



ARTICLE

Intranasal naloxone rapidly occupies brain mu-opioid receptors in human subjects

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Nasal spray formulations of naloxone, a mu-opioid receptor (MOR) antagonist, are currently used for the treatment of opioid overdose. They may have additional therapeutic utility also in the absence of opioid agonist drugs, but the onset and duration of action at brain MORs have been inadequately characterized to allow such projections. This study provides initial characterization of brain MOR availability at high temporal resolution following intranasal (IN) naloxone administration to healthy volunteers in the absence of a competing opioid agonist. Fourteen participants were scanned twice using positron emission tomography (PET) and [¹¹C]carfentanil, a selective MOR agonist radioligand. Concentrations of naloxone in plasma and MOR availability (relative to placebo) were monitored from 0 to 60 min and at 300–360 min post naloxone. Naloxone plasma concentrations peaked at ~20 min post naloxone, associated with slightly delayed development of brain MOR occupancy (half of peak occupancy reached at ~10 min). Estimated peak occupancies were 67 and 85% following 2 and 4 mg IN doses, respectively. The estimated half-life of occupancy disappearance was ~100 min. The rapid onset of brain MOR occupancy by IN naloxone, evidenced by the rapid onset of its action in opioid overdose victims, was directly documented in humans for the first time. The employed high temporal-resolution PET method establishes a model that can be used to predict brain MOR occupancy from plasma naloxone concentrations. IN naloxone may have therapeutic utility in various addictions where brain opioid receptors are implicated, such as gambling disorder and alcohol use disorder.

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INTRODUCTION

Naloxone is well established as an antidote for the reversal of respiratory depression in the management of opioid overdose. Recently, formulations of naloxone have become available for intranasal (IN) administration, as the US Food and Drug Administration (FDA) approved 2 and 4 mg doses of IN naloxone for the emergency treatment of known or suspected opioid overdose [1].

Of the opioid receptor subtypes present in the central nervous system (CNS), naloxone binds with the greatest affinity to mu-opioid receptors (MOR) [2]. When administered in usual doses and in the absence of opioid agonists, it exhibits essentially no pharmacologic activity [3].

In addition to management of opioid overdose, IN naloxone may have therapeutic utility in other conditions. In particular, IN naloxone has been proposed for the treatment of pathological gambling (PG), a disorder currently recognized as a “behavioral addiction” (Diagnostic and Statistical Manual of Mental Disorders, Fifth edition). The biological, psychological, social, and cultural background factors behind PG are largely similar to those behind other addiction disorders [4]. Thus, the principles used in the treatment of alcohol dependence may also be largely applied to the treatment of PG. To date, two orally administered opioid antagonist drugs that are used in the treatment of alcohol

dependence, naltrexone and nalmefene, have been investigated in subjects with PG [5–10]. The results of placebo-controlled trials have so far failed to demonstrate statistically significant differences in outcomes between the treatment arms. These disappointing results, together with a growing body of evidence for the pivotal role of the brain’s opioidergic systems in addictions [11–13], highlight the need to investigate the possible utility of IN naloxone in the treatment of PG.

As a possible explanation for their lack of efficacy in PG, naltrexone and nalmefene have rather long durations of action; the drugs remain for a long time in the systemic circulation (nalmefene’s elimination half-life in plasma is 12.5 h, naltrexone’s half-life is 4 h; [14]), and MOR occupancy in the brain is maintained at high levels even longer than one would predict from plasma concentrations [15, 16]. Possible advantages offered by transient MOR antagonism would be the ensuing targeted reduction in MOR availability, and the possibility to maintain important physiological reward functions unimpaired when addiction cues are not present. Gambling disorder as a behavioral addiction is characterized by a very rapid onset of craving that can arise without any significant external stimulus. In this context, for a pathological gambler, a self-administered and short-acting MOR antagonist might be capable of dampening the reward and thus

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reduce the risk of gambling. For accurate cue-targeted timing of the drug therapy, IN naloxone administration might be useful both because of the rapid entry of the drug into the systemic circulation and subsequently into the CNS, and for its short elimination half-life in plasma (2 h; [17]).

Pharmacokinetic (PK) investigations of novel IN formulations of naloxone indicate that the drug is rapidly absorbed into the bloodstream from the nasal mucosa, with peak plasma concentrations in plasma reached in 20–30 min [17, 18]. Efficient MOR blockade in the brain after IN naloxone has been documented in non-human primates with positron emission tomography (PET) imaging [19], but without detailed information about the time course. Clinical evidence obtained from opioid overdose victims treated with IN naloxone suggests rapid entry of the drug into the CNS, as over 70% of successful rescues occurred in <5 min after dosing [1, 20].

Based on these findings, we hypothesized that IN naloxone readily enters the brain and promptly occupies MORs to a sufficient extent to attain targeted antagonistic effects, while its duration of action would be significantly shorter than that of the orally administered opioid antagonists nalmefene and naltrexone. To test this hypothesis, we characterized the early (0–60 min) and late (300–360 min) phases of brain MOR occupancy in healthy, young male volunteers following IN administration of two doses of naloxone (2 and 4 mg) approved for the treatment of opioid overdose.

MATERIALS AND METHODS

Subjects

Fourteen healthy, young (mean age 25 years, range 20–31 years) male subjects participated after written informed consent; eight in Part 1 of the study, assessing emergence of brain MOR occupancy (0–60 min after dosing of IN naloxone), and six in Part 2 of the study, assessing its disappearance (300–360 min after dosing). Inclusion criteria included good general health, body weight 60–90 kg, and body mass index 18–28 kg m⁻². Exclusion criteria included intake of any medication that could affect the outcome of the study, within 2 weeks prior to the tracer administration (2 months for enzyme-inducing drugs like rifampicin or carbamazepine), or <5 times the half-life of the medication, history of drug abuse or positive result in urine drug screen, high alcohol consumption, and regular smoking. Subject candidates with high scores (>2) on the Finnish version of the South Oaks Gambling Screen (SOGS-R) were excluded [21]. The study protocol was approved by the Ethics Committee of South-West Finland Hospital District and the Finnish Medicines Agency (Fimea). Relevant regulations and guidance for biomedical research involving human subjects, such as the Declaration of Helsinki and GCP were adhered to. The study was registered with the EudraCT registration number 2015-002681-23.

Study outline

The study was double blind and placebo controlled, consisting of two parts, each with different participants. Each participant received one IN dose of naloxone (2 mg, *n* = 7, or 4 mg, *n* = 7; the 4 mg doses were delivered by administering one 0.1 mL puff into each nostril; 2 mg into either the left or right nostril) and one placebo treatment in a balanced, randomized order. Doses (spray formulation of naloxone hydrochloride developed by Lightlake Therapeutics Inc., UK, 20 mg mL⁻¹, or matching placebo) were given to supine subjects with a Pfeiffer Bidose liquid device. The two sessions were held at least 5 days apart in order to eliminate possible treatment-related effects on MOR availability. In Part 1 of the study, the participants received their IN doses of placebo and naloxone (2 mg, *n* = 4, or 4 mg, *n* = 4) during the PET scan, 20 min after the [¹¹C]carfentanil tracer injection. PET scan data were collected until 60 min after the IN drug administration. In Part 2 of

the study, placebo and naloxone (2 mg, *n* = 3, or 4 mg, *n* = 3) were administered 5 h before [¹¹C]carfentanil and the start of PET scanning.

Structural magnetic resonance imaging (MRI) of the brain was conducted to exclude subjects with anatomical brain abnormalities and to collect anatomical reference data for PET data analysis. T1-weighted MRI scans were acquired using a 3 T scanner (Philips Ingenuity TF PET/MR, Philips Medical Systems, Cleveland, OH, USA).

PK assessments

Venous blood samples were collected for determination of naloxone concentrations in plasma. Samples were collected prior to dosing and at 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 80, and 100 min, as well as at 2, 3, 4, and 6 h after IN dosing (see Supplementary Methods for sample preparation and analysis). The lower limit of quantitation was 0.10 ng mL⁻¹.

Non-compartmental analysis with WinNonlin[®] Phoenix 6.4 software (Pharsight Corporation, Mountain View, CA, USA) was used to determine the PK parameters peak concentration (*C*_{max}), time to peak (*T*_{max}), area under the concentration by time curve (AUC) to 6 h (AUC_{0–6}), AUC to the last measurable concentration (AUC_{0–t}), elimination half-life (*t*_{1/2}), and AUC extrapolated to infinity (AUC_{0–∞}). Terminal time points were selected based on best-fit criteria and review of the selected points. The linear trapezoidal rule was used to estimate AUC.

PET assessments

The PET tracer, [¹¹C]carfentanil, was produced [22] at the radiochemistry laboratory of Turku PET Center on the day of PET imaging. The target radioactivity of each administered bolus injection was 500 MBq, and the carfentanil mass was not allowed to exceed 2.0 µg per injected dose. PET data were acquired with a High-Resolution Research Tomograph (HRRT, Siemens Medical Solutions, Knoxville, TN, USA), and image reconstruction and preprocessing of the data were conducted as reported earlier [23] (see Supplementary Information for details).

Automated regions-of-interest (ROIs) were generated with the help of individual T1 MRI anatomical data and FreeSurfer software (version 5.3; <http://surfer.nmr.mgh.harvard.edu/>; [24]), and using Statistical Parametric Mapping (SPM8; Wellcome Institute, London, UK; [25]) to generate atlas-based subcortical ROIs, resulting in 60 ROIs [26]. An aggregate average ROI was generated over the regions listed in Table 1 to best reflect the global effect of naloxone in the brain. All analyzed ROIs are additionally reported as Supplementary Information. The lateral occipital cortex served as a reference region, being practically devoid of MORs [27].

ROI-based binding potential as estimated using reference tissue models (BP_{ND}; [28]) was considered as the primary outcome parameter in PET modeling. Multilinear reference tissue modeling (MRTM) with fixed *k*₂' [29, 30] was conducted for BP_{ND} estimation in placebo scans and late (Part 2) naloxone scans, while an additional time-dependent term [31] was required for successful modeling of early (Part 1) naloxone scans, as indicated by model selection criteria (Supplementary Information). Model fits derived with the most compatible model were generally of high quality (cf. Figure 1c), whereas visually apparent deviations were observed when poorly complying models were applied for the early naloxone scans (data not shown). Subsequently, dynamic BP_{ND}s were calculated in early naloxone scans as described before [32], yielding dynamic receptor occupancies (RO(*t*)) as defined in Eq. (1),

$$RO(t) = \left(1 - \frac{BP_{ND}^{Naloxone}(t)}{BP_{ND}^{Placebo}} \right) \times 100\%, \quad (1)$$

where *t* is the time of BP_{ND} estimation under the naloxone treatment. Dynamic receptor occupancies were analyzed to

Table 1. Fifteen most receptor-dense brain regions in a ROI-based analysis using MRTM (with the occipital cortex as reference) and all 14 PET scans with [¹¹C]carfentanil and IN placebo administration

ROI	BP _{ND} , mean (SD)
Prefrontal thalamus	2.79 (0.34)
Limbic striatum	2.42 (0.34)
Temporal thalamus	1.85 (0.22)
Amygdala	1.75 (0.21)
Temporal striatum	1.61 (0.30)
Executive striatum	1.49 (0.28)
Posterior parietal thalamus	1.23 (0.21)
Dorsal raphe	1.16 (0.15)
Caudal anterior cingulate	1.15 (0.20)
Rostral motor striatum	1.14 (0.24)
Insula	1.12 (0.15)
Sensory thalamus	1.10 (0.18)
Caudal motor striatum	1.06 (0.24)
Rostral anterior cingulate	1.06 (0.17)
Primary motor thalamus	1.05 (0.16)

BP_{ND} is the binding potential of [¹¹C]carfentanil relative to the uptake of the tracer in the non-displaceable compartment
IN intranasal, ROI region-of-interest, MRTM multilinear reference tissue model, PET positron emission tomography

determine peak receptor occupancy (RO_{max}), and time to reach half of RO_{max} (t_{RO/2}). Late naloxone scans were analyzed for static receptor occupancy using Eq. (1) without time dependence.

Relationships between PK and PD data

The relationship of the time courses of naloxone concentrations in plasma and brain MOR occupancy was investigated by plotting (GraphPad Software Inc., La Jolla, CA, USA) the log-linear receptor occupancy estimates and drug concentrations in plasma according to each in-scan PK sampling time point (3, 6, 9, 12, 15, 20, 25, 30, 40, 50, and 60 min). Departure of the plots of the early time points from the log-linear regression line of the last (60 min post naloxone) measurement was interpreted as hysteresis in brain MOR occupancy, while the earliest measurement time point exhibiting similar log-linear relationship with the 60 min data was deemed as the start time of equilibrium between plasma naloxone concentrations and brain MOR occupancy.

The drug concentration associated with half-maximal effect (EC₅₀) was estimated using GraphPad by fitting the plasma naloxone concentrations (C) at all in-scan measurement time points after equilibrium (see above) to the estimated occupancy (RO):

$$RO = RO' \left(\frac{C}{C + EC_{50}} \right),$$

where RO' represents the model-derived maximal occupancy. Theoretically, RO' should be fixed at 100%, but to account for some uncertainty in the RO estimates, a non-fixed RO' parameter was employed.

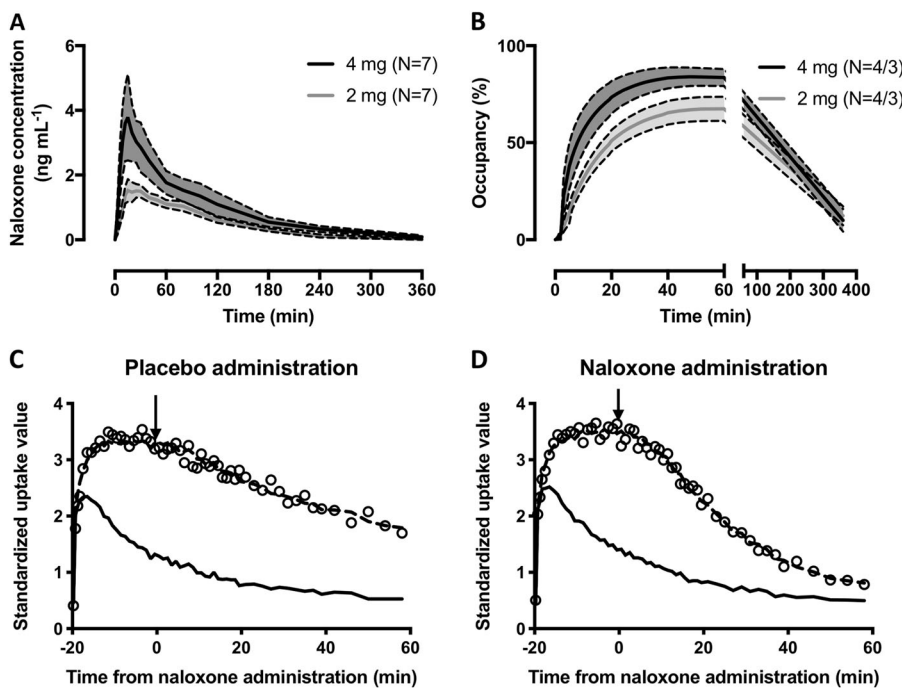


Fig. 1 **a** Plasma naloxone concentrations (mean ± SEM) after intranasal (IN) naloxone administration in healthy male volunteers (n = 7 for 4 mg and n = 7 for 2 mg doses). **b** Mu-opioid receptor (MOR) occupancies (%) (mean ± SEM) after IN naloxone administration in healthy male volunteers (n = 4 (3) for 4 mg and n = 4 (3) for 2 mg doses in 0–60 min (300–360 min) time intervals) as estimated from [¹¹C]carfentanil-derived BP_{ND} data under the placebo and naloxone conditions (see Methods), using linearized-parametric neurotransmitter positron emission tomography (PET) (lp-ntPET) analysis (see Methods). **c**, **d** [¹¹C]carfentanil PET-derived standardized time-activity course (TAC) data during the placebo and naloxone conditions in one individual subject (101) after 4 mg dosing of naloxone. Solid lines depict the radioactivity observations from the reference region (occipital cortex), and circles the ones from a relevant brain ROI with high MOR availability (the limbic striatum, average of both sides), and dashed lines depict the best lp-ntPET model fits to the data. Application of the nasal spray formulation occurred 20 min after [¹¹C]carfentanil administration and initiation of PET data acquisition (vertical arrow). Appearance of MOR occupancy by naloxone is observable as a perturbation of the [¹¹C]carfentanil signal pattern in the naloxone scan as compared to the expected pattern observed during the placebo scan

Table 2. Summary of observed and calculated PK and PET variables after intranasal naloxone administration by dose level ($n = 7$ per dose level)

	PK variables					PET variables					
	C_{max}	$t_{1/2}$	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	BP_{ND}^{PLC}	BP_{ND}^{NLX}	$\%RO_{max}$	$t_{RO/2}$		
102	1.81	49	30	204	215	1.08	0.20	82	12		
103	1.14	51	25	113	130	1.38	0.53	61	14		
106	1.94	58	25	235	255	1.57	0.42	73	11		
107	1.28	59	80	135	149	1.24	0.57	54	13		
109	3.24	92	15	233	246	1.62	1.26	22			
110	1.49	57	20	117	132	1.55	1.44	7			
111	1.91	41	20	126	133	1.23	1.12	8			
Mean (SD)	1.83 (0.70)	58 (16)	25 (16)	166 (55)	180 (57)	1.38 (0.21)	0.43 (0.17)	1.27 (0.16)	67 (12)	13 (9)	12 (11)
101	3.09	54	25	260	274	1.25	0.19	85	10		
104	5.22	50	15	284	295	1.44	0.18	87	6		
105	11.13	82	15	683	706	1.48	0.06	96	5		
108	3.25	86	100	515	552	1.44	0.42	71	13		
112	3.38	65	12	229	245	1.33	1.20	10			
113	1.02	79	15	77	88	1.51	1.52	0			
114	3.23	116	15	257	285	1.76	1.42	20			
Mean (SD)	4.33 (3.24)	76 (22)	15 (12)	329 (202)	349 (208)	1.46 (0.16)	0.21 (0.15)	1.38 (0.16)	85 (10)	10 (10)	8 (5)

C_{max} (ng mL⁻¹), maximal observed concentration of naloxone in plasma; $t_{1/2}$ (min) terminal half-life; T_{max} (min) time to reach C_{max} ; AUC_{0-t} (min ng mL⁻¹) area under the naloxone plasma concentration curve from time zero to last measurable concentration at time t ; $AUC_{0-\infty}$ (min ng mL⁻¹) area under the naloxone plasma concentration curve from time zero to infinity; BP_{ND} binding potential calculated using an estimate of non-displaceable binding as reference; $\%RO_{max}$ maximum MOR occupancy by naloxone in the given time interval relative to placebo; $t_{RO/2}$ (min) time to reach half of maximal occupancy; PLC = placebo; NLX = naloxone

Data from subjects 101–108 (Part 1) depict early (0–60 min post naloxone) brain binding characteristics and data from subjects 109–114 (Part 2) depict the late (300–360 min post naloxone) binding characteristics

PET positron emission tomography, PK pharmacokinetic, MOR mu-opioid receptor

Statistical analysis

The small number of observations per naloxone dose level and time point precluded formal statistical testing of the primary PK and PET (PD) variables. The results of PK and PET data analysis are presented in terms of individual values and descriptive statistics including mean and standard deviation (SD) or median and range calculated with Microsoft Excel (Microsoft Office Professional Plus 2010, Version 14.0).

RESULTS

Naloxone is rapidly absorbed into the bloodstream after IN spray dosing

Naloxone was readily absorbed after IN administration. Its peak concentration in plasma was reached in 15 to 80 min (median T_{max} 25 min) after the 2 mg dose and in 12 to 100 min (median T_{max} 15 min) after the 4 mg dose (Table 2 and Fig. 1a). Subjects 107 and 108 (Table 2) showed significantly (>3-fold difference from median) delayed absorption of naloxone, but they were, nevertheless, included in the subsequent analysis if not otherwise mentioned. The means (SD) of the observed C_{max} concentrations in plasma after 2 and 4 mg IN naloxone were 1.83 (0.70) and 4.33 (3.24) ng mL⁻¹, respectively; the means (SD) of AUC_{0-t} were 166 (55) and 329 (202) min ng mL⁻¹; and the means (SD) of $AUC_{0-\infty}$ were 180 (57) and 349 (208) min ng mL⁻¹, suggesting dose proportionality of the achieved concentrations in plasma. The means (SD) of the elimination half-lives ($t_{1/2}$) after 2 and 4 mg IN naloxone were 58 (16) and 76 (22) min, respectively, indicating that naloxone was rapidly cleared after IN administration.

IN spray dosing of naloxone results in rapid and sustained displacement of [¹¹C]carfentanil

Within the first 60 min after IN naloxone administration, a marked decline was observed in [¹¹C]carfentanil binding (see Fig. 1c), while

administration of placebo failed to elicit any observable perturbation in the [¹¹C]carfentanil signal (see Fig. 1d); this was also confirmed by model selection criteria (Supplementary Information).

Maximum MOR occupancy by naloxone was observed within the first 60 min in a dose-dependent manner, in the range of 54–82% following 2 mg, and in the range of 71–96% following 4 mg doses (Table 2). Receptor occupancy was maintained at these high levels until the end of the 60-min observation period (Fig. 1b). The time taken to achieve 50% occupancy ($t_{RO/2}$) ranged from 11 to 14 min after 2 mg and from 5 to 13 min after 4 mg doses (Table 2), indicating rapid displacement of [¹¹C]carfentanil from MORs after IN naloxone. Markedly lower MOR occupancy by naloxone was observed at 300–360 min after IN naloxone administration—ranging from 7 to 22% following 2 mg, and from 0 to 20% after dosing of 4 mg (Table 2), suggesting relatively rapid clearance of naloxone from the brain. Based on 60 and 360 min post-dose receptor occupancies, the half-life of disappearance of MOR occupancy was estimated to be ~100 min. There were no clear differences between the brain ROIs in either the time course or magnitude of MOR occupancy (Supplementary Information), supporting the use of a compound ROI for subsequent analysis.

Average spatially normalized maps of $BP_{placebo}$ and $BP_{naloxone}$ pooled according to the dose level and time after administration, and the corresponding MOR occupancy images (Fig. 2) confirmed global brain MOR blockade following IN naloxone; this had almost disappeared by 5.5 h after drug administration.

Naloxone plasma concentrations predict brain MOR occupancy in a time-dependent manner

The log-linear relationships between naloxone plasma concentrations and MOR occupancy are presented in Fig. 3a. In this analysis, data were pooled from both dose levels and grouped by the post-dose time points of PK and MOR occupancy data collection. Figure 3a demonstrates that plasma naloxone concentrations

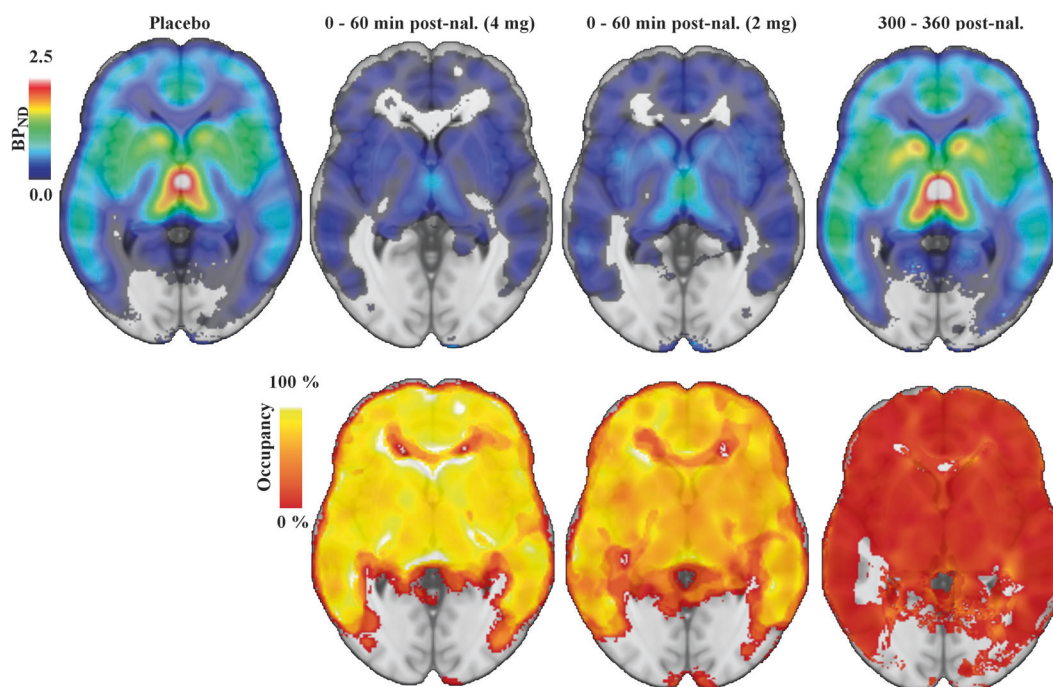


Fig. 2 Average BP_{ND} images (top) of [^{11}C]carfentanil overlaid on a magnetic resonance imaging (MRI) template image, after placebo (first column; $n = 14$), after 4 mg of naloxone at 0–60 min after administration (second column; $n = 4$), after 2 mg of naloxone at 0–60 min after administration (third column; $n = 4$), and after 2 or 4 mg of naloxone at 300–360 min after administration (last column; $n = 6$). Corresponding occupancy (%) images relative to the placebo condition are shown in the bottom row

consistently predicted the brain MOR occupancy, while a departure from the 60 min regression line was evident at sampling time points earlier than 30 min. This observation was supported by a marked rightward shift in the MOR occupancy time course as compared to the time course of naloxone concentrations in plasma, as depicted in Fig. 3b. This was particularly evident in those subjects with fast naloxone absorption, while in those subjects with slow naloxone absorption, the time courses of naloxone in plasma and MOR occupancy in the brain were in good synchrony. Nevertheless, data collected before 30 min after IN naloxone were excluded from the dose-occupancy model calculation shown in Fig. 3c, which yielded an average EC_{50} estimate of $0.70 \pm 0.12 \text{ ng mL}^{-1}$ and an average RO' estimate of $105 \pm 5\%$.

DISCUSSION

Here, we provide preliminary evidence of fast systemic uptake of naloxone after IN administration in a small dose volume (0.1 mL), associated with a rapid displacement of the high-affinity MOR agonist radioligand [^{11}C]carfentanil in the brain. The ensuing MOR occupancy by naloxone reached high average levels of 67 and 85%, respectively, following IN administration of clinically relevant (2 mg and 4 mg) doses. A slight lag of ~20 min was observed in maximal MOR occupancy relative to the naloxone concentrations in plasma, even if [^{11}C]carfentanil displacement started to emerge promptly after naloxone administration, in accord with the clinical observations of opioid overdose patients being resuscitated within minutes after IN naloxone [20]. It should, however, be noted that our study was not designed to explore the reversion of opioid overdosing, and thus no direct inferences should be made to such conditions. The presence of high concentrations of opioid agonists would be expected to shorten the duration of brain MOR occupancy by naloxone.

If the results obtained from two outlier subjects with slow uptake of naloxone into the circulation are not considered, the present PK results are well in line with those of two earlier PK studies on IN naloxone [17, 18]. For most participants (12 out of 14), the time to reach peak naloxone concentrations in plasma was in the range of 15–30 min, that is, in good concordance with the earlier reports of Tylleskar et al. [18] (range 11–24 min) and Krieter et al. [17] (range 12–60 min). The delayed peak times of 80 and 100 min observed in two participants could be explained by delayed absorption due to sub-optimal delivery of the dose too close to the orifice of the nostril, with subsequent slow absorption. This interpretation is supported by the non-discordant total AUCs of these two outliers (Table 2). Furthermore, total AUCs suggested dose proportionality between the 2 and 4 mg doses, in good concordance with earlier findings [17, 18].

The employed time-sensitive PET analysis technique allowed the assessment of brain MOR occupancy by naloxone at unprecedentedly high temporal resolution. The minute-level temporal resolution of the BP_{ND} estimates yielded nearly instantaneous depiction of brain MOR occupancy by naloxone, as compared to average estimates of MOR occupancy in conventional PET studies that typically span over 1–2 h post drug. Nevertheless, the peak occupancies observed in the present study were well in line with earlier estimates [19, 33]. The present estimate of 67% peak MOR occupancy after 2 mg of IN naloxone was in accord with an average MOR occupancy of 80% measured after 2 mg of intravenous (IV) naloxone in humans at 5–65 min post naloxone [33], allowing for higher expected occupancy after IV drug delivery. In non-human primates, Saccone et al. [19] reported 56–66% average MOR occupancy at 10–70 min after IN delivery of naloxone (0.032 mg kg^{-1}), also in good concordance with the present results when considering that peak occupancy is expected to be higher compared to the average occupancy over this time interval. Yet, it should be noted that the anesthetics

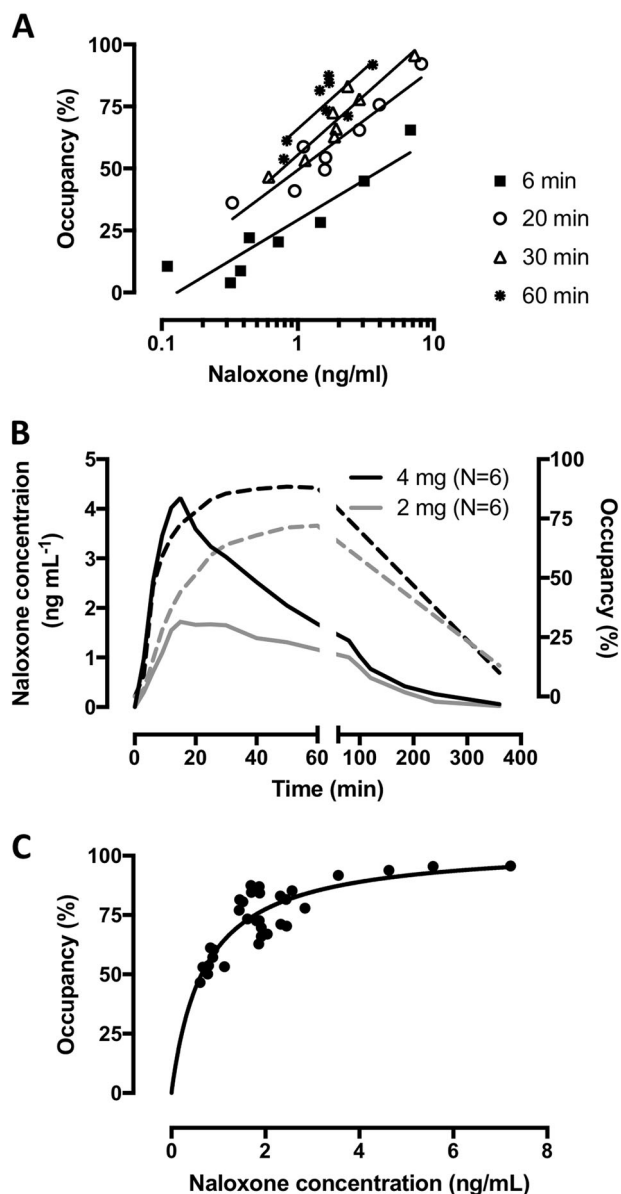


Fig. 3 **a** Log-linear relationships between naloxone plasma concentrations and mu-opioid receptor (MOR) occupancy (%) data, pooled by the time of measurement. Symbols represent individual data points from the indicated post dose time and lines represent log-linear fit results. **b** Plasma naloxone concentrations (mean, left axis, solid lines) after intranasal (IN) naloxone (2 mg, $n=6$, gray line or 4 mg, $n=6$, black line) dosing in healthy male volunteers, and the corresponding occupancy (%) estimates (mean, right axis, lines; broken lines). **c** MOR occupancy determined as a function of the naloxone plasma concentration at all post-dose time points later than 30 min. The solid line represents the best fit to the two-parameter model indicating an estimated plasma drug concentration associated with half-maximal effect (EC_{50}) of $0.70 \pm 0.12 \text{ ng mL}^{-1}$

(ketamine and sevoflurane) administered to the non-human primate study subjects of Saccone et al. [19] may have directly or indirectly affected MOR availability, possibly complicating the comparison.

The present experimental results add evidence to the hypothesized behavior of naloxone in the brain after IN dosing in the presence of negligible agonist concentrations, thus supporting its

use as a fast-acting and transient MOR antagonist in the treatment of behavioral addictions such as PG. A growing body of evidence has linked personality traits predisposing to addictions with relative differences in brain MOR availability [34, 35], and individuals with PG have shown blunted opioidergic responses to pharmacological stimuli [36]. These findings indicate that clinical benefit could be derived from opioid antagonist treatment in PG. Thus, even if the long-lasting MOR antagonists naltrexone and nalmefene have failed to show consistent clinical efficacy in PG [5–10], targeted IN administration of naloxone may prove clinically feasible because of its ease of delivery, fast brain uptake and relatively short duration of MOR blockade, as shown in the present study. Neuropharmacological treatments of PG have gained increasing interest after a reclassification of PG under the “Substance-related and addictive disorders” section of DSM-5, and future studies are expected to shed light on the neurochemical underpinnings of this particular behavioral addiction that might serve to improve our understanding of other related disorders. In particular, converging evidence highlighting the role of MORs in human emotions (see [37] for a review), in regulation of food intake [38], in eating disorders [22, 23] and even in the pleasure related to physical exercise [39] underlines the importance of better understanding of the function and dysfunction of this system in order to develop new efficacious treatments for various addictions.

At present, IN naloxone is being investigated as treatment of PG in a placebo-controlled, double-blinded study in similar doses (2 or 4 mg) as in the present study, although not using exactly the same nasal spray device (ClinicalTrials.gov Identifier: NCT03430180). Regardless of the outcome of the ongoing clinical trial, it might be motivated to extend the present work to PG populations and larger cohorts in the future, to further investigate the robustness of IN drug delivery and to elaborate the required naloxone dosage for desired MOR occupancy levels in the brain. Nevertheless, pending these experiments, a preliminary dose-occupancy model with an $EC_{50} = 0.70 \pm 0.12 \text{ ng mL}^{-1}$ naloxone concentration in plasma was established in the present study, which together with naloxone plasma concentration data derived from future studies may fairly robustly predict the attained brain MOR occupancy levels.

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ADDITIONAL INFORMATION

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