NLX-112 could attenuate parkinsonian symptoms in some patients and/or have some L-DOPA dose-sparing activity. Nevertheless, such interpretations require further investigation, preferably in a clinical setting. In conclusion, in view of the accumulated molecular, neurochemical and behavioral data pointing to efficacious and potent activity of NLX-112, it would be desirable to conduct appropriately-designed clinical trials to determine its utility as a pharmacotherapeutic for patients suffering from Parkinson's disease or, potentially, other movement disorders.

Declaration of competing interest

AN-T, MAV and RD are employees and/or stockholders of Neurolixis Inc. The other authors have no disclosures.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2020.107997.

References

Aubert, Y., Gustison, M.L., Gardner, L.A., Bohl, M.A., Lange, J.R., Allers, K.A., Sommer, B., Datson, N.A., Abbott, D.H., 2012. Flibanserin and 8-OH-DPAT implicate serotonin in association between female marmoset monkey sexual behavior and changes in pair-bond quality. J. Sex. Med. 9 (3), 694–707. https://doi.org/10.1111/j.1743-6109.2011.02616. PMID: 22304661.

Bardin, L., Assie, M.B., Pelissou, M., Royer-Urios, I., Newman-Tancredi, A., Ribet, J.P., Sautel, F., Koek, W., Colpaert, F.C., 2005. Dual, hyperalgesic, and analgesic effects of the high-efficacy 5-hydroxytryptamine 1A (5-HT1A) agonist F 13640 [(3-chloro-4-fluoro-phenyl)-[4-fluoro-4-{[(5-methyl-pyridin-2-ylmethyl)-am ino]-methyl}piperidin-1-yl]methanone, fumaric acid salt]: relationship with 5-HT1A receptor occupancy and kinetic parameters. J. Pharmacol. Exp. Therapeut. 312 (3), 1034–1042. https://doi.org/10.1124/jpet.104.077669. PMID: 15528450.

Bezard, E., Tronci, E., Pioli, E.Y., Li, Q., Porras, G., Bjorklund, A., Carta, M., 2013. Study of the antidyskinetic effect of eltoprazine in animal models of levodopa-induced dyskinesia. Movement disorders. Mov Disord 28 (8), 1088–1096. https://doi.org/10.1002/mds.25366. PMID: 23389842.

Bishop, C., Krolewski, D.M., Eskow, K.L., Barnum, C.J., Dupre, K.B., Deak, T., Walker, P.D., 2009. Contribution of the striatum to the effects of 5-HT1A receptor stimulation in L-DOPA-treated hemiparkinsonian rats. J. Neurosci. Res. 87 (7), 1645–1658. https://doi.org/10.1002/jnr.21978. PMID: 19115412.

Brys, I., Halje, P., Scheffer-Teixeira, R., Varney, M., Newman-Tancredi, A., Petersson, P., 2018. Neurophysiological effects in cortico-basal ganglia-thalamic circuits of anti-dyskinetic treatment with 5-HT$_{1A}$ receptor biased agonists. Exp. Neurol. 302, 155–168. https://doi.org/10.1016/j.expneurol.2018.01.010. PMID: 29339052.

Buritova, J., Berrichon, G., Cathala, C., Colpaert, F., Cussac, D., 2009. 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), 350–361. https://doi.org/10.1016/j.neuropharm.2008.09.004. PMID: 18809418.

Caccia, S., Muglia, M., Mancinelli, A., Garattini, S., 1983. Disposition and metabolism of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in the rat. Xenobiotica 13 (3), 147–153. https://doi.org/10.3109/00498258309052248. PMID: 6613160.

Carlsson, T., Carta, M., Munoz, A., Mattsson, B., Winkler, C., Kirik, D., Bjorklund, A., 2009. Impact of grafted serotonin and dopamine neurons on development of L-DOPA-induced dyskinesia in parkinsonian rats is determined by the extent of dopamine neuron degeneration. Brain: J. Neurol. 132 (Pt 2), 319–335. https://doi.org/10.1093/brain/awn305. PMID: 19039008.

Carlsson, T., Carta, M., Winkler, C., Bjorklund, A., Kirik, D., 2007. Serotonin neuron transplants exacerbate L-DOPA-induced dyskinesias in a rat model of Parkinson's disease. J. Neurosci. : the official journal of the Society for Neuroscience 27 (30), 8011–8022. https://doi.org/10.1523/JNEUROSCI.2079-07.2007. PMID: 17652591.

Carta, M., Carlsson, T., Kirik, D., Bjorklund, A., 2007. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain: J. Neurol. 130 (pt 7), 1819–1833. https://doi.org/10.1093/brain/awm082. PMID: 17452372.

Carta, M., Carlsson, T., Munoz, A., Kirik, D., Bjorklund, A., 2008. Serotonin-dopamine interaction in the induction and maintenance of L-DOPA-induced dyskinesias. Prog. Brain Res. 172, 465–478. https://doi.org/10.1016/S0079-6123(08)00922-9. PMID: 18772046.

Cenci, M.A., Riggare, S., Pahwa, R., Eidelberg, D., Hauser, R.A., 2019. Dyskinesia matters. Mov. Disord. https://doi.org/10.1002/mds.27959. PMID: 31872501.

Colom, M., Costes, N., Redoute, J., Dailler, F., Gobert, F., Le Bars, D., Billard, T., Newman-Tancredi, A., Zimmer, L., 2020. ^{18}F-F13640 PET imaging of functional receptors in humans. Eur. J. Nucl. Med. Mol. Imag. 47 (1), 220–221. https://doi.org/10.1007/s00259-019-04473-7. PMID: 31414208.

Colpaert, F.C., 2006. 5-HT$_{1A}$ receptor activation: new molecular and neuroadaptive mechanisms of pain relief. Curr. Opin. Invest. Drugs 7, 40–47 PMID: 16425670.

Colpaert, F.C., Tarayre, J.P., Koek, W., Pauwels, P.J., Bardin, L., Xu, X.J., Wiesenfeld-Hallin, Z., Cosi, C., Carilla-Durand, E., Assie, M.B., Vacher, B., 2002. Large-amplitude 5-HT1A receptor activation: a new mechanism of profound, central analgesia. Neuropharmacology 43 (6), 945–958. https://doi.org/10.1016/s0028-3908(02)00119-3. PMID: 12423664.

Eskow, K.L., Dupre, K.B., Barnum, C.J., Dickinson, S.O., Park, J.Y., Bishop, C., 2009. The role of the dorsal raphe nucleus in the development, expression, and treatment of L-dopa-induced dyskinesia in hemiparkinsonian rats. Synapse 63, 610–620. https://doi.org/10.1002/syn.20630. PMC2741636.

FDA, 2005. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. In: (CDER), C.f.D.E.a.R. (Ed.), U.S. Department of Health and Human Services, Food and Drug Administration (FDA).

Gerber, R., Altar, C.A., Liebman, J.M., 1988. Rotational behavior induced by 8-hydroxy-DPAT, a putative 5HT-1A agonist, in 6-hydroxydopamine-lesioned rats.

Psychopharmacology (Berlin) 94, 178–182. https://doi.org/10.1007/bf00176841. PMID: 2965396.

Gregoire, L., Samadi, P., Graham, J., Bedard, P.J., Bartoszyk, G.D., Di Paolo, T., 2009. Low doses of sarizotan reduce dyskinesias and maintain antiparkinsonian efficacy of L-Dopa in parkinsonian monkeys. Park. Relat. Disord. 15, 445–452. https://doi.org/10.1016/j.parkreldis.2008.11.001. PMID: 19196540.

Huot, P., 2018. 5-HT1A agonists and dyskinesia in Parkinson's disease: a pharmacological perspective. Neurodegener. Dis. Manag. 8, 207–209. https://doi.org/10.2217/nmt-2018-0017. PMID: 30040029.

Huot, P., Johnston, T.H., Fox, S.H., Newman-Tancredi, A., Brotchie, J.M., 2015. The highly-selective 5-HT(1A) agonist F15599 reduces L-DOPA-induced dyskinesia without compromising anti-parkinsonian benefits in the MPTP-lesioned macaque. Neuropharmacology 97, 306–311. https://doi.org/10.1016/j.neuropharm.2015.05.033. MID: 26071982.

Iderberg, H., McCreary, A.C., Varney, M.A., Cenci, M.A., Newman-Tancredi, A., 2015a. Activity of serotonin 5-HT receptor 'biased agonists' in rat models of Parkinson's disease and l-DOPA-induced dyskinesia. Neuropharmacology 93C, 52–67. https://doi.org/10.1016/j.neuropharm.2015.01.012. PMID: 25645393.

Iderberg, H., McCreary, A.C., Varney, M.A., Kleven, M.S., Koek, W., Bardin, L., Depoortère, R., Cenci, M.A., Newman-Tancredi, A., 2015b. NLX-112, a novel 5-HT1A receptor agonist for the treatment of L-DOPA-induced dyskinesia: behavioral and neurochemical profile in rat. Exp. Neurol. 271, 335–350. https://doi.org/10.1016/j.expneurol.2015.05.021. PMID: 26037043.

Iravani, M.M., Tayarani-Binazir, K., Chu, W.B., Jackson, M.J., Jenner, P., 2006. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates, the selective 5-hydroxytryptamine 1a agonist (R)-(+)-8-OHDPAT inhibits levodopa-induced dyskinesia but only with increased motor disability. J. Pharmacol. Exp. Therapeut. 319, 1225–1234. https://doi.org/10.1124/jpet.106.110429. PMID: 16959959.

Jackson, M.J., Al-Barghouthy, G., Pearce, R.K., Smith, L., Hagan, J.J., Jenner, P., 2004. Effect of 5-HT1B/D receptor agonist and antagonist administration on motor function in haloperidol and MPTP-treated common marmosets. Pharmacol. Biochem. Behav. 79, 391–400. https://doi.org/10.1016/j.pbb.2004.07.015. PMID: 15582011.

Jenner, P., McCreary, A.C., Scheller, D.K., 2011. Continuous drug delivery in early- and late-stage Parkinson's disease as a strategy for avoiding dyskinesia induction and expression. J. Neural. Transm. 118, 1691–1702. https://doi.org/10.1007/s00702-011-0703-9. PMID: 21881838.

Levigoureux, E., Vidal, B., Bouillot, C., Billard, T., Newman-Tancredi, A., Zimmer, L., 2019a. PET imaging of functional 5-HT1A receptors in hemiparkinsonian rats. NeuroFrance 2019 Marseille, France, Poster P2.094.

Levigoureux, E., Vidal, B., Fieux, S., Bouillot, C., Emery, S., Newman-Tancredi, A., Zimmer, L., 2019b. Serotonin 5-HT1A receptor biased agonists induce different cerebral metabolic responses: a [18F]FDG PET study in conscious and anesthetized rats. ACS Chem. Neurosci. 10, 3108–3119. https://doi.org/10.1021/acschemneuro.8b00584. PMID: 30576601.

Lindenbach, D., Palumbo, N., Ostock, C.Y., Vilceus, N., Conti, M.M., Bishop, C., 2015. Side effect profile of 5-HT treatments for Parkinson's disease and L-DOPA-induced dyskinesia in rats. Br. J. Pharmacol. 172, 119–130. https://doi.org/10.1111/bph.12894. PMID: 25175895.

Llado-Pelfort, L., Assie, M.B., Newman-Tancredi, A., Artigas, F., Celada, P., 2012. In vivo electrophysiological and neurochemical effects of the selective 5-HT1A receptor agonist, F13640, at pre- and postsynaptic 5-HT1A receptors in the rat. Psychopharmacology 221, 261–272. https://doi.org/10.1007/s00213-011-2569-9. PMID: 22147258.

Matsubara, K., Shimizu, K., Suno, M., Ogawa, K., Awaya, T., Yamada, T., Noda, T., Satomi, M., Ohtaki, K., Chiba, K., Tasaki, Y., Shiono, H., 2006. Tandospirone, a 5-HT1A agonist, ameliorates movement disorder via non-dopaminergic systems in rats with unilateral 6-hydroxydopamine-generated lesions. Brain Res. 1112, 126–133. https://doi.org/10.1016/j.brainres.2006.07.003. PMID: 16884702.

McCreary, A.C., Varney, M.A., Newman-Tancredi, A., 2016a. The novel 5-HT1A receptor agonist, NLX-112 reduces l-DOPA-induced abnormal involuntary movements in rat: a chronic administration study with microdialysis measurements. Neuropharmacology 105, 651–660. https://doi.org/10.1016/j.expneurol.2015.05.021. PMID: 26037043.

McCreary, A.C., Varney, M.A., Newman-Tancredi, A., 2016b. The novel 5-HT receptor agonist, NLX-112 reduces L-DOPA-induced abnormal involuntary movements in rat: a chronic administration study with microdialysis measurements. Neuropharmacology 105, 651–660. https://doi.org/10.1016/j.neuropharm.2016.01.013. PMID: 26777281.

Meadows, S.M., Chambers, N.E., Conti, M.M., Bossert, S.C., Tasber, C., Sheena, E., Varney, M., Newman-Tancredi, A., Bishop, C., 2017. Characterizing the differential roles of striatal 5-HT1A auto- and hetero-receptors in the reduction of l-DOPA-induced dyskinesia. Exp. Neurol. 292, 168–178. https://doi.org/10.1016/j.expneurol.2017.03.013. PMID: 28342749.

Munoz, A., Li, Q., Gardoni, F., Marcello, E., Qin, C., Carlsson, T., Kirik, D., Di Luca, M., Bjorklund, A., Bezard, E., Carta, M., 2008. Combined 5-HT1A and 5-HT1B receptor agonists for the treatment of L-DOPA-induced dyskinesia. Brain : J. Neurol. 131, 3380–3394. https://doi.org/10.1093/brain/awn235. PMID: 18952677.

Nair, A.B., Jacob, S., 2016. A simple practice guide for dose conversion between animals and human. J. Basic Clin. Pharm. 7, 27–31. https://doi.org/10.4103/0976-0105.177703. PMID: 27057123.

Newman-Tancredi, A., Bardin, L., Auclair, A., Colpaert, F., Depoortere, R., Varney, M.A., 2018a. NLX-112, a highly selective 5-HT1A receptor agonist, mediates analgesia and antidepressant-like activity in rats via spinal cord and prefrontal cortex 5-HT1A receptors, respectively. Brain Res. 1688, 1–7. https://doi.org/10.1016/j.brainres.2018.

03.016. PMID: 29555239.

Newman-Tancredi, A., Depoortere, R., Carilla-Durand, E., Tarayre, J.P., Kleven, M., Koek, W., Bardin, L., Varney, M.A., 2018b. NLX-112, a highly selective 5-HT1A receptor agonist: effects on body temperature and plasma corticosterone levels in rats. Pharmacol. Biochem. Behav. 165, 56–62. https://doi.org/10.1016/j.pbb.2017.11.002. PMID: 29126856.

Newman-Tancredi, A., Martel, J.C., Cosi, C., Heusler, P., Lestienne, F., Varney, M.A., Cussac, D., 2017. Distinctive in vitro signal transduction profile of NLX-112, a potent and efficacious serotonin 5-HT1A receptor agonist. J. Pharm. Pharmacol. 69, 1178–1190. https://doi.org/10.1111/jphp.12762. PMID: 28612503.

Paillard, B.D.F.L., Brunner, V., Newman-Tancredi, A., Varney, M., 2016. A method for treating movement disorders with befiradol. In: Pierre Fabre Medicament, WO2016/005527A1.

Pearce, P.K., et al., 1995. Chronic L-DOPA administration induces dyskinesias in the 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (Callithrix Jacchus). Mov Disord 10 (6), 731–740 PMID: 8749992.

Peroutka, S.J., 1985. Selective interaction of novel anxiolytics with 5-hydroxytryptamine1A receptors. Biol. Psychiatr. 20, 971–979. https://doi.org/10.1016/0006-3223(85)90194-5. PMID: 2862927.

Pilleri, M., Antonini, A., 2015. Therapeutic strategies to prevent and manage dyskinesias in Parkinson's disease. Expert Opin. Drug Saf. 14, 281–294. https://doi.org/10.1517/14740338. PMID: 25483147.

Politis, M., Wu, K., Loane, C., Brooks, D.J., Kiferle, L., Turkheimer, F.E., Bain, P., Molloy, S., Piccini, P., 2014. Serotonergic mechanisms responsible for levodopa-induced dyskinesias in Parkinson's disease patients. J. Clin. Invest. 124, 1340–1349. https://doi.org/10.1172/JCI71640. PMID: 24531549.

Prinssen, E.P., Koek, W., Colpaert, F.C., Kleven, M.S., 2000. Repeated treatment with 8-OH-DPAT induces tolerance to its ability to produce the 5-HT1A behavioural syndrome, but not to its ability to attenuate haloperidol-induced catalepsy. Behav. Pharmacol. 11, 299–305. https://doi.org/10.1097/00008877-200006000-00013. PMID: 11103884.

Rabiner, E.A., Gunn, R.N., Wilkins, M.R., Sedman, E., Grasby, P.M., 2002. Evaluation of EMD 128 130 occupancy of the 5-HT1A and the D2 receptor: a human PET study with [11C]WAY-100635 and [11C]raclopride. J. Psychopharmacol. 16, 195–199. https://doi.org/10.1177/026988110201600301. PMID: 12236624.

Rana, A.Q., Kabir, A., Jesudasan, M., Siddiqui, I., Khondker, S., 2013. Pain in Parkinson's disease: analysis and literature review. Clin. Neurol. Neurosurg. 115, 2313–2317. https://doi.org/10.1016/j.clineuro.2013.08.022. PMID: 24075714.

Rana, A.Q., Qureshi, A.R., Rahman, L., Jesudasan, A., Hafez, K.K., Rana, M.A., 2016. Association of restless legs syndrome, pain, and mood disorders in Parkinson's disease. Int. J. Neurosci. 126 (2), 116–120. https://doi.org/10.3109/00207454.2014.994208. PMID: 25469455.

Roussakis, A.A., Politis, M., Towey, D., Piccini, P., 2016. Serotonin-to-dopamine transporter ratios in Parkinson disease: relevance for dyskinesias. Neurology 86, 1152–1158. https://doi.org/10.1212/WNL.0000000000002494. PMID: 26920358.

Schapira, A.H., Bezard, E., Brotchie, J., Calon, F., Collingridge, G.L., Ferger, B., Hengerer, B., Hirsch, E., Jenner, P., Le Novere, N., Obeso, J.A., Schwarzschild, M.A., Spampinato, U., Davidai, G., 2006. Novel pharmacological targets for the treatment of Parkinson's disease. Nat. Rev. Drug Discov. 5, 845–854. https://doi.org/10.1038/nrd2087. PMID: 17016425.

Sellnow, R.C., Newman, J.H., Chambers, N., West, A.R., Steece-Collier, K., Sandoval, I.M., Benskey, M.J., Bishop, C., Manfredsson, F.P., 2019. Regulation of dopamine neurotransmission from serotonergic neurons by ectopic expression of the dopamine D2 autoreceptor blocks levodopa-induced dyskinesia. Acta Neuropathol Commun 7, 8. https://doi.org/10.1186/s40478-018-0653-7. PMID: 30646956.

Smith, L.M., Peroutka, S.J., 1986. Differential effects of 5-hydroxytryptamine1a selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24, 1513–1519. https://doi.org/10.1016/0091-3057(86)90477-6. PMID: 2942947.

Tronci, E., Lisci, C., Stancampiano, R., Fidalgo, C., Collu, M., Devoto, P., Carta, M., 2013. 5-Hydroxy-tryptophan for the treatment of L-DOPA-induced dyskinesia in the rat Parkinson's disease model. Neurobiol. Dis. 60, 108–114. https://doi.org/10.1016/j.nbd.2013.08.01. PMID: 24004632.

Veyres, N., Hamadjida, A., Huot, P., 2018. Predictive value of parkinsonian primates in pharmacologic studies: a comparison between the macaque, marmoset, and squirrel monkey. J. Pharmacol. Exp. Therapeut. 365, 379–397. https://doi.org/10.1124/jpet.117.247171. PMID: 29523699.

Vidal, B., Fieux, S., Colom, M., Billard, T., Bouillot, C., Barret, O., Constantinescu, C., Tamagnan, G., Newman-Tancredi, A., Zimmer, L., 2018. (18)F-F13640 preclinical evaluation in rodent, cat and primate as a 5-HT1A receptor agonist for PET neuroimaging. Brain Struct. Funct. 223, 2973–2988. https://doi.org/10.1007/s00429-018-1672-7. PMID: 29730825.

Vidal, B., Sebti, J., Verdurand, M., Fieux, S., Billard, T., Streichenberger, N., Troakes, C., Newman-Tancredi, A., Zimmer, L., 2016. Agonist and antagonist bind differently to 5-HT1A receptors during Alzheimer's disease: a post-mortem study with PET radiopharmaceuticals. Neuropharmacology 109, 88–95. https://doi.org/10.1016/j.neuropharm.2016.05.009. PMID: 27183968.

Yokoyama, C., Mawatari, A., Kawasaki, A., Takeda, C., Onoe, K., Doi, H., Newman-Tancredi, A., Zimmer, L., Onoe, H., 2016. Marmoset serotonin 5-HT1A receptor mapping with a biased agonist PET probe 18F-F13714: comparison with an antagonist tracer 18F-MPPF in Awake and anesthetized states. Int. J. Neuropsychopharmacol. 19 (12), 1–12. https://doi.org/10.1093/ijnp/pyw079. PMID: 27608810.