RESEARCH ARTICLE

NYX-458 Improves Cognitive Performance in a Primate Parkinson's Disease Model

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ABSTRACT: BACKGROUND: NYX-458 is a N-methyl-D-aspartate receptor (NMDAR) modulator that enhances synaptic plasticity. Dopaminergic cell loss in Parkinson's disease (PD) leads to NMDAR dysregulation in the cortico-striato-pallidal-thalmo-cortical network and altered plasticity in brain regions important to cognitive function. We hypothesize that targeting the NMDAR may be an efficacious approach to treating cognitive impairment in PD.
OBJECTIVES: NYX-458 was evaluated in 2 nonhuman primate models of PD. The first, a chronic low-dose 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)–administration model, was used to assess the effects of NYX-458 on cognitive domains impacted early in PD including attention, working memory, executive function, and visuospatial learning. The second, a high-dose MPTP-administration model, was used to assess potential for NYX-458 induced change in motor symptoms.
METHODS: NYX-458 was evaluated in the chronic low-dose MPTP model using the variable delayed response measure to assess attention and working memory and simple discrimination reversal to assess executive function. NYX-458 was also assessed in the high-dose MPTP model as a monotherapy and in combination with low-dose or high-dose levodopa to assess potential impact on motor symptoms.
RESULTS: NYX-458 administration resulted in rapid and long-lasting improvement in cognitive function across the domains of attention, working memory, and executive function. Dose levels effective in improving cognitive performance had no effect on PD motor symptoms, the anti-parkinsonian benefit of levodopa, or dyskinesia.
CONCLUSIONS: NYX-458 provides benefit in specific domains known to be impaired in PD in a dopamine depletion model of PD-like cognitive impairment. These data support the continued evaluation of NYX-458 as a potential therapeutic for cognitive decline in PD. © 2020 International Parkinson and Movement Disorder Society

Although Parkinson's disease (PD) is the most common movement disorder,[1] nonmotor symptoms, particularly neuropsychological and cognitive symptoms, are increasingly recognized as central to PD. PD cognitive impairment (PD-CI) often involves deficits in attention, executive function, and visuospatial and working memory.[2] Approximately 15% to 25% of newly diagnosed PD patients meet the criteria for mild CI (PD-MCI),[3] which often progresses to the development of PD dementia.[2,4-6] Dopamine is a key regulator of N-methyl-D-aspartate receptor (NMDAR) function, and a loss of dopaminergic input decouples the modulatory role of dopamine in NMDAR activation, which may lead to NMDAR dysregulation and dysfunction in both long-term potentiation and depression.[7,8] This could contribute to cognitive deficits in PD patients,[9] and compounds that normalize NMDAR function may be effective therapies for the treatment of PD-CI,

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potentially by reducing the threshold of dopaminergic activity needed for NMDAR function, particularly early in the disease course.

NYX-458 is a small-molecule NMDAR modulator derived from a novel chemistry platform.[10] Compounds from this platform uniquely modulate NMDAR activity to facilitate long-term potentiation and induce long-lasting changes in metaplasticity and dendritic spine morphology in cortical regions. These compounds also enhance cognitive performance in rodent models of learning and memory.[10] Accordingly, drugs such as NYX-458 may be beneficial in improving cognitive function in PD patients.

In the present study, chronic low-dose 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered to cynomolgus macaques as a model of PD-MCI. The dopaminergic depletion in the macaques results in a clear, stable, and long-term deficit in cognition,[11-16] with a minimal impact on motor function,[17] enabling the assessment of cognitive function without interference of motor complications. The exact mechanism driving the dichotomy between cognitive and motor performance in these animals is unclear[17]; however, the cognitive domains affected in this dopamine-depletion model are the same domains first affected in PD-MCI patients,[11-16] making this an ideal model for the assessment of therapeutics in early PD-CI. We also tested NYX-458 as a monotherapy and in combination with levodopa (L-dopa) in an acute high-dose MPTP motor-impairment model to assess the impact on PD motor symptoms.[18-20]

Methods

NMDAR Subtype-Expressing Human Embryonic Kidney (HEK) Cell Membrane Preparation and Assay

NYX-458 was synthesized by Sai Life Sciences (Hyderabad, India). N-methyl-D-aspartate receptor subunit 2A (NMDAR2A) and 2B were coexpressed with postsynaptic density protein 95 (PSD95) in HEK cells and crude membrane extracts were prepared and assessed for NMDAR2 subtype glutamate coagonist activity using an tritiated MK-801 ([^{3}H]MK-801) potentiation assay, as previously described.[10]

Nonhuman Primate Studies

Procedures on cynomolgus macaques (*Macaca fascicularis*; Suzhou Xishan Zhongke Laboratory Animal Company, Xishan Island, Jiangsu province, People's Republic of China) were conducted by Atuka, Inc. (Toronto, Ontario, Canada) under approved Institutional Animal Care and Use Committee (IACUC) protocols (IP20170226PD04 for pharmacokinetics (PK), IP20161121PD29 for cognition, and IP20171128PD31 for motor studies). Animals were housed individually and were subject to a controlled light–dark cycle (12 hours on from 7 AM) and temperature (20–28°C).

Pharmacokinetic Study of NYX-458

Macaques (N = 3 males, 6–7 years old, 3–4.5 kg) were fasted from 5 PM on the day prior to plasma sampling until 4 hours after NYX-458 treatment. Plasma samples were taken from saphenous or cephalic veins 5 minutes prior to NYX-458 treatment (0.5 mg/kg, oral gavage) and 1, 2, 4, 6, 8, 12, and 24 hours post-administration on day 1 and following 7 days of NYX-458 daily administration (on day 8). Plasma levels were quantified using an API-4000 mass spectrometer (Sciex, Concorde, Ontario, Canada) with analyte chromatographic separation achieved with a Zorbax XDB-C18 column (Agilent, Santa Clara, CA).

Chronic Low-Dose MPTP-Lesioned Macaque Model of PD-CI

The PD-CI model was used to determine the effect of acute (0.03, 0.10, and 1.0 mg/kg, PO) and chronic (1.0 mg/kg, PO, daily, for 10 days) NYX-458 treatment on cognitive dysfunction caused by MPTP-induced dopaminergic depletion. Macaques (N = 5 males, 6–7 years old, 3–4.5 kg) were trained to achieve a stable baseline performance in the variable delayed response (VDR) and the simple discrimination and reversal learning (SDR) tasks prior to MPTP administration, as described previously.[21,22] Animals received MPTP injections (doses ranged from 0.05–0.30 mg/kg, intravenously, based on individual sensitivity to MPTP), 2 to 3 times per week, over several months until stable cognitive deficits (at least a 15% decrease in cognitive performance from pre-MPTP levels for at least 3 months across assays) with minimal parkinsonian motor impairment were observed. Animals had access to water ad libitum and were maintained on a food restriction schedule that did not change throughout the study and was optimized per animal to maintain stable performance.

VDR assessment. During VDR assessment, a white circle (cue) appeared on a computer screen. After a variable delay, animals were presented with 2 red circles and must select (touch) the red circle in the same location as the cue to receive a reward pellet (nutritionally complete flavored pellet). Each testing session included 75 trials (15 at each of the 5 delay intervals) randomly distributed in blocks throughout a daily testing session. Delay lengths were customized depending on individual performance during initial training sessions. Delays 1 to 5 were 2, 5, 10, 25 to 40, and 35 to 50 seconds, respectively. Percent correct responses on trials with different delay durations were analyzed using a 2-way, repeated-measures analysis of variance (ANOVA), Fisher's least significant difference (FLSD) test.

SDR assessment. During SDR assessment, animals were presented with 2 images on a computer screen and were required to identify a randomly designated "correct" image, the touch of which resulted in delivery of a reward pellet, on 14/16 consecutive trials to reach criterion in the simple discrimination component of the task. Animals were then required to shift responses to the other image, with the performance criterion of 14/16 consecutive correct responses in the SDR phase of the task. The measures recorded for each session were the total number of trials needed to (1) learn the initial simple discrimination and (2) the reverse, or shift, responses to the other image (SDR). The number of trials to criterion for simple discrimination and SDR were analyzed using a 1-way ANOVA, FLSD.

Testing phase. At the outset of the study, cognitive assessments after vehicle administration alone were used to assess the effect of oral gavage (PO) on behavior. Animals were then dosed with 3 dose levels of NYX-458 (0.03, 0.10, and 1.0 mg/kg, PO); see Figure 1A for the dosing and testing schedules. VDR was tested on days 1, 6, 15, and 21 postadministration of NYX-458 (0.03 mg/kg, PO), and SDR was initially tested 18 days after this first administration of NYX-458. At 26 days following the first dose of NYX-458, the animals were administered a second dose of NYX-458 (0.03 mg/kg, PO) and assessed for cognitive performance in both the VDR (3 and 10 days postdose) and SDR (2 hours and 8 days postdose). The animals then went without the drug for 2 weeks before the next dose of NYX-458 (0.1 mg/kg, PO), after which they were assessed for VDR (3 and 12 days postdose) and SDR (2 hours and 11 days postdose). To assess the effect of higher dose levels of NYX-458, additional MPTP was administered for up to 53 days until cognitive deficits were reestablished. The animals were then dosed with 0.1 mg/kg (PO) NYX-458 and tested in VDR (24 hours postdose) and SDR (2 hours postdose). The animals were administered 1.0 mg/kg (PO) NYX-458 2 weeks later and tested again in VDR (24 hours postdose) and SDR (2 hours postdose).

To assess the impact of repeat dosing on cognitive performance, the animals considered responders (N = 4 of 5) in previous experiments went through a 40-day washout followed by daily dosing of 1.0 mg/kg (PO) NYX-458 for 10 days. VDR (assessed 24 hours postdose) and SDR (assessed 2 hours postdose) performance was measured after daily doses 1, 5, and 10. Following the last of the 10-daily repeat doses, the animals were assessed for the duration of drug effect and were tested in VDR and SDR out to 132 days postdose.

Parkinsonism and Dyskinesia in the MPTP-Lesioned Macaque (PD Motor) Model

In a separate cohort of macaques (N = 7 females, 9.2–14.9 years of age, 3.7–5.2 kg), the animals were given daily doses of MPTP (0.2 mg/kg, subcutaneously) for 8 to 12 days and observed for approximately 30 days until moderate to marked parkinsonian symptoms stabilized. Additional MPTP was given to some animals to ensure similar degrees of parkinsonism across the group. In total, the animals received an average of 29 ± 5.7 (mean ± standard error the mean) mg of MPTP. Animals recovered for an additional 30 days minimum to ensure stable parkinsonism. The animals were then given L-dopa (25 mg/kg) and Madopar (Roche, Basel, Switzerland) (L-dopa, carbidopa) twice daily for 2 months to treat PD motor symptoms and to prime L-dopa–induced dyskinesia.[23,24]

Using an ascending-dose paradigm starting with vehicle administration, NYX-458 was tested as a monotherapy (0.03 and 1 mg/kg, PO) in combination with a low dose of L-dopa (mean dose of 13.1 mg/kg) and in combination with a high dose of L-dopa (mean dose of 30.6 mg/kg). A minimum of 3 days was left between treatments. The animals were video recorded for 6 hours post–drug administration, and behavior was assessed post hoc by a neurologist specializing in movement disorders and blind to treatment condition. PD motor signs were rated for 5 minutes, every 10 minutes, for the 6-hour period. Parkinsonian disability was assessed using the Primate Parkinsonian Disability Rating Scale, as described previously.[18] The Nonhuman Primate Dyskinesia Rating Scale was used to assess dyskinesia.[25] The effects of treatment on the duration and quality of any antiparkinsonian benefit were measured as the total *on* time (time without bradykinesia) and particularly "good" *on* time (time without either bradykinesia or dyskinesia). Overall locomotor activity was measured via infrared-sensors.

Results

NYX-458 is an Orally Bioavailable NMDAR Modulator

When tested in a [^{3}H]MK-801 binding assay, NYX-458 demonstrated NMDAR modulatory activity with preferential activity at N-methyl-D-aspartate receptor subunit 2B (NMDAR2B) over NMDAR2A (Table 1).

The pharmacokinetic study confirmed drug availability in plasma after oral dosing in macaques. NYX-458 (0.5 mg/kg, PO) had a plasma peak concentration of 271 ± 36 ng/ml, time to peak concentration at 1 hour following an acute single dose, and 307 ± 30 ng/ml at 1 hour following the last of 7 daily doses (0.5 mg/kg, PO, mean ± standard error of the mean, N = 3; Table 1). Repeated dosing did not impact plasma levels of NYX-458 (*t* test, 2-tailed for each time point), and there were no observed adverse events following either the single or 7 daily doses of NYX-458.

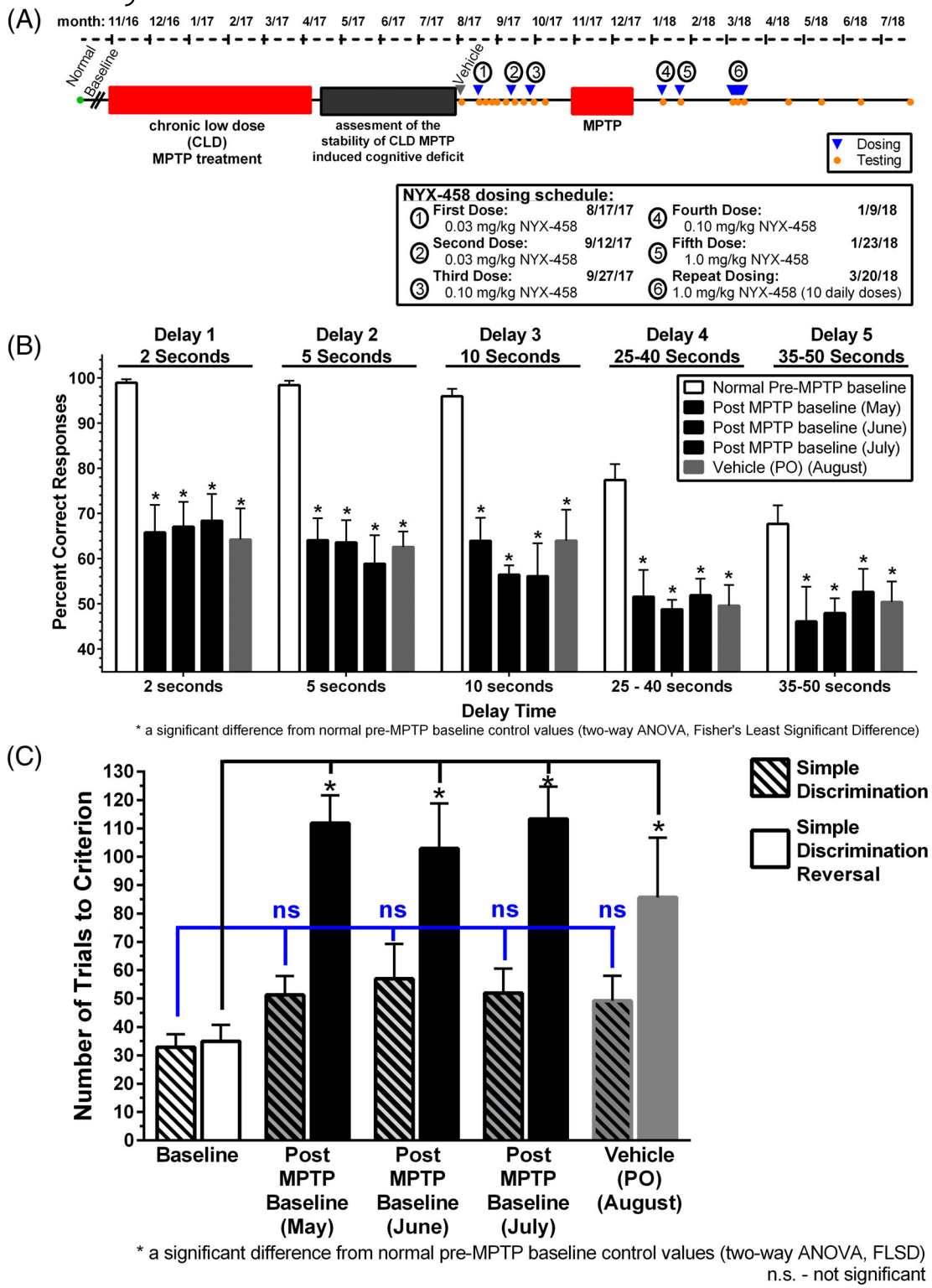

FIG. 1. Cognitive impairment study design timeline (**A**). After administration of CDL-MPTP, macaques showed a stable variable delayed response (**B**) and simple discrimination and reversal learning (**C**) cognitive deficit that was not affected by oral vehicle administration. ANOVA, analysis of variance; CLD, chronic low dose; FLSD, Fisher's Least Significant Difference; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PO, oral gavage. [Color figure can be viewed at wileyonlinelibrary.com]

Chronic Low Dose MPTP Administration Resulted in Stable Cognitive Deficits

Macaques trained in VDR and SDR assays were given low-dose MPTP over 5 months to induce stable cognitive deficits.[15] Once deficits were established, MPTP administration was discontinued, and the animals were evaluated for an additional 3 months to ensure stability of the deficits (Fig. 1A). Spontaneous

TABLE 1. NYX-458 is orally bioavailable and facilitates NMDAR2B channel opening

A. [^{3H}]MK-801 binding activity of NYX-458 in PSD95 and NMDAR-expressing HEK cells[a]	% Activity	EC_{50}
NMDAR2A	39.7 (2.5)	1.97 pM
NMDAR2B	85.6 (3.9)	209 fM

B. Plasma pharmacokinetics after oral gavage dosing of 0.5 mg/kg NYX-458 daily for 8 days in cynomolgus macaques[b]	$T_{1/2}$ h	t_{max} h	C_{max} ng × mL^{-1}	AUC_{0-t} h × ng × mL^{-1}
Day 1	1.7 (0.3)	1.0 (0.0)	271 (63.2)	900 (214)
Day 8	1.6 (0.1)	1.0 (0.0)	307 (52.5)	892 (264)

[a]Data are mean (standard error of mean) % maximal [^{3}H]MK-801 binding activity relative to that of 1 mM glycine and 50 μM glutamate and potency (EC_{50}), N = 18 to 20.
[b]Data are mean (standard error of mean) plasma exposure levels following acute and subacute oral administration of NYX-458 for a period of 8 days with samples analyzed on days 1 and 8 (N = 3).
NMDAR2B, N-methyl-D-aspartate receptor subunit 2B; [^{3H}]MK-801, tritiated MK-801; NMDAR, N-methyl-D-aspartate receptor; EC_{50}, concentration of drug that gives a half-maximal response; PSD95, postsynaptic density protein 95; NMDAR2A, N-methyl-D-aspartate receptor subunit 2A; HEK, human embryonic kidney; pM, picomolar; fM, femtomolar; $T_{1/2}$, half-life; t_{max}, time to maximum concentration; C_{max}, maximum concentration; AUC_{0-t}, area under the curve.

recovery of cognitive deficits was not observed in VDR or SDR. Using a within-subjects design, prior to administering NYX-458, the animals were tested once with vehicle (PO) so that each animal served as its own control, and a significant and stable deficit compared to the pre-MPTP baseline remained in the VDR (Fig. 1B; $F_{4,16} = 31.93$, $P < 0.0001$; repeated measures 2-way ANOVA, FLSD) and SDR (Fig. 1C; $F_{4,16} = 7.94$, $P < 0.001$; 1-way ANOVA, FLSD) tests postvehicle.

NYX-458 Resulted in a Long-Lasting Reversal of Cognitive Deficits Induced by Low-Dose MPTP

The animals dosed with NYX-458 (0.03 mg/kg, PO) showed significant improvement in VDR relative to the average post-MPTP-treatment baseline across all time delays (2–50 seconds; Fig. 2A; $F_{4,16} = 15.09$, $P < 0.0001$; RM 2-way ANOVA, FLSD). This improvement occurred as early as 24 hours postdosing and continued for at least 3 weeks after a single dose. The initial plan was to measure SDR performance following a second administration of drug that would be given after a drug washout. However, because of the long duration of effect in VDR, it was decided to measure SDR performance 18 days after the first administration of NYX-458. Indeed, a single dose of NYX-458 (0.03 mg/kg) resulted in a significant improvement in SDR compared to post-MPTP baseline when assessed 18 days postadministration (Fig. 3A; 1-way ANOVA, FLSD; $F_{3,16} = 6.11$, $P = 0.005$).

The improvement in cognitive performance continued after a second dose of NYX-458 (0.03 mg/kg, PO) given 26 days after the first dose, and after a third, higher dose of NYX-458 (0.1 mg/kg, PO) given 15 days after the second dose. Improvement in VDR was seen from the first to the third dose (Fig. 2A; $F_{4,16} = 15.09$, $P < 0.0001$; RM 2-way ANOVA, FLSD) and across time postdose (Fig. 2A; $F_{4,16} = 6.4$, $P < 0.0001$; RM 2-way ANOVA, FLSD). SDR also improved after the second dose of 0.03 mg/kg (PO) NYX-458 when measured 2 hours postdosing, with a trend toward but nonsignificant improvement 8 days postdosing (Fig. 3A; 1-way ANOVA, FLSD; $P = 0.018$ and 0.27, respectively), and after the third, higher dose of NYX-458 (0.10 mg/kg, PO) when measured 2 hours and 11 days postdosing (Fig. 3A; 1-way ANOVA, FLSD; $P = 0.036$ and 0.048, respectively).

Additional MPTP Induced Further CI, Which Was Reversed with Additional Administration of NYX-458

The long-lasting effect of NYX-458 (Figs. 2A and 3A) precluded the assessment of a potential dose response within subjects. To assess whether higher doses of NYX-458 would contribute to further benefit, the animals were given a second round of low-dose MPTP to cause additional dopaminergic cell loss and further cognitive deficit. The second MPTP administration session resulted in deficits in both VDR and SDR, to a similar level as the first round of MPTP administration. Once deficits were stable, NYX-458 was administered. A single dose of 0.1 mg/kg (PO) NYX-458 improved VDR performance at short and medium delay lengths (Fig. 2B; 2-way ANOVA, FLSD; $P < 0.005$ and 0.026 for delays 1 and 3, respectively), and a subsequent higher dose of 1 mg/kg (PO) NYX-458 resulted in improvements in VDR performance at all delay lengths (Fig. 2B; 2-way ANOVA, FLSD; $P < 0.02$ for all delay lengths). Significant improvement in SDR was also observed following dosing with 0.1 and 1 mg/kg (PO) NYX-458 (Fig. 3B; 1-way ANOVA, FLSD; $P = 0.008$ and 0.004, respectively).

The Cognitive Enhancement by NYX-458 Was Maintained After Daily Dosing and Persisted for 3 Months

To understand the effect of repeat daily dosing, the animals were assessed following daily administration of 1 mg/kg (PO) NYX-458 for 10 consecutive days. Only

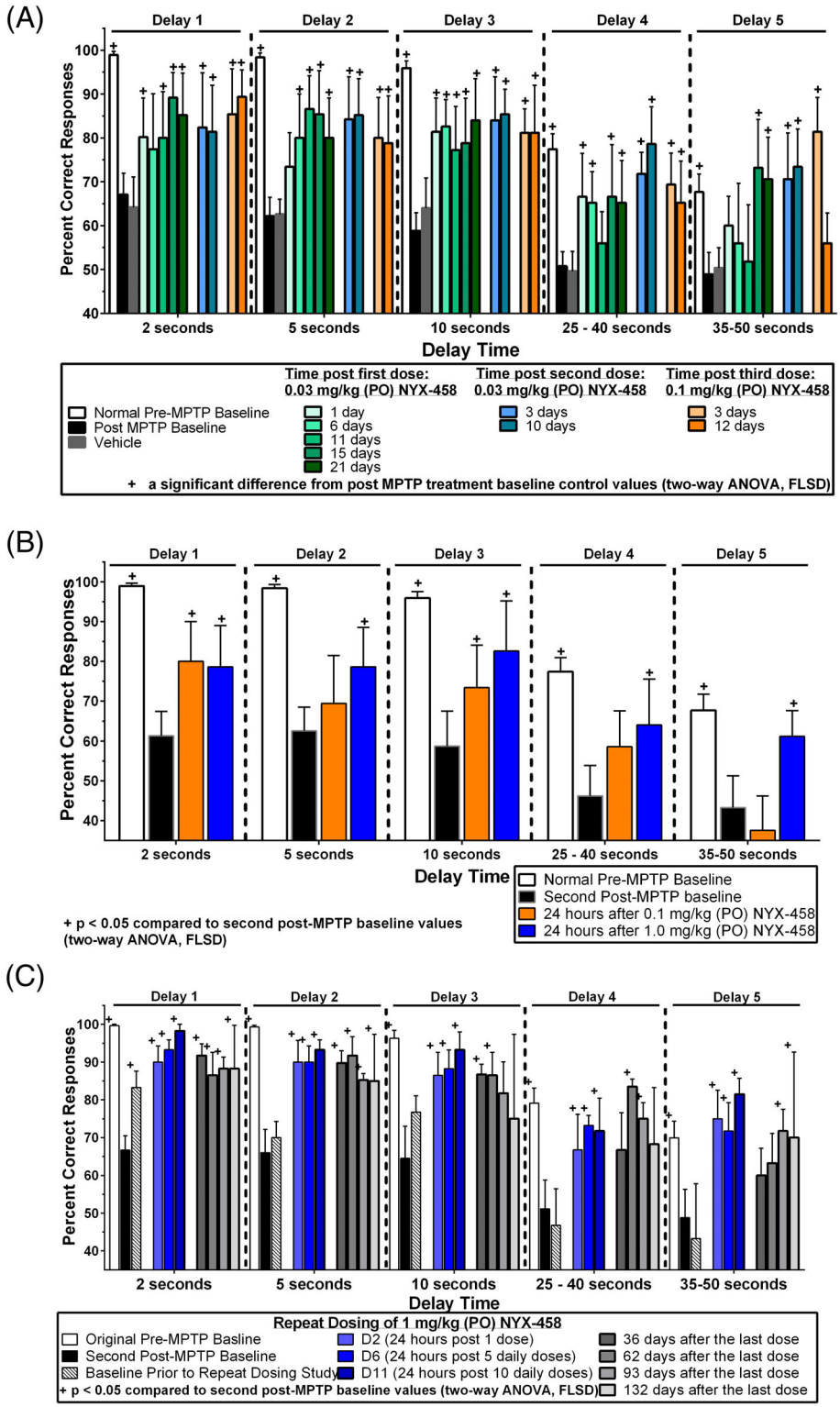

FIG. 2. NYX-458 effect on variable delayed response after CLD-MPTP-induced cognitive deficits. A low dose of NYX-458 (0.03–0.1 mg/kg, PO) improved performance across all delay lengths (**A**), which was reproduced after additional MPTP (**B**) and maintained long after repeated daily dosing (**C**). ANOVA, analysis of variance; CLD, chronic low dose; FLSD, Fisher's Least Significant Difference; MPTP, 1-methyl-4-phenyl-1,-2,3,6-tetrahydropyridine; PO, oral gavage.

macaques that demonstrated consistent improvement in VDR and SDR after NYX-458 treatment in previous experiments (4/5 animals) were included. Animals underwent a 40-day washout period and were tested to assess any residual effect of previous dosing; except for the shortest VDR delay length, the animals returned to

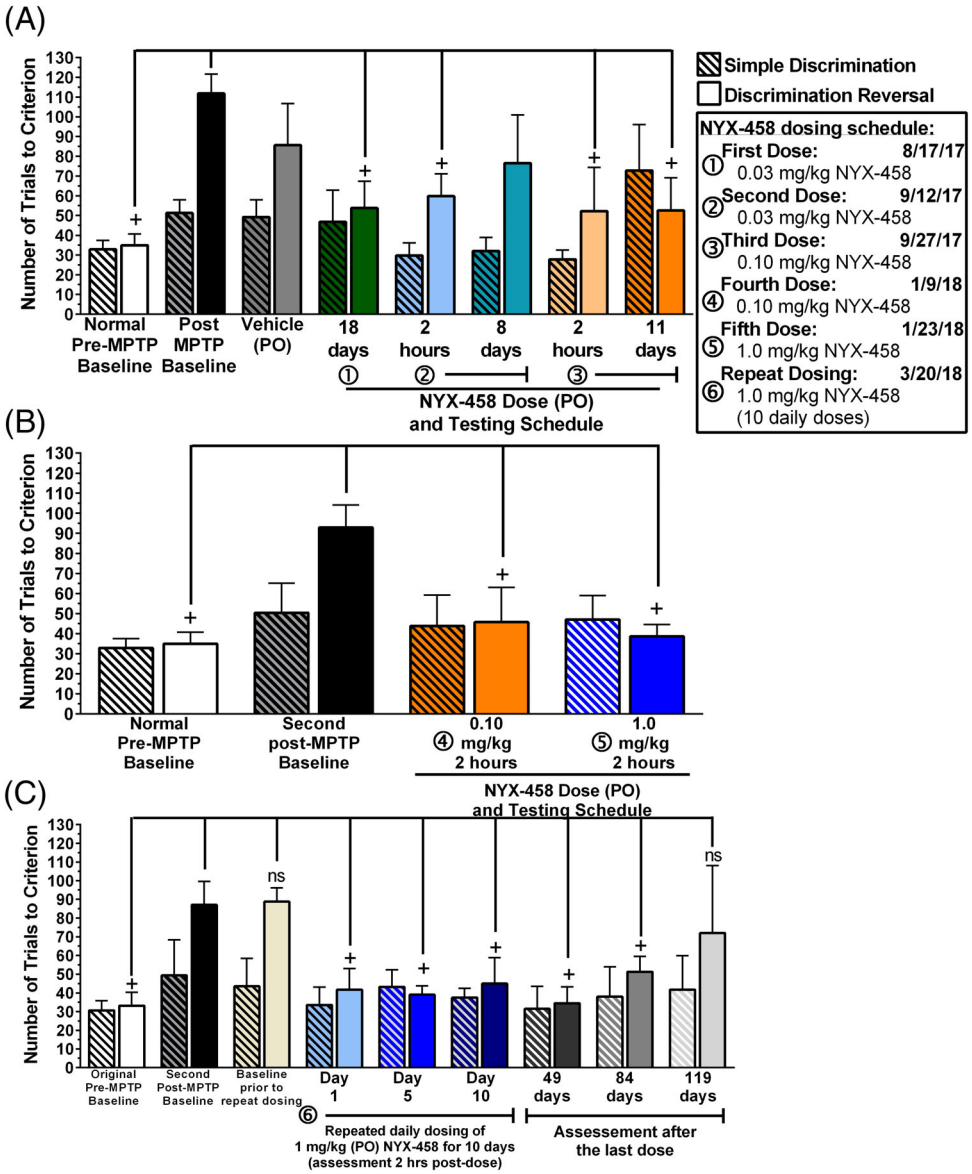

FIG. 3. NYX-458 effect on simple discrimination and reversal learning after CLD-MPTP-induced cognitive deficits. A low dose of NYX-458 resulted in long-lasting improvement of reversal learning (**A**) and was reproduced after additional MPTP (**B**) and was maintained long after repeated daily dosing of 1 mg/kg (**C**). ANOVA, analysis of variance; CLD, chronic low dose; FLSD, Fisher's Least Significant Difference; MPTP, 1-methyl-4-phenyl-1,-2,3,6-tetrahydropyridine; PO, oral gavage.

baseline deficit levels (baseline prior to repeat dosing; Figs. 2C and 3C).

Following daily dosing of NYX-458 (1 mg/kg), performance at all delay lengths was improved in the VDR assay when measured 24 hours after 1, 5, and 10 daily doses when compared with the second post-MPTP baseline (Fig. 2C; 2-way ANOVA, FLSD; $P < 0.021$ for all delays). SDR also improved when measured 2 hours after 1, 5, and 10 daily doses of NYX-458 (1 mg/kg, PO) compared with the second post-MPTP baseline (Fig. 3C; 1-way ANOVA, FLSD; $P = 0.018$, 0.014, 0.025, respectively). Overall, improvement in cognitive performance was maintained after repeated administration (10 daily doses) of 1.0 mg/kg (PO) NYX-458.

To assess the duration of effect, the animals were tested approximately monthly for 4 months following the last of 10 daily doses: improved VDR (Fig. 2C) and SDR (Fig. 3C) performance persisted for at least 3 months with animals starting to return to baseline-deficit levels after approximately 4 months postdosing (Fig. 2C; 2-way ANOVA, FLSD; $P < 0.05$; Fig. 3C, 1-way ANOVA, FLSD; $P < 0.05$).

NYX-458 Had No Impact on PD Motor Symptoms or on L-Dopa's Antiparkinsonian Effect in High-Dose MPTP-Lesioned Macaques

To determine whether NYX-458, at doses previously shown to improve PD-CI (0.03 and 1 mg/kg, PO), had

TABLE 2. NYX-458 had no adverse effect on motor symptoms when given as a monotherapy or in combination with low and high doses of L-dopa: NYX-458 effect on parkinsonism and dyskinesia in an MPTP-lesioned macaque model of Parkinson's disease

Treatment and Measure	Vehicle	0.03 mg/kg, NYX-458	1 mg/kg, NYX-458
Monotherapy			
Parkinsonian Disability Score	178 (21)	196 (27)	217 (28)
Dyskinesia score	0 (0)	0 (0)	0 (0)
Total activity counts	3399 (879)	3091 (910)	2704 (869)
Low-dose Madopar (L-dopa)			
Parkinsonian Disability Score	122 (18)	116 (21)	140 (27)
Dyskinesia score	31 (4)	32 (3)	32 (6)
Total *on* time, min	139 (15)	139 (12)	131 (23)
"Good" *on* time, min	100 (27)	90 (13)	93 (15)
Total activity counts	5302 (642)	5788 (693)	5094 (942)
High-dose Madopar (L-dopa)			
Parkinsonian Disability Score	86 (9)	68 (12)	104 (4)
Dyskinesia score	76 (4)	81 (6)	69 (8)
Total *on* time, min	241 (13)	254 (18)	199 (21)
"Good" *on* time, min	67 (14)	70 (3)	35 (8)
Total activity counts	6065 (945)	7171 (1108)	5109 (806)

Data are mean (standard error of mean) sum of the hourly scores for PDS and dyskinesia, number of minutes for *on* time, beam crosses for activity, over the 6 hours. No significant difference between vehicle and either drug treatment group (1-way analysis of variance, Fisher's Least Significant Difference, N=7)
L-dopa, levodopa; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

any effect on PD motor symptoms, a separate cohort of animals was given high doses of MPTP to induce PD motor deficits. Parkinsonian disability scores (PDS) following treatment with vehicle alone were moderate during the 0 to 6-hour period of observation. NYX-458, when given as a monotherapy (0.03 and 1 mg/kg, PO), did not change mean PDS or total activity cumulated during the 6-hour period (1-way ANOVA, FLSD; $P > 0.05$; Table 2). Furthermore, at no time did either dose of NYX-458 (0.03 and 1 mg/kg, PO), when given alone, induce dyskinesia.

A low dose of L-dopa improved PDS accumulated during the 6-hour period as compared to vehicle alone (*t* test; $P < 0.05$; Table 2). A high dose of L-dopa also significantly improved PDS (*t* test, $P < 0.05$; Table 2), but also significantly increased dyskinesia (*t* test; $P < 0.05$; Table 2). NYX-458 (0.03 and 1 mg/kg, PO), when given in combination with either low-dose or high-dose L-dopa, had no added nor detrimental effect on PDS, dyskinesia, total *on* time, total "good" *on* time, or total activity (1-way ANOVA, FLSD; $P > 0.05$; Table 2). NYX-458, at dose levels that improve cognitive performance, did not worsen PD motor symptoms or interfere with the antiparkinsonian effect of L-dopa.

Discussion

The objective of this study was to evaluate the effect of a novel NMDAR modulator on cognitive and motor functions in primate dopamine-depletion models of PD. In the CI model, the animals administered chronic low-dose MPTP showed clear deficits in attention and working memory, as measured by the VDR assay, and in executive function, as measured by the SDR assay. Importantly, there was no simple-discrimination deficit, demonstrating that dopamine depletion resulted in deficits specific to cognitive domains affected in early PD and that the motivational state of the animal was not impacted. The long-term stability of cognitive deficits seen using this specific primate strain and dosing paradigm has been characterized previously,[13,17] and indeed, in this study a stable deficit was monitored for 3 months after the withdrawal of MPTP before administering vehicle or NYX-458.

A within-subjects design was used with all animals receiving vehicle prior to NYX-458 administration. Importantly, vehicle did not improve performance in any measure, whereas all dose levels of NYX-458 (0.03–1 mg/kg, PO) provided significant, rapid, and long-lasting improvement in VDR with short-to-medium delay lengths, demonstrating the ability of this compound to improve acute and sustained attention. Higher doses of NYX-458 (0.1 and 1 mg/kg, PO) also improved performance after longer delay lengths, further establishing the ability of this compound to improve working memory. In comparison, the NMDAR partial agonist D-cycloserine has demonstrated improvement in short, but not long, delay lengths in the VDR assay using the same model.[22]

NYX-458, at all doses, also rapidly (within 2 hours) and significantly improved performance in reversal learning (SDR), a measure of executive function.

For both VDR and SDR, the procognitive benefit of NYX-458 administration persisted for at least 3 weeks after a single dose. This sustained effect of cannot be explained by continued drug action, as the half-life of NYX-458 in primate plasma is short. In addition, unpublished rat and human PK studies with NYX-458 have confirmed drug presence in the cerebrospinal fluid and support a short half-life of this compound in the brain. Recent studies with other compounds from this unique class of NMDAR modulators, including NYX-2925, have shown long-lasting effects on behavior, metaplasticity, and dendritic morphology that persist after the drug is no longer present.[10,26] The improvement in cognition seen with NYX-458 was reproduced after administering animals additional MPTP, further attributing the specificity of the cognitive improvement to NYX-458 and not to spontaneous recovery. The animals continued to see improved cognition out to 132 days post their last dose in at least 3 delay lengths in the VDR assay and out to 93 days postdose in the SDR assay. By day 132, some animals clearly reverted to a CI state in both VDR and SDR, suggesting that NYX-458 drives a long-lasting, but not permanent, improvement in cognitive function. Overall, these data support the hypothesis that NYX-458 mediates long-lasting changes in glutamatergic signaling and synaptic plasticity processes that underly improvements in cognitive performance.

Executive-function and working-memory deficits are frequently observed in PD patients and are characteristic of PD-MCI. The precise basis of cognitive deficits in PD is unknown; however, NMDAR function in the cortico-striato-pallidal-thalmo-cortical loop circuitry is critical for cognitive processes, and dopaminergic cell loss in PD leads to NMDAR dysfunction.[27-30] Indeed, the selective NMDAR2B antagonist (CP-101,606) has been shown to impair reversal learning in rodents,[31] visual paired associate learning tasks requiring working memory in nonhuman primates,[32] and executive-function and working-memory deficits by causing amnesia and abnormal thinking in PD patients, specifically.[33] These studies imply an important role for NMDAR2B-mediated activity in the development of PD-CI and suggest that the NMDAR may act as an important therapeutic target for the treatment of cognitive decline. NYX-458 is a highly potent NMDAR2B modulator, and the positive modulation of NMDAR2B may account for the NYX-458–mediated benefits observed in our study.

Early CI in PD contributes to poorer quality of life even when motor symptoms are well controlled,[34] and given that there are no approved therapies for PD-MCI, this is a major unmet medical need. A therapy for PD-MCI should not only improve cognitive performance but also have limited detrimental effects on PD motor symptoms either as a monotherapy or in combination with L-dopa. Although the effect of repeated daily administration of NYX-458 on motor deficits was not examined, the data suggest that dose levels of NYX-458 that improve cognitive function will not worsen motor symptoms, interfere with L-dopa's anti-parkinsonian effect, or induce or worsen dyskinesia. NYX-458 administration did not alleviate dyskinesia. This is not surprising considering that NYX-458 is a positive NMDAR modulator and L-dopa–induced motor complications, including L-dopa–induced dyskinesia, are associated with an increase in NMDAR2B expression and NMDAR antagonists improve LIDs, particularly late in the disease course.[33,35,36]

There are some important limitations to this study. For one, the animals used in this study have not yet been euthanized, and their brains have not been examined. However, the neurochemical and histopathological impact of this MPTP-dosing paradigm in macaques has been characterized previously.[17,37] This model results in substantial dopaminergic loss in the caudate and putamen and neurochemical changes in the noradrenergic, cholinergic, and serotonergic systems similar to that seen in early PD. Another limitation is that a within-subjects study design was used instead of a separate placebo control group, which calls into question the potential for spontaneous recovery or practice effect. However, the stable cognitive deficit seen prior to vehicle or NYX-458 administration, the lack of improvement seen with vehicle, the further decline in function seen with additional MPTP administration, and the reproducible improvement seen after NYX-458 administration all support that the effect seen was NYX-458 mediated.

Overall, although additional studies are needed to understand the mechanism in which NYX-458 mediates prolonged improvement in cognitive symptoms, the cognitive improvement seen in this small primate study and the lack of drug-induced motor impairment or dyskinesia seen in the primate motor study support the continued development of NYX-458 as a potential therapeutic for CI in early PD. ■

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