

## REGULAR RESEARCH ARTICLE

# A Randomized Controlled Trial of Intranasal Neuropeptide Y in Patients With Major Depressive Disorder

Aleksander A. Mathé, Miranda Michaneck, Elisabeth Berg,  
Dennis S. Charney, James W. Murrough

Center for Psychiatric Research, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden (Dr Mathé, Dr Michaneck, and Ms Berg); Office of the Dean, Icahn School of Medicine at Mount Sinai, New York, New York (Dr Charney); Depression and Anxiety Center for Discovery and Treatment, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, New York (Drs Charney and Murrough); Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, New York (Dr Murrough).

D.S.C. and J.W.M. contributed equally to this work.

Correspondence: Aleksander Mathé, MD, PhD, Department of Clinical Neuroscience, Tomtebodavägen 18A Karolinska Institutet, 17176 Stockholm, Sweden ([aleksander.mathe@ki.se](mailto:aleksander.mathe@ki.se)).

## Abstract

**Background:** Since about one-third of patients with major depressive disorder (MDD) do not respond adequately to available antidepressants, there is a need for treatments based on novel mechanisms of action. Neuropeptide Y (NPY), a normal brain constituent, is reduced in cerebrospinal fluid of patients with MDD and post-traumatic stress disorder and in corresponding rodent models. Moreover, NPY administered centrally or intranasally rescues pathophysiology in these models. Consequently, we conducted the first, to our knowledge, controlled trial of NPY as a treatment for MDD.

**Methods:** Thirty MDD patients on a stable dose of a conventional antidepressant insufflated 6.8 mg NPY ( $n=12$ ) or placebo ( $n=18$ ) in a double blind randomized fashion. Effects were assessed at baseline, +1 hour, +5 hours, +24 hours, and +48 hours. The primary outcome was change in depression severity measured with the Montgomery-Åsberg Depression Rating Scale (MADRS).

**Results:** NPY was superior to placebo at +24 hours (change  $-10.3$  [95% CI:  $-13.8$ ;  $-6.8$ ] vs  $-5.6$  (95% CI:  $-8.4$ ;  $-2.7$ ); group\*time  $F = 3.26$ ,  $DF = (1,28)$ ,  $P = .04$ ; Cohen's  $d = 0.67$ ). At +5 hours MADRS decreased  $-7.1$  ([95% CI:  $-10.0$ ;  $-4.2$ ] vs  $-3.5$  [95% CI:  $-5.8$ ;  $-1.2$ ]; group\*time  $F = 2.69$ ,  $DF = (1,28)$ ,  $P = .05$ ; Cohen's  $d = 0.61$ ). MADRS reduction at +48 hours was not significant.

**Conclusions:** Since no results regarding the trajectory of NPY effects existed prior to this study we extrapolated from the known NPY biology and predicted the effects will occur 5–48 hours post insufflation. We chose +48 hours as the primary endpoint and +1, +5, and +24 hours as secondary endpoints. The results, the first of their kind, indicate that insufflated NPY is antidepressant, despite not meeting the primary outcome, and call for dose ranging and repeated NPY insufflation trials.

**Clinical Trial Registration:** EudraCT Number: 2014-000129-19.

**Key Words:** Depression, antidepressant, neuropeptide Y, intranasal, resilience

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## Significance Statement

Major depressive disorder is the predominant cause of years of life lived with disability and years of life lost because of premature death. The gravity of the problem is increasing due to growing population longevity. Understanding the disease pathophysiology is limited, and 30%–40% of depressed patients do not respond adequately to conventional monoamine-targeting agents. Preclinical evidence shows reduced NPY, a normal brain constituent, in both depression and PTSD animal models, findings supported by studies revealing decreased NPY in MDD and PTSD patients. Since NPY administration rescues pathology in animal models, we hypothesized that NPY will alleviate MDD and PTSD symptoms in patients. Our first study (Sayed et al., 2018) demonstrated that intranasal NPY insufflation decreased PTSD symptomatology, and here we show that intranasal NPY insufflation reduces symptom severity in MDD. Consequently, NPY constitutes a novel target for development of depression and PTSD treatments.

## Introduction

Mood disorders, including major depressive disorder (MDD), are the major cause of years of life lived with disability and years of life lost because of premature death. The lifetime depression risk is estimated at 20%–25% for women and 7%–12% for men (ECNP/European Brain Council, 2011; WHO, 2017). The gravity of the problem is increasing due to lengthening longevity of the population (Catalá-López et al., 2013). Understanding the disease pathophysiology remains limited (Svensson and Mathé, 2002; McEwen et al., 2015), and in the recent decades research focus has, in part, shifted from the monoaminergic system and the hypothalamic pituitary adrenal axis to neuropeptides, for example, neuropeptide Y (NPY), glutamatergic signaling, neurotrophic factors, and inflammation (Heilig, 2004; Hascup et al., 2009; Wu et al., 2011; Haroon et al., 2017; Thorsell and Mathé, 2017). Optimal treatments are lacking and 30%–40% of depressed patients fail to respond adequately (Tundo et al., 2015). Consequently, there exists a major unmet medical need to develop more efficient treatments.

NPY, evolutionarily a well-preserved peptide, is found in brain of all mammals and is distributed in regions of relevance for depression, anxiety, and vegetative functions. In rodents, we have found reduced brain NPY mRNA and protein in (1) genetic and environmental models of depression, (2) posttraumatic stress disorder (PTSD) model, and (3) animals exposed to chronic stress and maternal separation (Jiménez-Vasquez et al., 2000a, 2000b, 2001, 2007; Heilig, 2004; Heilig et al., 2004; Cohen et al., 2012, 2015, 2018). Consistent with the animal data, NPY is reduced in depressed patients exposed to childhood trauma and in postmortem brains from bipolar patients who committed suicide (Rasmusson et al., 2000; Caberlotto and Hurd, 2001; Heilig et al., 2004; Sah and Geraciotti, 2013; Kautz et al., 2017). Lastly, NPY in cerebrospinal fluid (CSF) from euthymic bipolar patients is a risk marker for suicide (Sandberg et al., 2014).

With regard to treatment effects, all antidepressant procedures tested preclinically to date, such as electroconvulsive stimuli, selective serotonin reuptake inhibitors, lithium, ketamine, and exercise (voluntary running) increase brain NPY in rodent models (Stenfors et al., 1994; Mathé et al., 1998, 2014; Husum et al., 2000; Jiménez-Vasquez et al., 2000a, 2000b; Husum and Mathé 2002; Bjørnebekk et al., 2006). Furthermore, central NPY administration of, for example, 20 µg (Heilig et al., 1989), 6 µg (Husum et al., 2000), 12.76 µg (Redrobe et al., 2002), 5 µg and 10 µg (Cohen et al., 2012, 2015) as well as 1.0 nmol/0.5 mL of neuropeptide S via NPY-Y1 receptor (Cohen et al., 2018) had antidepressant and PTSD-alleviating effects in rodents. Furthermore, seminal work in rodents by Serova and Sabban and their collaborators demonstrated that NPY administered intranasally enters the brain and has pronounced direct and prophylactic

antidepressant and PTSD-alleviating effects. Of particular importance regarding the dynamics of NPY uptake into the brain and relationship to changes in CSF and blood were the findings that following intranasal administration of vehicle, 50 µg NPY, or 90 µg NPY, respectively, blood NPY levels did not change while levels in CSF that were not detectable following vehicle were increased to 0.4 µg/mL and 2.8 µg/mL, respectively. In separate cohorts of animals, intranasal administration of 150 µg NPY had significant anxiolytic and antidepressant effects. Lastly, NPY entry into brain was confirmed using fluorescent-labeled NPY (Serova et al., 2013, 2014; Sabban et al., 2015). Since NPY was infused under isoflurane anesthesia, which has antidepressant and anxiolytic effects both in rodents and depressed patients (Langer et al., 1985; Hoffmann et al., 1991; Weeks et al., 2013), it should be of interest to test nose-to-brain NPY delivery with a control compound or without a general anesthesia.

Translationally consistent with these preclinical findings, NPY was increased in CSF in patients following electroconvulsive therapy, the selective serotonin reuptake inhibitor citalopram, and the atypical antipsychotic quetiapine (Mathé et al., 1995/1996; Nikisch et al., 2005, 2012; Nikisch and Mathé, 2008). In line with these findings, transgenic rats overexpressing hippocampal NPY show decreased depression and anxiety-like behaviors (Thorsell et al., 2000). Collectively, these data indicate that the NPY system is dysregulated in depression and PTSD and that 1 shared feature of all antidepressant treatments so far tested is upregulation of NPY expression and NPY-Y1 receptors (Wu et al., 2011; Cohen et al., 2012, 2018), suggesting that NPY upregulation may be a common pathway of effective antidepressant therapies.

The rationale for this proof-of-concept trial was our hypothesis that NPY will have antidepressant effects in patients with MDD based on the (1) cumulative preclinical and clinical results showing decreased brain NPY expression in depression and PTSD, (2) results showing that all treatments that increase NPY alleviate depression and PTSD symptoms (the decrease correlating to increase in NPY), and (3) central administration of NPY rescues altered behavior in rodents, that is, experiments consistently demonstrating that NPY injected centrally had antidepressant, anxiolytic, and PTSD-alleviating effects (Heilig et al., 1989; Husum et al., 2000; Redrobe et al., 2002; Cohen et al., 2012, 2015) (for review, see Wu et al., 2011).

A pivotal question was that of an appropriate administration method. Oral administration is not feasible due to breakdown of NPY in the gastrointestinal tract. Of note, investigations in rodents demonstrated that several peptides, including labeled NPY, cross the blood-brain barrier and enter the brain albeit at a very low rate (Kastin and Akerstrom, 1999). Since NPY half-life

in circulation is <30 minutes and uptake is <1%, and considering possible systemic side-effects, the i.v. route was deemed not to be a viable option. In another area of research, studies with a variety of compounds, including chitosan and transportan, have been conducted with the aim to increase uptake of potential therapeutics into cells (Thorne and Frey, 2001; Copolovici et al., 2014; Ramsey and Flynn, 2015). Drawing on that work, we explored the possibility in our Flinders Sensitive Line rats, a well validated model of depression (Jiménez-Vasquez et al., 2000a, 2000b, 2001, 2007; Björnebekk et al., 2006) to increase NPY uptake into brain by binding it to a carrying molecule, transportan, and investigated uptake using i.v. and i.p. administration. The experiments were not successful (Mathé, Eféndic, Langel, unpublished data). In contrast, preclinical and clinical experiments have shown that intranasal administration is feasible for a variety of compounds, and these techniques are now accepted as a method for direct delivery of protein therapeutics into the CNS. Studies with inhalation of prostaglandins in patients with bronchial asthma (Mathé 1976; Mathé et al., 1977a, 1977b) and pioneering work by Born and coworkers (Born et al., 2002) using healthy male and female subjects to “sniff peptides” followed by other researchers using human participants (Hallschmid et al., 2003; Craft et al., 2012; Illum, 2012; Lochhead and Thorne, 2012; Chapman et al., 2013) led to our decision to conduct a proof-of-concept trial of NPY employing the insufflation procedure that takes advantage of a direct nose-to-brain transport of compounds.

## Methods

### Study Participants

The protocol and the Investigational Medicinal Product Dossier were approved by the institutional review board (EPN, Regionala Etikprövningsnämnden i Stockholm, Dnr 2014/582-31/2 and 2015/1233-32) and the Swedish Regulatory Agency (Läkemedelsverket [LMV]). The trial is registered at <https://www.clinicaltrialsregister.eu>: EudraCT Number: 2014-000129-19. All procedures were carried out in compliance with the Karolinska Institute’s regulations and Declaration of Helsinki-The World Health Organization (2001). Participants were recruited by the Karolinska Trial Alliance, who also monitored the trial. All participants signed the informed consent form. Subsequent to signing informed consent, participants underwent medical and psychiatric screening by a trained psychiatrist. Inclusion criteria

were recurrent MDD according to DSM-V confirmed with the Structural Clinical Interview Swedish version), a history of at least 1 previous episode, and currently in a major depressive episode of at least moderate severity with a minimum Montgomery Åsberg Depression Rating Scale (MADRS) score of 25 or greater. Patients remained on their antidepressant medication and no medication changes were allowed during the study. Exclusion criteria were pregnancy, cardiovascular disease, epilepsy or other neurological diseases, and uncontrolled hypertension or other serious somatic diseases. Participants with a suicide risk, current substance abuse, a history of manic or mixed affective episode, psychosis, or schizophrenia were not included. Lastly, patients on lithium, antipsychotics, or benzodiazepines were excluded.

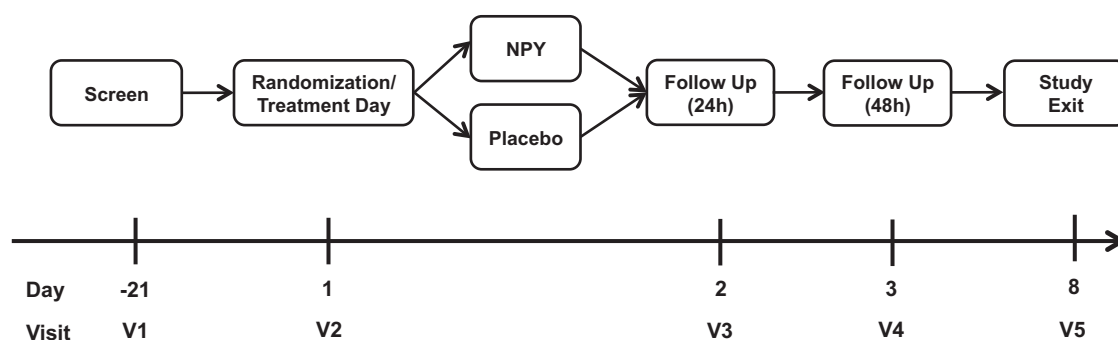
### Study Design

The detailed study design is presented in [supplemental Material](#) (Consort checklist; Consort diagram; study design figure and text; [supplemental Tables 1–3](#); adverse events definition). The time trajectory is shown in [Figure 1](#).

This randomized, placebo controlled, double blind trial consisted of 5 visits, each starting at 9 AM: visit 1 (V1), screening; visit 2 (V2), the randomization/treatment visit (1:1 allocation between NPY and matching placebo) within 14 to max 21 days post-screening; visit 3 (V3), assessment 24 hours following treatment; visit 4 (V4), assessment 48 hours following treatment; visit 5 (V5), assessment 7 days following treatment and study exit. At the screening visit, participants met the study physician who took the medical history and performed a physical examination. ECG and vital signs were recorded; urine toxicology and an alcohol breath test were taken. Safety blood samples were drawn and a pregnancy test taken. Prior and concomitant medications were documented. The CONSORT diagram depicting the flow of patients is shown in [supplemental Material](#).

### Outcome Measures

The primary outcome measure was depression severity measured by the MADRS. As there were no results whatsoever regarding effects of NPY administration in MDD patients before the study reported herein, the exact time trajectory of possible changes could not be predicted. However, based on known NPY biology and the preclinical results, we hypothesized that the antidepressant effects will occur between 5 and 48 hours post



**Figure 1.** Montgomery Åsberg Depression Rating Scale (MADRS) score change over time following a single insufflation of 6.8 mg neuropeptide Y (NPY) or placebo in major depressive disorder (MDD) patients. Data shown as mean (error bars 95% CI) MADRS score change from baseline in the NPY group (n=12) and placebo group (n=18). NPY was superior to placebo in reducing MADRS score at 24 hours posttreatment; decrease of 10.34 points (95% CI: -13.5; -6.8) vs decrease of 5.55 points (95% CI: -8.4; -2.7), respectively (group\*time interaction  $F=3.26$   $DF=(1,28)$ ,  $P=.04$ ; Cohen's  $d=0.67$ ), and at +5 hours (decrease of 7.1 points (95% CI: -10.0; -4.2) vs decrease of 3.5 points (95% CI: -5.8; -1.2); (group\*time interaction  $F=2.69$ ,  $DF=(1,28)$ ,  $P=.05$ ; Cohen's  $d=0.61$ ).

NPY insufflation and chose the time point for primary outcome at +48 hours and the secondary outcomes at +1 hour, +5 hours, and +24 hours post-dosing. Additional secondary outcomes included Quick Inventory of Depressive Symptomatology-Self Rated (QIDS-SR), Clinical Global Impression-Severity scale (CGI-I), and Profile of Mood States (POMS) scores at +1 hour, +5 hours, +24 hours, and +48 hours (supplemental Tables 1–3). Efficacy assessments were also completed at 7 days post-dosing, although no observable treatment effect was anticipated at that time point. Sleepiness was assessed throughout the trial with the Epworth Sleepiness scale based on a theoretical risk of sedation or tiredness associated with NPY effects.

### Safety and Tolerability

All participants completed the study. There were no side effects, and no signs or symptoms due to NPY/placebo insufflation emerged except for the changes as assessed by MADRS. The procedure was well tolerated and no adverse events occurred.

### Study Drug and Randomization

NPY was purchased from Bachem AG (Bubendorf, Switzerland) as dry substance in sealed sterile glass vials. On the day of the experiment, the designated pharmacist who was not otherwise involved in any of the study procedures (APL, Pharmacy, Steriltillverkningen Karolinska Solna) prepared the NPY or placebo in sterile water and delivered the unmarked syringes to the person responsible for experimental solution administration with the ViaNase Electronic Atomizer (Kurve Technology Inc, Bothel, WA). This device has been tested for nose-to-brain delivery of peptides for other CNS conditions, including a study of insulin and NPY (Craft et al., 2012; Sayed et al., 2018). NPY dissolves completely in the solution and there is no odor or taste; therefore, the NPY and placebo conditions appeared identical to study staff and patients. The list of random treatment assignments was set up according to the random permuted blocks method with blocks of 10 patients. NPY and placebo were prepared by an independent person not otherwise involved in any of the study procedures. A randomization list was only available to the independent person preparing the study drug. All other study personnel were blind to treatment assignment. Code envelopes were available at the study site in case the treatment blind needed to be broken for emergency reasons.

### Statistical Analyses

**Determination of sample size**—Assuming a 20% MADRS score mean reduction in the placebo-treated group and a 50% mean reduction in the NPY group, a sample size of 30 patients in each group will have 80% power to detect this difference with a 0.05 1-sided significance level assuming that the SD is 45%. In view of our hypothesis, based on a wealth of preclinical and clinical results consistently showing that brain NPY expression is reduced in depression and PTSD and that all efficient treatments enhance NPY commensurate with altered behavior, and considering that this was an early-phase study with the aim of reducing the risk of the type-2 error, we found it justified to employ 1-tailed test.

**Analytic approach**—Two-way ANOVA for repeated measures, Mann-Whitney U test, and Wilcoxon Matched Pairs test were used. Homogeneity of variances was examined using box-

plots and Levene's test. Non-parametric statistics were used to compare CGI-I scores.  $P < .05$  was considered significant. Since this was designed as an early-phase proof of concept study, there was no adjustment planned for multiplicity. Effect size for treatment effects was calculated according to Cohen's  $d$  (Cohen 1988).

## Results

### Demographic and Clinical Characteristics

Thirty participants were enrolled and all completed the trial; 12 were treated with NPY and 18 were treated with placebo under randomized, double blind conditions. Demographic and clinical characteristics of the sample are shown in Table 1. Individuals were enrolled between November 2015 and October 2017. Enrollment ended early, at  $n=30$ , due to unanticipated limitations in drug supply. Participants were on the following concomitant antidepressant medication, listed in alphabetical order: agomelatine, bupropion, citalopram, duloxetine, escitalopram, fluoxetine, mirtazapine, paroxetine, sertraline, venlafaxine, vortioxetine. In view of the small number of participants and a large variety of antidepressants, the influence of specific concomitant medications on treatment outcome was not estimated.

### Efficacy

**Primary Outcome: MADRS**—Changes in MADRS score by group within the 48 hours post NPY insufflation are presented in Figure 1 and Tables 2 and 3. NPY was superior to placebo in reducing MADRS score at 24 hours posttreatment; decrease of 10.3 points [95% CI: -13.5; -6.8] vs decrease of 5.6 points [95% CI: -8.4; -2.7], respectively (group\*time interaction  $F=3.26$   $DF=(1,28)$ ,  $P=.04$ ; Cohen's  $d=0.67$ ). There was a similar trend toward benefit of NPY over placebo at +1 hour (decrease of 5.7 points [95% CI: -8.5; -2.8] vs decrease of 3.8 points [95% CI: -6.2; -1.5]; group\*time interaction  $F=0.71$   $DF=(1,28)$ ,  $P=.20$ ) and at +5 hours (decrease of 7.1 points [95% CI: -10.0; -4.2] vs decrease of 3.5 points [95% CI: -5.8; -1.2]; group\*time interaction  $F=2.69$ ,  $DF=(1,28)$ ,  $P=.05$ ; Cohen's  $d=0.61$ ). At +48 hours there was no longer a separation between NPY and placebo (decrease of 8.3 [95% CI: -12.5; -4.3] vs 8.5 points [95% CI: -11.8; -5.2]; group\*time interaction  $F=0.0001$ ,  $DF=(1,28)$ ,  $P=.49$ ).

**Secondary Outcomes: QIDS-SR, CGI, POMS**—Changes in QIDS-SR, CGI, and POMS are considered exploratory only and are shown in the supplemental Material. There was a trend favoring NPY compared with placebo as measured by QIDS-SR, CGI, and

**Table 1.** Demographics and Clinical Characteristics of Study Sample

	NPY (n=12)	Placebo (n=18)
Age, mean (SD)	57.0 (14.0)	52.8 (12.5)
Female, n	6	14
MADRS	30.2 (3.1)	28.1 (4.9)
QIDS-SR	18.4 (2.4)	16.7 (3.3)
CGI-S	4.7 (0.5)	4.3 (0.7)
POMS	97.1 (47.1)	94.9 (37.5)

Abbreviations: CGI-I, Clinical Global Impression-Severity; MADRS, Montgomery Åsberg Depression Rating Scale; NPY, neuropeptide Y; POMS, profile of mood states; QIDS-SR, Quick Inventory of Depressive Symptomatology-Self Report. Data presented as mean  $\pm$  SD at baseline.



**Table 2.** MADRS Scores Over Time Following a Single NPY Intranasal Dose of 6.8 mg or Placebo in MDD Patients

MADRS	NPY (n=12)		Placebo (n=18)	
	Mean	SD	Mean	SD
Baseline	30.1	3.1	28.1	4.9
+1 h	24.5	7.6	24.3	8
+5 h	23.1	7.5	24.6	7.9
+24 h	19.8	7.2	22.6	7.8
+48 h	21.8	7.4	19.6	10.2

Abbreviations: MADRS, Montgomery Åsberg Depression Rating Scale; NPY, neuropeptide Y.

POMS. There were no differences between the NPY and placebo groups on the Epworth Sleepiness scale (data not shown).

### Discussion

This double blind, placebo controlled trial of a single dose of intranasal NPY is the first report, to our knowledge, showing that NPY decreases symptoms of MDD. Since no data regarding NPY effects in MDD patients existed prior to our trial, the time trajectory of possible changes could not be predicted with any degree of precision. However, based on preclinical results, we estimated the antidepressant effects to occur between 5 and 48 hours post NPY insufflation. Consequently, the time point for primary outcome was chosen at +48 hours and secondary outcomes at +1 hour, +5 hours, and +24 hours. While the primary outcome was not achieved at the a priori hypothesized +48 hours post insufflation, the pivotal finding confirmed our hypothesis that insufflated NPY has an antidepressant effect. Notably, the trajectory of MADRS score differences (5.7 vs 3.8 [at ±1 hour] // 7.1 vs 3.5 [at ± 5 hours] // 10.3 vs 5.5 [at ± 24 hours] // 8.4 vs 8.5 [at ± 48 hours]) showed that NPY is antidepressant and that the onset of the antidepressant effect is faster than originally surmised. In view of these results and the dose-ranging intranasal NPY insufflation trial in participants diagnosed with PTSD (Sayed et al., 2018), we are preparing an investigation of NPY dose-response as well as repeated intranasal administration in MDD patients.

Intranasal administration is a feasible technique for direct delivery of protein therapeutics to the CNS, effectively bypassing the blood-brain barrier and reaching the brain by 2 possible routes: (1) absorption of the drug across the nasal epithelium to the submucosa, whereupon the drug can either directly access the CSF or undergo extracellular transport within perineuronal channels into the CNS; and (2) internalization of drug into primary neurons of the olfactory epithelium followed by intracellular transport into the olfactory bulb and subsequent distribution into other brain regions. Using 125-I-labeled compounds, intranasal uptake into brain was shown to be 2–3 orders of magnitude larger than i.v. administration (Thorne and Frey II, 2001). Thus entry into brain of, for example, angiotensin, arginine-vasopressin, IGF-1, insulin, leptin, melatonin, NPY, oxytocin, rhNGF, and vasointestinal polypeptide has been demonstrated (for reviews, see Alam et al., 2010; Dhuria et al., 2010; Illum, 2012; Lochhead and Thorne, 2012; Chapman et al., 2013). Furthermore, Born and coworkers (Born et al., 2002) demonstrated that following intranasal delivery of melanocortin, vasopressin, and insulin, concentrations of each peptide in CSF started to increase 10 minutes after administration, reaching peak values after 30 minutes for melanocortin and insulin and

**Table 3.** MADRS Score Changes Over Time Following a Single Insufflation of 6.8 mg NPY or Placebo in MDD Patients

Baseline change to	Mean NPY	Mean placebo	n NPY	n Placebo	Mean difference NPY-placebo	SD		Cohen's d	Pooled SD	95% LL d	95% UL d
						Difference NPY	Difference placebo				
MADRS +1 h	5.667	3.833	12	18	1.833	5.516	6.042	0.314	5.841	-0.421	1.049
MADRS +5 h	7.083	3.500	12	18	3.583	6.052	5.732	0.612	5.860	-0.135	1.358
MADRS +24 h	10.333	5.556	12	18	4.778	6.527	7.438	0.673	7.094	-0.077	1.424
MADRS +48 h	8.417	8.500	12	18	-0.083	7.025	9.044	-0.010	8.310	-0.740	0.720

80 minutes for vasopressin. Of note, NPY showed significant changes in cortical direct current potentials but no changes in electro-oculogram or electro-myogram (Born et al., 2002; Hallschmid 2003).

A major issue was to determine the appropriate initial dose for this first proof-of-concept trial of intranasal NPY in MDD. A wealth of publications regarding the dose conversion between animal species has used body weight and surface area as determining indices, and some have also considered pharmacokinetics of a given drug (USFDA, 2005; Blanchard et al., 2015; Nair and Jacob, 2016). Experiments on rodents and trials in humans using i.v. or intranasal NPY administration as well as direct injection into CNS have not found untoward effects and were thus useful with regard to the issue of safety and tolerability. That information was of limited use in the context of our trial as it did not address the question of the initial dose of NPY that would mitigate MDD and PTSD symptoms. However, findings that central NPY administration (cf Introduction) had antidepressant and PTSD-alleviating effects in rodents, subsequently confirmed by Serova and Sabban and coworkers (Serova et al., 2013, 2014; Sabban et al., 2015), allowed for a reasonable extrapolation that the starting NPY dosages could be between 3 and 15 mg. Using a translational dose calculation from rodents to humans, based on the work of Reagan-Shaw and collaborators (Reagan-Shaw et al., 2008), the NPY dose was 7.3 mg and the LMV approved a single fixed dose of 6.8 mg of NPY for this first proof-of-concept trial.

The meta-analysis by Tural and Iosifescu (2020) of papers reporting NPY levels (measured using different assay procedures starting in 1987 and extending over several decades) in postmortem brain tissue, as well as plasma and CSF from cohorts of male and female participants diagnosed with PTSD, depression, and exposed to chronic stress, has added important knowledge regarding vicissitudes of NPY in plasma and CSF. Since to our knowledge no paper dealt with the question of possible modification of effects of administered NPY by endogenous NPY, this aspect will be investigated in future trials. The limitation of this study is that we enrolled 30 patients, which was only a subset of the original target sample size of 60. This was due to unanticipated limitations in the availability of NPY approved for human use by the European Medical Agency. In addition, there were more patients randomized to placebo ( $n=18$ ) than NPY ( $n=12$ ) because the randomization scheme was designed in blocks of 10 and the study ended earlier than expected. Based on the early-phase proof-of-concept nature of the study, the statistical plan did not call for adjustment for multiplicity in order to guard against type II error. Given that the study sample did not reach its planned enrollment target, the findings are in need of replication.

In summary, this first controlled study, to our knowledge, of intranasal NPY administration to patients with MDD demonstrated the tolerability and safety of the procedure and indicated that NPY, consistent with the evidence from preclinical experiments, may have rapid antidepressant effects. The early-phase nature of the current study precludes definitive conclusions with regard to generalization of findings to large populations of individuals diagnosed with MDD. Currently, we are planning trials to determine the dose response for NPY as well as effects of repeated NPY administrations necessary to establish intranasal NPY as a novel target to treat MDD.

## Supplementary Materials

Supplementary data are available at *International Journal of Neuropsychopharmacology (IJNPPY)* online.

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## Statement of Interest

In the past 5 years, Dr Murrough has provided consultation services to Otsuka, Clexio Biosciences, FSV7, Boehringer Ingelheim, Sage Therapeutics, Novartis, Allergan, Fortress Biotech, Janssen Research and Development, Genentech, Medavante-Prophase, and Global Medical Education (GME) and has received research support from Avanir Pharmaceuticals, Inc. Drs Murrough and Charney are named on a patent pending for neuropeptide Y as a treatment for mood and anxiety disorders (U.S. Serial No. 14/889 746 and related foreign patent applications). In addition, Dr Charney discloses patents as follows: patent US 9592 207—Intranasal Administration of Ketamine to Treat Depression (issued March 14, 2017) licensed to Janssen Pharmaceutical, Inc., a patent US 9539 220—Methods for Treating Suicidal Ideation (issued January 10, 2017) licensed to Janssen Pharmaceutical, Inc., a patent US 8785 500—Intranasal Administration of Ketamine to Treat Depression (issued July 22, 2014) licensed to Janssen Pharmaceuticals, Inc., a patent WO 2016/049234—Systems and Methods for Treating a Psychiatric Disorder licensed to Click Therapeutics, a patent U.S. Serial No. 14/783 686 and related foreign patent applications—Ketamine—As a Rapid Treatment for Post-Traumatic Stress Disorder (PTSD) pending, a patent WO 2016/172672—Method of Reducing Risk of Suicidal Ideations with Combined Ketamine/Lithium Therapy pending, and a patent U.S. Serial No. 15/379 013 and U.S. Serial No. 15/417 689—Intranasal Administration of Ketamine to Treat Depression (Continuation patent applications in the same patent family as issued U.S. Patents 8785 500 and U.S. 9539 220 and U.S. 9592 207 pending). All other authors declare no conflicts.

## References

- Alam MI, Beg S, Samad A, Baboota S, Kohli K, Ali J, Ahuja A, Akbar M (2010) Strategy for effective brain drug delivery. *Eur J Pharm Sci* 40:385–403.
- Bjørnebekk A, Mathé AA, Brené S (2006) Running has differential effects on NPY, opiates, and cell proliferation in an animal model of depression and controls. *Neuropsychopharmacology* 31:256–264.
- Blanchard OL, Smoliga JM (2015) Translating dosages from animal models to human clinical trials--revisiting body surface area scaling. *FASEB J* 29:1629–1634.
- Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL (2002) Sniffing neuropeptides: a transnasal approach to the human brain. *Nat. Neurosci* 5:514–516.

- Caberlotto L, Hurd YL (2001) Neuropeptide Y Y(1) and Y(2) receptor mRNA expression in the prefrontal cortex of psychiatric subjects. Relationship of Y(2) subtype to suicidal behavior. *Neuropsychopharmacology* 25:91–97.
- Catalá-López F, Gênova-Maleras R, Vieta E, Tabarés-Seisdedos R (2013) The increasing burden of mental and neurological disorders. *Eur Neuropsychopharmacol* 23:1337–1339.
- Chapman CD, Frey WH 2nd, Craft S, Danielyan L, Hallschmid M, Schiöth HB, Benedict C (2013) Intranasal treatment of central nervous system dysfunction in humans. *Pharm Res* 30:2475–2484.
- Cohen H, Liu T, Kozlovsky N, Kaplan Z, Zohar J, Mathé AA (2012) The neuropeptide Y (NPY)-ergic system is associated with behavioral resilience to stress exposure in an animal model of post-traumatic stress disorder. *Neuropsychopharmacology* 37:350–363.
- Cohen H, Vainer E, Zeev K, Zohar J, Mathé AA (2018) Neuropeptide S in the basolateral amygdala mediates an adaptive behavioral stress response in a rat model of posttraumatic stress disorder by increasing the expression of BDNF and the neuropeptide YY1 receptor. *Eur Neuropsychopharmacol* 28:159–170.
- Cohen J (1988) *Statistical power analysis for the behavioral sciences*. 2nd Edition. Hillsdale, MI: Lawrence Erlbaum.
- Cohen S, Vainer E, Matar MA, Kozlovsky N, Kaplan Z, Zohar J, Mathé AA, Cohen H (2015) Diurnal fluctuations in HPA and neuropeptide Y-ergic systems underlie differences in vulnerability to traumatic stress responses at different zeitgeber times. *Neuropsychopharmacol* 40:774–790.
- Copolovici DM, Langel K, Eriste E, Langel Ü (2014) Cell-penetrating peptides: design, synthesis, and applications. *ACS Nano* 8:1972–1994.
- Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, Arbuckle M, Callaghan M, Tsai E, Plymate SR, Green PS, Leverenz J, Cross D, Gerton B (2012) Intranasal insulin therapy for Alzheimer disease and amnesic mild cognitive impairment: a pilot clinical trial. *Arch Neurol* 69:29–38.
- Dhuria SV, Hanson LR, Frey WH 2nd (2010) Intranasal delivery to the central nervous system: mechanisms and experimental considerations. *J Pharm Sci* 99:1654–1673.
- ECNP/European Brain Council (2011) ECNP/European Brain Council report 2011. *Eur Neuropsychopharmacol* 21:715–779.
- Hallschmid M, Gais S, Meinert S, Born J (2003) NPY attenuates positive cortical DC-potential shift upon food intake in man. *Psychoneuroendocrinology* 28:529–539.
- Haroon E, Miller AH, Sanacora G (2017) Inflammation, glutamate, and glia: a trio of trouble in mood disorders. *Neuropsychopharmacology* 42:193–215.
- Hascup KN, Hascup ER, Gerhardt GA. (2009) Neuropeptide Y modulation of the glutamate system in the Flinders Sensitive Line rat: a rat model of depression. *Eur Neuropsychopharmacol* 21:S41–S42.
- Heilig M (2004) The NPY system in stress, anxiety and depression. *Neuropeptides* 38:213–224.
- Heilig M, Söderpalm B, Engel JA, Widerlöv E (1989) Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology (Berl)* 98:524–529.
- Heilig M, Zachrisson O, Thorsell A, Ehnvall A, Mottagui-Tabar S, Sjögren M, Asberg M, Ekman R, Wahlestedt C, Agren H (2004) Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. *J Psychiatr Res* 38:113–121.
- Hoffmann O, Johnson L, Mebius C, Stenfors C, Vinnars E, Mathé AA (1991) Isoflurane and electroconvulsive treatment in depression: a pilot study. In: *Pharmacotherapies, psychotherapies, other therapies* (Stefanis CN, Soldatos GR, Rabavilas AD, eds), pp480–486. Toronto: Excerpta Medica.
- Husum H, Mathé AA (2002) Early life stress changes concentrations of neuropeptide Y and corticotropin-releasing hormone in adult rat brain. Lithium treatment modifies these changes. *Neuropsychopharmacology* 27:756–764.
- Husum H, Mikkelsen JD, Hogg S, Mathé AA, Mørk A (2000) Involvement of hippocampal neuropeptide Y in mediating the chronic actions of lithium, electroconvulsive stimulation and citalopram. *Neuropharmacology* 39:1463–1473.
- Illum L (2012) Nasal drug delivery - recent developments and future prospects. *J Control Release* 161:254–263.
- Jiménez Vasquez PA, Salmi P, Ahlenius S, Mathé AA (2000a) Neuropeptide Y in brains of the Flinders Sensitive Line rat, a model of depression. Effects of electroconvulsive stimuli and d-amphetamine on peptide concentrations and locomotion. *Behav Brain Res* 111:115–123.
- Jiménez-Vasquez PA, Overstreet DH, Mathé AA (2000b) Neuropeptide Y in male and female brains of Flinders Sensitive Line, a rat model of depression. Effects of electroconvulsive stimuli. *J Psychiatr Res* 34:405–412.
- Jiménez-Vasquez PA, Mathé AA, Thomas JD, Riley EP, Ehlers CL (2001) Early maternal separation alters neuropeptide Y concentrations in selected brain regions in adult rats. *Brain Res Dev Brain Res* 131:149–152.
- Jiménez-Vasquez PA, Diaz-Cabiale Z, Caberlotto L, Bellido I, Overstreet D, Fuxe K, Mathé AA (2007) Electroconvulsive stimuli selectively affect behavior and neuropeptide Y (NPY) and NPY Y(1) receptor gene expressions in hippocampus and hypothalamus of Flinders Sensitive Line rat model of depression. *Eur Neuropsychopharmacol* 17:298–308.
- Kastin AJ, Akerstrom V (1999) Nonsaturable entry of neuropeptide Y into brain. *Am J Physiol* 276:E479–E482.
- Kautz M, Charney DS, Murrough JW (2017) Neuropeptide Y, resilience, and PTSD therapeutics. *Neurosci Lett* 649:164–169.
- Langer G, Neumark J, Koinig G, Graf M, Schönbeck G (1985) Rapid psychotherapeutic effects of anesthesia with isoflurane (ES narcotherapy) in treatment-refractory depressed patients. *Neuropsychobiology* 14:118–120.
- Lochhead JJ, Thorne RG (2012) Intranasal delivery of biologics to the central nervous system. *Adv Drug Deliv Rev* 64:614–628.
- Mathé AA (1976) Studies on actions of prostaglandins in the lung. *Acta Physiol Scand Suppl* 441:1–55.
- Mathé AA, Hedqvist P, Strandberg K, Leslie CA (1977a) Aspects of prostaglandin function in the lung (first of two parts). *N Engl J Med* 296:850–855.
- Mathé AA, Hedqvist P, Strandberg K, Leslie CA (1977b) Aspects of prostaglandin function in the lung (second of two parts). *N Engl J Med* 296:910–914.
- Mathé AA, Rudorfer MV, Stenfors C, Manji HK, Potter WZ, Theodorsson E (1995/1996) Effects of electroconvulsive treatment on somatostatin, neuropeptide Y, endothelin, and neurokinin A concentrations in cerebrospinal fluid of depressed patients: a pilot study. *Depression* 3:250–256.
- Mathé AA, Jimenez PA, Theodorsson E, Stenfors C (1998) Neuropeptide Y, neurokinin A and neurotensin in brain regions of Fawn Hooded “depressed”, Wistar, and Sprague Dawley rats. Effects of electroconvulsive stimuli. *Prog Neuropsychopharmacol Biol Psychiatry* 22:529–546.
- Mathé AA, Nikosjokov A, Sousa VC, Weide-Fischer C, Lennartsson A, Wegener G, Svenningsson P (2014) Ketamine is antidepressant and enhances neuropeptide Y expression in the prefrontal cortex and hippocampus of a sero-

- tonin transporter knock out rat model, American College of Neuropsychopharmacology (ACNP) Annual Meeting December 6-10; poster.
- McEwen BS, Bowles NP, Gray JD, Hill MN, Hunter RG, Karatsoreos IN, Nasca C (2015) Mechanisms of stress in the brain. *Nat Neurosci* 18:1353–1363.
- Nair AB, Jacob S (2016) A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm* 7:27–31.
- Nikisch G, Agren H, Eap CB, Czernik A, Baumann P, Mathé AA (2005) Neuropeptide Y and corticotropin-releasing hormone in CSF mark response to antidepressive treatment with citalopram. *Int J Neuropsychopharmacol* 8:403–410.
- Nikisch G, Baumann P, Liu T, Mathé AA (2012) Quetiapine affects neuropeptide Y and corticotropin-releasing hormone in cerebrospinal fluid from schizophrenia patients: relationship to depression and anxiety symptoms and to treatment response. *Int J Neuropsychopharmacol* 15:1051–1061.
- Nikisch G, Mathé AA (2008) CSF monoamine metabolites and neuropeptides in depressed patients before and after electroconvulsive therapy. *Eur. Psychiatry* 23:356–359.
- Ramsey JD, Flynn NH (2015) Cell-penetrating peptides transport therapeutics into cells. *Pharmacol Ther* 154:78–86.
- Rasmuson AM, Hauger RL, Morgan CA, Bremner JD, Charney DS, Southwick SM (2000) Low baseline and yohimbine-stimulated plasma neuropeptide Y (NPY) levels in combat-related PTSD. *Biol. Psychiatry* 47:526–539.
- Reagan-Shaw S, Nihal M, Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* 22:659–661.
- Redrobe JP, Dumont Y, Fournier A, Quirion R (2002) The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. *Neuropsychopharmacology* 26:615–624.
- Sabban EL, Serova LI, Alaluf LG, Laukova M, Peddu C (2015) Comparative effects of intranasal neuropeptide Y and HS014 in preventing anxiety and depressive-like behavior elicited by single prolonged stress. *Behav Brain Res* 295:9–16.
- Sah R, Geraciotti TD (2013) Neuropeptide Y and posttraumatic stress disorder. *Mol. Psychiatry* 18:646–655.
- Sandberg JV, Jakobsson J, Pålsson E, Landén M, Mathé AA (2014) Low neuropeptide Y in cerebrospinal fluid in bipolar patients is associated with previous and prospective suicide attempts. *Eur Neuropsychopharmacol* 24:1907–1915.
- Sayed S, Van Dam NT, Horn SR, Kautz MM, Parides M, Costi S, Collins KA, Iacoviello B, Iosifescu DV, Mathé AA, Southwick SM, Feder A, Charney DS, Murrough JW (2018) A randomized dose-ranging study of Neuropeptide Y in patients with posttraumatic stress disorder. *Int J Neuropsychopharmacol* 21:3–11.
- Serova LI, Tillinger A, Alaluf LG, Laukova M, Keegan K, Sabban EL (2013) Single intranasal neuropeptide Y infusion attenuates development of PTSD-like symptoms to traumatic stress in rats. *Neuroscience* 236:298–312.
- Serova LI, Laukova M, Alaluf LG, Pucillo L, Sabban EL (2014) Intranasal neuropeptide Y reverses anxiety and depressive-like behavior impaired by single prolonged stress PTSD model. *Eur Neuropsychopharmacol* 24:142–147.
- Stenfors C, Mathé AA, Theodorsson E (1994) Repeated electroconvulsive stimuli: changes in neuropeptide Y, neurotensin and tachykinin concentrations in time. *Prog Neuropsychopharmacol Biol Psychiatry* 18:201–209.
- Svensson TH, Mathé AA (2002) Biological psychiatry. In: Monoaminergic transmitter systems (D'haenen H, den Boer JA, Willner P, eds), pp45–66. UK: Wiley.
- Thorne RG, Frey WH 2nd (2001) Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. *Clin Pharmacokinet* 40:907–946.
- Thorsell A, Mathé AA (2017) Neuropeptide Y in alcohol addiction and affective disorders. *Front Endocrinol (Lausanne)* 8:178.
- Thorsell A, Michalkiewicz M, Dumont Y, Quirion R, Caberlotto L, Rimondini R, Mathé AA, Heilig M (2000) Behavioral insensitivity to restraint stress, absent fear suppression of behavior and impaired spatial learning in transgenic rats with hippocampal neuropeptide Y overexpression. *Proc Natl Acad Sci U S A* 97:12852–12857.
- Tundo A, de Filippis R, Proietti L (2015) Pharmacologic approaches to treatment resistant depression: evidences and personal experience. *World J Psychiatry* 5:330–341.
- Tural U, Iosifescu DV (2020) Neuropeptide Y in PTSD, MDD, and chronic stress: a systematic review and meta-analysis. *J Neurosci Res* 98:950–963.
- US Food and Drug Administration (USFDA) (2005) Guidance for industry: estimating the maximum safe starting dose in adult healthy volunteer. Rockville, MD: US Food and Drug Administration.
- Weeks HR 3rd, Tadler SC, Smith KW, Iacob E, Saccoman M, White AT, Landvatter JD, Chelune GJ, Suchy Y, Clark E, Cahalan MK, Bushnell L, Sakata D, Light AR, Light KC (2013) Antidepressant and neurocognitive effects of isoflurane anesthesia versus electroconvulsive therapy in refractory depression. *PLoS One* 8:e0175668.
- World Health Organization (2017) Depression and other common mental disorders: global health estimates. Geneva, Switzerland: World Health Organization. License: CC BY-NC-SA 3.0 IGO.
- World Medical Association (2001) World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bull World Health Organ* 79:373–374.
- Wu G, Feder A, Wegener G, Bailey C, Saxena S, Charney D, Mathé AA (2011) Central functions of neuropeptide Y in mood and anxiety disorders. *Expert Opin Ther Targets* 15:1317–1331.