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Methodology and effects of repeated intranasal delivery of DNSP-11 in awake Rhesus macaques

M.J. Stenslik[a], A. Evans[a], F. Pomerleau[a], R. Weeks[a], P. Huettl[a], E. Foreman[a], J. Turchan-Cholewo[a], A. Andersen[b], W.A. Cass[a], Z. Zhang[a], R.C. Grondin[a], D.M. Gash[a], G.A. Gerhardt[a], L.H. Bradley[a,c,*]

[a] Department of Neuroscience and Brain Restoration Center, University of Kentucky College of Medicine, United States
[b] Department of Magnetic Resonance Imaging and Spectroscopy Center, University of Kentucky College of Medicine, United States
[c] Department of Molecular & Cellular Biochemistry and Center of Structural Biology, University of Kentucky College of Medicine, United States

HIGHLIGHTS

- DNSP-11 was repeatedly delivered intranasally in awake Rhesus over 10-weeks.
- Neurochemical analysis of the striatum provided evidence for target engagement.
- No observed behavioral side-effects following repeated delivery or dose-escalation.
- Evidence supports direct nose-to-brain transport after a single dose.

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ABSTRACT

Background: To determine if the intranasal delivery of neuroactive compounds is a viable, long-term treatment strategy for progressive, chronic neurodegenerative disorders, such as Parkinson's disease (PD), intranasal methodologies in preclinical models comparable to humans are needed.
New method: We developed a methodology to evaluate the repeated intranasal delivery of neuroactive compounds on the non-human primate (NHP) brain, without the need for sedation. We evaluated the effects of the neuroactive peptide, DNSP-11 following repeated intranasal delivery and dose-escalation over the course of 10-weeks in Rhesus macaques. This approach allowed us to examine striatal target engagement, safety and tolerability, and brain distribution following a single ^{125}I-labeled DNSP-11 dose.
Results: Our initial data support that repeated intranasal delivery and dose-escalation of DNSP-11 resulted in bilateral, striatal target engagement based on neurochemical changes in dopamine (DA) metabolites-without observable, adverse behavioral effects or weight loss in NHPs. Furthermore, a ^{125}I-labeled DNSP-11 study illustrates diffuse rostral to caudal distribution in the brain including the striatum-our target region of interest.
Comparison with existing methods: The results of this study are compared to our experiments in normal and 6-OHDA lesioned rats, where DNSP-11 was repeatedly delivered intranasally using a micropipette with animals under light sedation.
Conclusions: The results from this proof-of-concept study support the utility of our repeated intranasal dosing methodology in awake Rhesus macaques, to evaluate the effects of neuroactive compounds on the NHP brain. Additionally, results indicate that DNSP-11 can be safely and effectively delivered intranasally in MPTP-treated NHPs, while engaging the DA system.

Abbreviations: BBB, blood-brain barrier; BCSFC, blood-cerebrospinal fluid barrier; CED, convection-enhanced delivery; CNS, central nervous system; CSF, cerebrospinal fluid; CPM, counts per min; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; DNSP-11, dopamine-neuron stimulating peptide-11; GDNF, glial cell line-derived neurotrophic factor; GFR, GDNF-family receptor; HPLC-EC, high performance liquid chromatography with electrical chemical detection; HVA, homovanillic acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride; MW, molecular weight; NHP, non-human primate; OB, olfactory bulb; OSN, olfactory sensory neuron; PD, Parkinson's disease; RP-HPLC, reverse phase-high performance liquid chromatography; SN, substantia nigra; 6-OHDA, 6-hydroxydopamine.

* Corresponding author at: MN220 Chandler Medical Center, 800 Rose Street, Lexington, KY 40536-0298, United States.
E-mail address: lhbradley@uky.edu (L.H. Bradley).

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1. Introduction

One of the major challenges in delivering large molecular weight (MW) compounds, such as peptides and proteins to the Central Nervous System (CNS), has been their targeted delivery to the brain (Thorne and Frey, 2001; Sullivan and O'Keeffe, 2016; Tachikawa et al., 2014; Wolak and Thorne, 2013). The presence of the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barriers (BCSFBs) greatly restricts the passive entry of large MW compounds into the CNS following oral and parenteral routes of administration (Tachikawa et al., 2014; Wolak and Thorne, 2013; Pardridge, 2005; Patel and Patel, 2017). Therefore, invasive surgical techniques are generally used to deliver compounds directly to the brain-often with varying degrees of success (Bartus and Johnson, 2017a; Bartus and Johnson, 2017b; Obeso et al., 2017; Peterson and Nutt, 2008; Siegel and Chauhan, 2000; Slevin et al., 2007; Slevin et al., 2005; Gill et al., 2003; Lang et al., 2006). For example, based on their ability to promote survival and growth in neuronal populations (Lin et al., 1993), neurotrophic factors have been extensively pursued as a possible disease-modifying treatment for Parkinson's disease (PD) (Sullivan and O'Keeffe, 2016; Bartus and Johnson, 2017a; Bartus and Johnson, 2017b; Obeso et al., 2017; Peterson and Nutt, 2008; Hegarty et al., 2017). Although preclinical studies have supported the efficacy of intraparenchymally infused neurotrophic factors into the nigrostriatal system of parkinsonian animal models (Gash et al., 2005; Grondin et al., 2003; Gash et al., 1996; Kearns et al., 1997; Kearns and Gash, 1995; Grondin et al., 2008; Hoffer et al., 1994; Kirik et al., 2000; Rosenblad et al., 1999); clinical trials examining the direct, surgical infusion of neurotrophic factors into targeted regions of the basal ganglia system have failed to meet primary end points (Obeso et al., 2017; Slevin et al., 2007; Slevin et al., 2005; Gill et al., 2003; Lang et al., 2006; Marks et al., 2010; Marks et al., 2008). This lack of clinical efficacy has been strongly attributed to insufficient biodistribution and/or bioavailability following direct infusion into the brain (Sullivan and O'Keeffe, 2016; Bartus and Johnson, 2017a; Bartus and Johnson, 2017b; Obeso et al., 2017; Hegarty et al., 2017; Salvatore et al., 2006). While additional efforts have been explored to improve distribution and/or bioavailability to key pathophysiological regions of the CNS following direct, surgical infusion (Obeso et al., 2017; Kordower et al., 2000; Kordower et al., 1999; Bankiewicz et al., 2016; Sherer et al., 2006; Kordower and Bjorklund, 2013), optimal delivery methodologies for neurotrophic factors have yet to be identified (Sullivan and O'Keeffe, 2016; Bartus and Johnson, 2017a; Bartus and Johnson, 2017b; Obeso et al., 2017; Hegarty et al., 2017; Sherer et al., 2006). Therefore, new avenues need to be examined that overcome challenges associated with delivering large MW compounds to the brain for the treatment of chronic, progressive neurodegenerative diseases and disorders (Thorne and Frey, 2001; Sullivan and O'Keeffe, 2016; Obeso et al., 2017). One possible alternative strategy currently under investigation is the discovery and development of new neuroactive compounds (Bradley et al., 2010; Fuqua et al., 2014; Kelps et al., 2011; Bradley et al., 2017) that can potentially be delivered to the CNS using non-invasive routes of administration, such as intranasal delivery (Thorne and Frey, 2001; Stenslik et al., 2015).

An emerging body of preclinical (Stenslik et al., 2015; Banks et al., 2004; Dhuria et al., 2009; Gozes et al., 2000; Migliore et al., 2014; Ross et al., 2004; Thorne et al., 2008; Thorne et al., 2004; Deadwyler et al., 2007; Yue et al., 2017) and clinical (Craft et al., 2017; Born et al., 2002; Parker et al., 2017; Chapman et al., 2013) evidence supports the intranasal delivery of compounds as a viable, non-invasive alternative route of administration that can potentially bypass the BBB and BCSFBs-directly targeting compounds to the CNS and cerebrospinal fluid (CSF) (Born et al., 2002; Chapman et al., 2013; Lochhead and Thorne, 2012; Hanson and Frey, 2008). Several preclinical studies have attributed the rapid nose-to-brain transport of intranasally-administered compounds to a combination of extracellular pathways including: perineural transport associated with the olfactory and trigeminal nerves, perivascular delivery by way of the cerebral vasculature, and perilymphatic system (Thorne and Frey, 2001; Lochhead and Thorne, 2012; Lochhead et al., 2015; Kumar et al., 2016; Lochhead and Thorne, 2014). However, the majority of intranasal studies investigating nose-to-brain transport mechanisms and/or the effects of neuroactive compounds on the CNS, have been conducted in rodents (Graff and Pollack, 2005; Illum, 1996). While the importance of these studies should not be underestimated, differences in rodent nasal anatomy (Lochhead and Thorne, 2012; Kumar et al., 2016; Lochhead and Thorne, 2014; Illum, 1996; Gizurarson, 1990; Harkema et al., 2006) and the typical use of sedation to optimize intranasal dosing to the olfactory region (Illum, 1996; Dhuria et al., 2010), may limit the translation of rodent studies into the clinic (Graff and Pollack, 2005; Illum, 1996; Barchet and Amiji, 2009). For example, our team has reported on the neuroactive effects of the synthetic, amidated 11-amino acid neuroactive peptide, DNSP-11 (dopamine neuron stimulating peptide-11) following repeated intranasal delivery in rats (Stenslik et al., 2015). However, the repeated use of light isoflurane sedation as chemical restraint appeared to enhance the toxicity of the 6-OHDA nigrostriatal lesion (Stenslik et al., 2015; Datla et al., 2006). Therefore, the development of intranasal dosing methodologies, amenable to repeated dosing paradigms in awake (unanesthetized) non-human primates (NHP), which are anatomically comparable to the human brain and olfactory system (Graff and Pollack, 2005; Illum, 1996; Gizurarson, 1990; Harkema et al., 2006), remains a critical step in the preclinical evaluation of neuroactive compounds intended for the treatment of chronic, progressive neurodegenerative diseases and disorders such as PD (Graff and Pollack, 2005; Barchet and Amiji, 2009).

Here we report an intranasal dosing methodology, using an atomizer in awake NHPs that can be implemented to evaluate the effects of neuroactive compounds, such as DNSP-11, on the brain following prolonged, repeated intranasal dosing. In this proof-of-concept study, Rhesus macaques were administered DNSP-11 (or vehicle) intranasally 4 consecutive days-per-week, with escalation of the DNSP-11 dose (0, 0.3, 1.0, 3.0, 10.0 mg/day) occurring biweekly over the course of 10-weeks. This dosing strategy allowed us to examine striatal target engagement, safety and tolerability of repeated dosing and dose-escalation, and brain distribution following a single ^{125}I-labeled DNSP-11 dose. Our data support that the repeated intranasal delivery and dose-escalation of DNSP-11 resulted in bilateral, target engagement based on changes in striatal tissue levels of DA and DA metabolites: 3,4–dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). In addition, there were no observable behavioral effects or weight loss following repeated intranasal delivery. Finally, we observed that DNSP-11 is rapidly transported to the CNS following a single, bilateral intranasal dose-as evident from a ^{125}I DNSP-11 distribution study. Collectively, these results demonstrate that DNSP-11 can be safely delivered intranasally at various concentrations over an extended period of time in awake NHPs, while maintaining its neuroactive properties in the striatum (Stenslik et al., 2015).

2. Methods

2.1. Ethics statement & animals

The animal facility at the University of Kentucky strictly follows the guidelines set by the National Institutes of Health (NIH), and are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International