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1 **Pharmacokinetic neuroimaging to study the dose-related brain kinetics and target**
2 **engagement of buprenorphine *in vivo***

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2 **Abstract (250 words)**

3 A wide range of buprenorphine doses are used for either pain management or maintenance
4 therapy in opioid addiction. The complex *in vitro* profile of buprenorphine, with affinity for μ -,
5 δ - and κ -opioid receptors (OR), makes it difficult to predict its dose-related
6 neuropharmacology *in vivo*. In rats, microPET imaging and pretreatment by OR antagonists
7 were performed to assess the binding of radiolabeled buprenorphine (microdose ^{11}C -
8 buprenorphine) to OR subtypes *in vivo* (n=4 per condition). The μ -selective antagonist
9 naloxonazine (10 mg/kg) and the non-selective OR-antagonist naloxone (1 mg/kg) blocked
10 the binding of ^{11}C -buprenorphine while pretreatment by the δ -selective (naltrindole, 3 mg/kg)
11 or the κ -selective antagonist (norbinaltorphimine, 10 mg/kg) did not. In four macaques, PET
12 imaging and kinetic modeling enabled description of the regional brain kinetics of ^{11}C -
13 buprenorphine, co-injected with increasing doses of unlabeled buprenorphine. No saturation
14 of the brain penetration of buprenorphine was observed for doses up to 0.11 mg/kg. Regional
15 differences in buprenorphine-associated receptor occupancy were observed. Analgesic
16 doses of buprenorphine (0.003 and 0.006 mg/kg) respectively occupied 20% and 49% of
17 receptors in the thalamus while saturating the low but significant binding observed in
18 cerebellum and occipital cortex. Occupancy >90% was achieved in most brain regions with
19 plasma concentrations >7 $\mu\text{g/L}$. PET data obtained after co-injection of an analgesic dose of
20 buprenorphine (0.003 mg/kg) predicted the binding potential of microdose ^{11}C -
21 buprenorphine. This strategy could be further combined with pharmacodynamic exploration
22 or pharmacological MRI to investigate the neuropharmacokinetics and neuroreceptor
23 correlate, at least at μ -OR, of the acute effects of buprenorphine in humans.

24

1 Introduction

2 The thebaine derivative buprenorphine is a semi-synthetic opioid of the phenanthrene family
3 [1]. Low-dose buprenorphine offers potent analgesia for the treatment of moderate to severe
4 pain in patients. Compared with other opioids, buprenorphine benefits from a unique safety
5 profile, with limited risk for respiratory depression and overdose. High-dose buprenorphine is
6 therefore approved for addiction maintenance therapy in the management of opioid use
7 disorders with a growing interest in the context of the current opioid crisis [1–3].

8 *In vitro*, buprenorphine is one of the most affine ligand of the human μ -opioid receptor (μ -OR,
9 $K_i=0.9$ nM) and was compared with other opioid such as naloxone ($K_i=14$ nM), morphine
10 ($K_i=74$ nM) or oxycodone ($K_i=780$ nM) in the same conditions [4,5]. *In vitro*, buprenorphine is
11 also far more potent than morphine at stimulating μ -OR, with half-maximal effective
12 concentration (EC_{50}) <0.1 nM and 130 nM for buprenorphine and morphine, respectively,
13 although buprenorphine shows lower maximum efficacy than morphine in mediating μ -OR
14 coupling [4]. Buprenorphine was therefore classified as a highly potent but partial agonist of
15 μ -OR [1,6]. Buprenorphine shows a slow dissociation rate from μ -OR, assumed to account
16 for prolonged occupancy and duration of action *in vivo* [7,8]. Buprenorphine is also described
17 as antagonist of κ -OR and δ -OR, and agonist of nociceptin/ORL-1 receptors [9,10]. It is
18 therefore difficult to predict the *in vivo* dynamics of the interaction of buprenorphine with its
19 CNS targets from this complex *in vitro* profile.

20 There are still discrepancies in the description of the neuropharmacology of buprenorphine
21 [9,11]. *In vivo*, buprenorphine benefits from limited respiratory effects at high doses [12]. A
22 “ceiling” or “inverted U-shape” analgesic dose-response has been described in animals [13].
23 However, in patients, buprenorphine shows a dose-dependent analgesic effect similar than
24 that of full agonists [11]. Peripheral pharmacokinetics of buprenorphine is well established in
25 humans [14]. Norbuprenorphine (*N*-dealkyl-buprenorphine) is the predominant metabolite
26 and shows negligible blood-brain barrier (BBB) penetration compared with buprenorphine
27 [15]. Its relatively short elimination half-life of ~ 3 h contrasts with its prolonged duration of
28 action [1,11,14], suggesting particular brain kinetics.

29 Pharmacological Positron Emission Tomography (PET) imaging uses target-specific
30 radioligands to capture the target engagement associated with one controlled plasma level of
31 the investigated drug [16]. The μ -OR-selective radioligand ^{11}C -carfentanil [17] was used in
32 healthy volunteers and heroin-dependent patients to estimate the extent and duration of μ -
33 OR occupancy associated with high-doses of buprenorphine (2-16 mg, sublingual route) [18–
34 20]. Data regarding receptor occupancy associated with acute administration of analgesic
35 doses of buprenorphine (0.3-0.6 mg) are still lacking. Interestingly, isotopic radiolabeling of

1 buprenorphine is feasible [21]. This provides a unique opportunity for direct determination of
2 the brain kinetics of buprenorphine, at its site of action, a strategy named pharmacokinetic
3 imaging [22]. Moreover, pharmacological doses of buprenorphine, instead of microdose
4 usually encountered in PET studies, can be safely used to mimic the clinical situation in
5 terms of pharmacokinetics and pharmacodynamics.

6 In the present study, pharmacokinetic imaging using ^{11}C -buprenorphine was performed to
7 explore the neuropharmacology of buprenorphine *in vivo*. Blocking experiments were
8 performed to address the binding of ^{11}C -buprenorphine to μ -, δ - and κ -OR in rats. ^{11}C -
9 buprenorphine PET imaging was then performed in macaques to assess the regional
10 neuropharmacokinetics and receptor occupancy of buprenorphine associated with a wide
11 range of buprenorphine doses, that covers its clinical use in both analgesia and addiction
12 maintenance.

13

1 **Material and Methods**

2 **1. Chemicals**

3 Buprenorphine hydrochloride for i.v. injection (0.3 mg/mL) was obtained from Axience
4 (Pantin, France). Naloxone hydrochloride for i.v. injection (0.4 mg/mL) was obtained from
5 Aguetant (Lyon, France). Naloxonazine and norbinaltorphimine were obtained from Sigma-
6 Aldrich (Saint-Quentin Fallavier, France) and naltrindole from Tocris (Noyal-Chatillon sur
7 Sèche, France). Ketamine was obtained from Virbac (Caros, France). Propofol was
8 purchased from Fresenius laboratory (Sèvres, France). Isoflurane was obtained from Abbvie
9 (Rungis, France). ¹¹C-Buprenorphine was synthesized in-house according to the method
10 described by Lever et al. [21] with slight modifications (see supplemental material).

11 **2. Animals**

12 All animal use procedures were in accordance with the recommendations of the European
13 Community for the care and use of laboratory animals (2010/63/UE) and the French National
14 Committees (French Decret 2013-118). Experimental protocols were validated by a local
15 ethics committee for animal use (CETEA/A15-002 and A18-065) and approved by the french
16 government. Rodent experiments were conducted in male Sprague-Dawley rats (224±43g).
17 Each rat underwent a single PET experiment. Four adult male rhesus macaques (*Macaca*
18 *Mulatta*; 8.4±3.4 kg in weight during the study) were obtained from Silabe (Simian Laboratory
19 Europe, France). A minimum interval of 2 weeks was respected between two scans in the
20 same individual.

21 **3. Binding of ¹¹C-buprenorphine to OR subtypes in rats**

22 *MicroPET imaging*

23 ¹¹C-buprenorphine brain PET acquisitions were performed using an Inveon microPET
24 scanner (Siemens Medical Solutions, France). Anesthesia was induced and thereafter
25 maintained using 3% and 1.5–2.5% isoflurane in O₂, respectively. A catheter was inserted in
26 a lateral caudal vein for intravenous (i.v.) injection of tested OR-antagonists when necessary.
27 Microdose ¹¹C-buprenorphine (34±7 MBq, 3±2 µg, mean molar activity at time of injection
28 MA_{inj}=8.2±4.4 GBq.µmol⁻¹) was then injected in the same catheter.

29 *Pharmacological challenges*

30 Blocking experiments were performed to investigate the binding of ¹¹C-buprenorphine to
31 different OR subtypes in the living brain (n=4 per condition). PET acquisitions were
32 performed without or after previously reported blocking conditions using the non-selective
33 OR antagonist naloxone (1 mg/kg i.v., 5 min before ¹¹C-buprenorphine injection) [23], the

1 selective μ -OR antagonist naloxonazine (10 mg/kg i.v., 5 min before ^{11}C -buprenorphine
2 injection) [24], the selective κ -OR antagonist norbinaltorphimine (10 mg/kg intraperitoneal
3 injection, 30 min before ^{11}C -buprenorphine injection) [25] and the selective δ -OR antagonist
4 natriindole (3 mg/kg i.v., 5 min before ^{11}C -buprenorphine injection) [26].

5 *Data analysis*

6 MicroPET images were reconstructed as previously described [27]. Late brain PET images
7 acquired 40-60min after ^{11}C -buprenorphine injection were coregistered to the Schiffer rat
8 brain template using PMOD software V3.9 (PMOD Technologies, Zürich, Switzerland).
9 Cerebellum was shown devoid of μ - and δ -OR with limited expression of κ -OR in rats [28].
10 Regional uptake ratios (region/cerebellum) were calculated in baseline and blocking
11 conditions to take any change in peripheral pharmacokinetics of ^{11}C -buprenorphine into
12 account.

13 **4. Target engagement of buprenorphine in macaques**

14 *Co-injection study*

15 Further PET experiments were performed in macaques to allow for accurate arterial blood
16 sampling during PET acquisition. First, microdose ^{11}C -buprenorphine was i.v. injected,
17 followed by a 90 min brain PET acquisition. Then, the dose-dependent receptor occupancy
18 associated with therapeutic doses was addressed using a co-injection strategy. Increasing
19 doses of unlabeled buprenorphine (0; 0.003; 0.006; 0.03; 0.06 and 0.011 mg/kg, equivalent
20 to human doses ranging from 0 to 8 mg/70 kg, n=4 per dose) were mixed in the syringe
21 containing microdose ^{11}C -buprenorphine ($8.34 \pm 3.85 \mu\text{g}$). The preparation was i.v. injected at
22 the start of dynamic PET acquisition (90 min).

23 *Acquisition procedure*

24 First, each monkey underwent an anatomical T1-weighted brain MR scan using an Achieva
25 1.5T scanner (Philips Healthcare, Suresnes, France) under ketamine anesthesia
26 (intramuscular injection, i.m.). PET acquisitions were performed on a HR+ Tomograph
27 (Siemens Healthcare, Knoxville, TN, USA) in anesthetized macaques as previously
28 described [29]. Briefly, the macaque received ketamine (10 mg/kg, i.m.) to induce
29 anesthesia. After intubation in supine position, venous catheters were inserted for radiotracer
30 injection (sural vein), propofol infusion (sural vein) and drug injection for the displacement
31 experiments (brachial vein). Another catheter was inserted into the femoral artery for arterial
32 blood sampling. Macaques were positioned under the camera before administration of a 2
33 mL i.v. bolus of propofol followed by a 1 mL/kg/h i.v. infusion under oxygen ventilation.
34 Macaques were i.v. injected with microdose ^{11}C -buprenorphine ($241 \pm 42 \text{ MBq}$, $\text{MA}_{\text{inj}} = 13.5 \pm 5.1$

1 GBq/ μmol). Increasing doses of unlabeled buprenorphine were added to ^{11}C -buprenorphine
2 microdose for the co-injection study. Physiological monitoring, including heart rate, oxygen
3 saturation (SpO_2), respiratory rate, and end-tidal CO_2 , was performed throughout the duration
4 of the PET scan.

5 *Imaging data reconstruction and segmentation*

6 A post-reconstruction method was performed on dynamic PET image for noise reduction and
7 improved spatial resolution (see supplemental material) [30,31]. PET data were then
8 analyzed using PMOD software. PET images were coregistered to corresponding T1-weighted
9 MR images for each macaque. A macaque T1-weighted MR template [32] was normalized
10 onto individual MR images. Transformation matrices were then applied to the segmentation
11 obtained from the template to generate time-activity curves in 12 selected brain structures.

12 **5. Arterial Input Function and Metabolism**

13 During PET acquisition, arterial blood samples (500 μL) were withdrawn at selected times
14 after radiotracer injection. Samples were centrifuged (5 min; 2,054g; 4°C) and the
15 supernatant (200 μL) was gamma-counted for total plasma radioactivity. Additional plasma
16 samples were withdrawn at 0; 5; 10; 15; 30; 60 and 90 min to measure both *i*) the percentage
17 of parent (unmetabolized) ^{11}C -buprenorphine using radio-HPLC and a state-of-the-art
18 methodology [33] and *ii*) the total concentration of buprenorphine in plasma using mass
19 spectrometry, after radioactive decay. The fraction of parent ^{11}C -buprenorphine in each
20 sample was used to generate the metabolite-corrected arterial input function for
21 pharmacokinetic modeling of each PET experiment (see supplemental material, Fig. S1).

22 **6. Pharmacokinetic modeling**

23 Kinetics of radioactivity in the brain and in plasma samples were decay-corrected and
24 expressed as the percentage of injected dose of radioactivity per volume ($\%ID.\text{cm}^{-3}$). Kinetic
25 modeling was performed considering the metabolite-corrected arterial input function. The
26 initial transfer rate of ^{11}C -buprenorphine from plasma into the brain (K_1) was estimated using
27 the graphical plot analysis, as previously described [27] (see supplemental material, Fig. S2).
28 The brain distribution of ^{11}C -buprenorphine (V_T ; $\text{mL}.\text{cm}^{-3}$) was estimated using the Logan plot
29 graphical method [34]. Parametric images (V_T unit) were generated using PMOD to display
30 the regional brain distribution of ^{11}C -buprenorphine in tested conditions (Fig. 2).

31 Brain data obtained with ^{11}C -buprenorphine co-injected with the maximal dose of unlabeled
32 buprenorphine (0.11 mg/kg) were used to estimate the non-specific binding of ^{11}C -
33 buprenorphine (saturation scan) and define the non-displaceable volume of distribution
34 ($V_{\text{ND,saturation}}$) in each region for each animal. Regional $V_{\text{ND,saturation}}$ were compared with

1 graphically estimated $V_{ND,graphical}$ (Table 1 and supplemental material, Fig. S3). For each scan,
2 the specific binding of ^{11}C -buprenorphine in each brain region was estimated as the binding
3 potential relative to plasma (BP_p) [35] with:

$$BP_p = V_T - V_{ND,saturation}$$

4
5 BP_p estimated in microdose scans ($BP_{p,microdose}$) was used to estimate the receptor occupancy
6 associated with each pharmacological dose of unlabeled buprenorphine as follow [35]:

$$\text{Receptor Occupancy (\%)} = (BP_{p,microdose} - BP_{p,dose}) / BP_{p,microdose} \times 100$$

7
8 The occipital cortex showed the lowest PET signal and was used as pseudo-reference tissue
9 to estimate the regional binding of ^{11}C -buprenorphine without arterial blood sampling (DVR,
10 Logan reference method) [36]. Occipital cortex commonly serves as a reference region for
11 quantification of PET radioligands targeting μ -OR in humans and monkeys [18,19,37,38]. BP_p
12 and DVR are unitless values.

13 For each region and for each scan, the receptor occupancy of ^{11}C -buprenorphine was fitted
14 to the corresponding plasma concentration of buprenorphine, measured from 60 to 90 min
15 post-injection. Occupancy associated with plasma concentrations of buprenorphine obtained
16 with the 0.11 mg/kg dose were set to 100%. A non-linear fit model of saturation with one
17 binding site was used to estimate *i*) the plasma concentration of buprenorphine associated
18 with regional half-maximum receptor occupancy (EC_{50}) and *ii*) the receptor occupancy
19 associated with selected plasma levels of buprenorphine (GraphPad Prism software V7.0,
20 San Diego, CA, USA) (see supplemental material, Fig S4, Table 1).

21 **7. Displacement experiments in nonhuman primates**

22 Additional experiments were performed to address the reversibility of ^{11}C -buprenorphine
23 binding to CNS targets. Displacement experiments were performed in 3 macaques and
24 consisted in the injection of unlabeled buprenorphine (0.03 mg/kg) or naloxone (0.22 mg/kg),
25 30 min after ^{11}C -buprenorphine injection. The selected dose of naloxone is the maximum
26 recommended dose as an antidote against opioid overdose in humans [39]. Methods and
27 results of displacement experiments are reported as supplemental material (Fig. S5).

28 **8. Statistical analysis**

29 Statistical comparison between conditions was performed using GraphPad Prism. Outcome
30 parameters were compared using a 2-way ANOVA and the Tukey's post-hoc test. A result
31 was deemed significant when a 2-tailed p value was less than 0.05.

32

1 Results

2 1. Binding of ^{11}C -buprenorphine to OR subtypes in rats

3 Baseline brain distribution of ^{11}C -buprenorphine showed high PET signal in the thalamus,
4 striatum and hypothalamus with the lowest PET signal in the cerebellum. Significant
5 differences in uptake ratios were observed across brain regions ($p < 0.001$, Fig. 1). Blocking
6 experiments using the non-selective OR antagonist naloxone, used as positive control,
7 significantly decreased ^{11}C -buprenorphine binding in most brain regions, reaching similar
8 levels than in the cerebellum ($p > 0.05$). Blocking by the selective μ -OR antagonist
9 naloxonazine produced similar effects than naloxone. The binding of ^{11}C -buprenorphine was
10 not significantly decreased by selective blocking of κ -OR (norbinaltorphimine) and δ -OR
11 (naltrindole) (Fig. 1).

12 2. Co-injection study in macaques

13 PET images obtained in monkeys injected with microdose ^{11}C -buprenorphine are shown in
14 Fig. S6. The PET signal slowly accumulated in OR-rich regions such as the putamen,
15 caudate and thalamus. The maximum brain concentration was $0.0255 \pm 0.0052 \text{ \%ID.cm}^{-3}$ at
16 $t_{\text{max}} = 22.5 \text{ min}$. Regions with minimal OR expression (cerebellum and occipital cortex)
17 reached their maximum concentration earlier ($t_{\text{max}} = 6.6 \text{ min}$) with faster decrease of the
18 radioactivity (Fig. S5).

19 Then, ^{11}C -buprenorphine was co-injected with increasing doses of unlabeled buprenorphine
20 up to 0.11 mg/kg (Fig. 2). Buprenorphine doses were well tolerated and no change in
21 physiological parameters was observed. Selected doses of unlabeled buprenorphine did not
22 impact the metabolism and plasma kinetics of ^{11}C -buprenorphine with no difference in
23 plasma exposure ($p > 0.05$, Fig S1). This suggests a linear pharmacokinetics for
24 buprenorphine in plasma within the tested dose range. The plasma concentrations of
25 unlabeled buprenorphine estimated from 60 to 90 min ranged from $0.10 \pm 0.08 \text{ }\mu\text{g/L}$
26 (microdose condition) to $11.56 \pm 2.94 \text{ }\mu\text{g/L}$ (0.11 mg/kg condition) and were significantly
27 correlated with injected dose (Fig. S7).

28 Kinetic modeling was performed to estimate ^{11}C -buprenorphine distribution to brain regions in
29 the presence of increasing doses of unlabeled buprenorphine. Co-injection of unlabeled
30 buprenorphine up to 0.11 mg/kg did not impact the K_1 of ^{11}C -buprenorphine from plasma into
31 the brain ($p > 0.05$, Fig. S3). There was no difference in K_1 between brain regions ($p > 0.05$).
32 Parametric mapping of V_T obtained using microdose ^{11}C -buprenorphine showed significant
33 differences in regional V_T between OR-rich brain regions such as the thalamus, striatal and

1 cortical regions and OR-poor regions such as the cerebellum and occipital cortex ($p < 0.01$,
2 Fig. 2).

3 The lowest dose of unlabeled buprenorphine (0.003 mg/kg) was sufficient to saturate ^{11}C -
4 buprenorphine binding in the cerebellum and occipital cortex. OR-rich regions showed a
5 dose-dependent decrease in V_T , with a maximal 2.5-fold decrease observed in the putamen
6 obtained using the 0.06 mg/kg dose (Fig. 2). Higher dose (0.11 mg/kg) did not further
7 decrease V_T , suggesting complete saturation of buprenorphine brain targets at the 0.06
8 mg/kg dose. ^{11}C -buprenorphine-associated radioactivity at doses higher than 0.06 mg/kg
9 predominantly reflected the non-specific binding of ^{11}C -buprenorphine and there was no
10 difference in regional V_T across brain regions at either 0.06 mg/kg or 0.11 mg/kg ($p > 0.05$).
11 We found a strong correlation between $V_{\text{ND,saturation}}$ and $V_{\text{ND,graphical}}$ ($p < 0.001$, $R^2 = 0.997$, Table
12 1, Fig. S3). V_T estimated at 0.11 mg/kg therefore provides a good estimate of the regional
13 non-displaceable volume of distribution (V_{ND}) of ^{11}C -buprenorphine for each individual (Fig.
14 2).

15 In Figure 3, regional V_T estimated for each dose of unlabeled buprenorphine was plotted to
16 microdose V_T according to the $V_{T,\text{dose}} = f(V_{T,\text{microdose}})$ equation. The lowest dose of unlabeled
17 buprenorphine (0.003 mg/kg) did not impact the slope of the equation which remained ~ 1.0 ,
18 suggesting negligible occupancy in most brain regions. Higher doses of unlabeled
19 buprenorphine did not further decrease V_T in the occipital cortex and cerebellum but induced
20 a dose-dependent decrease in the slope of the equation. Deviation of the slope from zero
21 was not significant ($p > 0.05$) for doses of buprenorphine ≥ 0.06 mg/kg, suggesting total
22 occupancy (Fig. 3) [40].

23 There was a strong correlation between regional V_T and BP_p obtained with ^{11}C -
24 buprenorphine ($R^2 = 0.98$, $p < 0.001$, Fig. 4). Thus, microdose V_T accurately predicted the total
25 specific binding of ^{11}C -buprenorphine. V_T and BP_p values estimated with kinetic modeling
26 were used as a gold-standard to test the reliability of the Logan reference method using the
27 occipital cortex as a pseudo-reference region [36] (Fig. 4). $\text{DVR}_{\text{microdose}}$ and $\text{DVR}_{0.003\text{mg/kg}}$ were
28 not significantly different ($p > 0.05$, paired t -test, Table 1, Fig. 4), suggesting similar relative
29 binding across brain regions. $\text{DVR}_{\text{microdose}}$ or $\text{DVR}_{0.003\text{mg/kg}}$ correlated with $V_{T,\text{microdose}}$ ($p < 0.001$;
30 $R^2 = 0.43$ and 0.54 , respectively, data not shown). Better correlation was found between
31 $\text{DVR}_{\text{microdose}}$ or $\text{DVR}_{0.003\text{mg/kg}}$ and $\text{BP}_{p,\text{microdose}}$ ($p < 0.001$; $R^2 = 0.64$ and 0.66 , respectively). This
32 suggests that DVR estimated using ^{11}C -buprenorphine/buprenorphine at either microdose or
33 0.003 mg/kg predicted the regional BP_p of microdose ^{11}C -buprenorphine (Fig. 4).

34 Plasma concentrations of buprenorphine associated with analgesic doses of buprenorphine
35 (0.003 mg/kg and 0.006 mg/kg) were 0.29 ± 0.04 $\mu\text{g/L}$ and 0.66 ± 0.22 $\mu\text{g/L}$, respectively (Fig.

1 S7). In the thalamus, corresponding receptor occupancy was 20% and 49%, respectively.
2 Regions with the lowest specific binding (cerebellum and occipital cortex) were fully occupied
3 at the lowest analgesic dose. Thus, poor fit and estimation of EC_{50} were obtained in these
4 regions (Table 1, Fig. S4). In most OR-rich regions, receptor occupancy >90% was achieved
5 with plasma concentrations of buprenorphine >7 $\mu\text{g/L}$ (Table 1, Fig. S4). Regional receptor
6 occupancies associated with a range of plasma concentrations of buprenorphine were
7 estimated (Table 1). Regional differences in receptor occupancy and EC_{50} could be noticed.

8

9

1 Discussion

2 PET imaging studies using μ -OR-targeting radioligands are classically used for estimation of
3 the interaction of opioids with μ -OR, with limited information on brain kinetics of investigated
4 compounds [41]. Pharmacokinetic PET studies using radiolabeled analogues of drugs are
5 increasingly used for direct determination of their BBB penetration or brain delivery [22]. This
6 microdose strategy does not however provide information regarding pharmacodynamics, as
7 compared with behavioral investigation or pharmacological MRI (phMRI) [42,43]. We used
8 complementary pharmacokinetic neuroimaging approaches using ^{11}C -buprenorphine to
9 directly assess its binding to OR subtypes *in vivo*, as well as the dose-related brain kinetics
10 and target engagement associated with clinically relevant doses of unlabeled buprenorphine.

11 ^{11}C -buprenorphine PET signal in brain regions depends on its non-specific binding, its affinity
12 for OR subtypes, their regional availability and corresponding association/dissociation
13 kinetics. Binding of buprenorphine to μ -OR, κ -OR and δ -OR has been compared in the same
14 *in vitro* conditions. Respective K_i of buprenorphine for μ -, κ - and δ -OR was 0.08, 0.44 and
15 0.82 nM (monkey), 0.08, 0.11 and 0.42 nM (rat) and 12.4, 108 and 154 nM (human).
16 Buprenorphine showed much lower affinity for ORL-1 ($K_i = 285$ nM in rats) [9,44]. Our
17 blocking experiments in rats suggest that the specific binding of ^{11}C -buprenorphine
18 predominantly reflects its interaction with μ -OR rather than κ - or δ -OR. This is consistent with
19 previous *ex vivo* data showing a single predominant high affinity binding site for ^3H -
20 buprenorphine in rat brain lysate, leading to linear Scatchard plot in saturation experiments
21 [45]. Frost et al. compared the regional binding specificity of the non-selective OR antagonist
22 ^{11}C -diprenorphine and μ -OR-selective agonist ^{11}C -carfentanil in humans using the thalamus,
23 a region with known predominance of μ -OR, as a normalization region [46]. Using the same
24 method with our macaque data, the regional binding of ^{11}C -buprenorphine obtained using
25 either microdose or co-injection of 0.003 mg/kg of unlabeled buprenorphine fits the regional
26 distribution of ^{11}C -carfentanil rather than that of ^{11}C -diprenorphine (Fig. S8, Table S1). In
27 pharmacotherapy, the affinity for κ - and δ -OR was shown to account for the
28 pharmacodynamics of high-dose buprenorphine [9,44]. However, from a molecular imaging
29 perspective, only the μ -OR component of the neuropharmacology of buprenorphine can be
30 estimated using ^{11}C -buprenorphine PET imaging.

31 Modest but significant specific binding of ^{11}C -buprenorphine was observed in the cerebellum
32 and occipital cortex in macaques, in both our co-injection and displacement experiments
33 (Fig. S5). Data regarding the expression of OR in these brain regions in monkeys are scarce
34 [47]. Although species differences in OR expression may exist, it was reported a low but
35 significant local expression of μ -, κ - but not δ -OR in the human cerebellum [48,49]. In the

1 human occipital cortex, expression of κ -, δ - but not μ -OR has been detected [50,51]. In other
2 regions with known μ -OR expression, unlabeled buprenorphine dose-dependently decreased
3 ^{11}C -buprenorphine V_T (Fig. 2 and 3). No saturation of the BBB penetration of ^{11}C -
4 buprenorphine was observed for doses up to 0.11 mg/kg (Fig. S2). Full saturation of
5 neuroreceptors, achieved with the highest doses of buprenorphine, revealed the
6 homogenous mapping of the non-specific binding of ^{11}C -buprenorphine (Fig. 2). Thus,
7 quantitative data regarding total specific binding potential of ^{11}C -buprenorphine to CNS
8 targets (BP_p) could be derived (Table 1).

9 Estimation of the dose-related receptor occupancy by buprenorphine using a target-specific
10 radioligand such as ^{11}C -carfentanil or ^{11}C -diprenorphine may depend on the affinity of the
11 selected probe for investigated OR [52]. Direct saturation experiments with ^{11}C -
12 buprenorphine/buprenorphine therefore provide a unique *in vivo* translation of *in vitro* binding
13 experiments [7]. Plasma levels associated with analgesic doses of buprenorphine (0.003 and
14 0.006 mg/kg) ranged from 0.29 ± 0.04 to 0.66 ± 0.22 $\mu\text{g/L}$, consistent with clinical
15 pharmacokinetic data in patients [14]. Corresponding plasma levels of buprenorphine
16 occupied <50% of the total binding in μ -OR-rich regions such as the thalamus. This suggests
17 that partial occupancy of μ -OR is sufficient to achieve effective analgesia, which may also
18 involve action on κ -OR and nociceptin/ORL-1 at the spinal level [53].

19 Buprenorphine for addiction maintenance is administered *via* sublingual route (bioavailability
20 ~70%) [54]. Buprenorphine plasma levels associated with the lowest dose used for addiction
21 maintenance (0.03 mg/kg), administered i.v., still partially occupied μ -OR. Full receptor
22 occupancy was achieved with doses ≥ 0.06 mg/kg. It was suggested that >50% of μ -OR
23 occupancy is required to ensure suppression of withdrawal syndrome. Moreover, μ -OR
24 occupancy >80% is assumed to protect against opioid overdose syndrome induced by
25 massive and unintended intake [20]. Our macaque data suggest that plasma concentrations
26 >7 $\mu\text{g/L}$ have to be maintained to ensure >90% occupancy of OR by buprenorphine in the
27 striatum. In patients, higher doses of buprenorphine may thus essentially maintain plasma
28 concentration over the targeted threshold to ensure sustained and effective maintenance
29 therapy [55]. This observation is consistent with previous ^{11}C -carfentanil PET data obtained
30 in heroin-dependent subjects showing that total μ -OR occupancy is prolonged by increasing
31 the doses of buprenorphine [19].

32 Compared with other opioids, buprenorphine overdoses are rare but their clinical
33 management is difficult, with poor efficacy of naloxone as antidote [56]. This is consistent
34 with the slow reversibility of ^{11}C -buprenorphine binding by high-dose naloxone (0.22 mg/kg)
35 observed in our study. Previous blocking experiments performed in macaques and using the

1 μ -OR-selective radioligand ^{11}C -carfentanil showed that ~85% occupancy of μ -OR was
2 achieved by a lower dose of naloxone (0.03 mg/kg, i.v., 10 min before PET) [38].

3 The occipital cortex and cerebellum are not proper reference tissue for ^{11}C -buprenorphine
4 because of low but significant specific binding was found in these regions. We nonetheless
5 evaluated the occipital cortex as a pseudo-reference tissue region to non-invasively estimate
6 ^{11}C -buprenorphine binding. Both $\text{DVR}_{\text{microdose}}$ and $\text{DVR}_{0.003 \text{ mg/kg}}$ similarly predicted microdose
7 BP_p (Fig. 4). In the absence of arterial input function, the binding potential of ^{11}C -
8 buprenorphine in brain regions, which mainly reflects baseline availability of μ -OR, can
9 therefore be estimated using either microdose or low-dose ^{11}C -buprenorphine
10 pharmacokinetic imaging using this simplified method.

11 For safety reasons, PET imaging is usually performed using microdose receptor antagonists
12 and low injected mass to avoid any adverse effects. In radiotracer development, co-injection
13 of radiotracers with pharmacological doses of corresponding unlabeled compounds is only
14 used to investigate the specific binding to brain regions [57]. We assume this strategy will
15 gain interest for multimodal pharmacological imaging protocols on simultaneous hybrid PET-
16 MR systems [58]. Using CNS-active dose, the time-course of PET-derived target
17 engagement can therefore be directly compared with the hemodynamic response assessed
18 using pharmacological MRI (phMRI) or other pharmacodynamic parameters in the same
19 individual [59]. Interestingly, the CNS effects of investigated doses of buprenorphine have
20 been studied using phMRI in both monkeys (0.03 mg/kg) [60] and humans (0.003 mg/kg)
21 [42]. In rhesus monkeys, buprenorphine increased the cerebral blood volume in brain regions
22 consistent with the binding of corresponding doses of buprenorphine to brain regions found
23 in our study [60].

24 **Conclusion**

25 Pharmacokinetic imaging provides a pragmatic method to explore the neuropharmacokinetic
26 and the μ -OR correlates of the CNS effects of buprenorphine. ^{11}C -buprenorphine co-injected
27 with low dose buprenorphine could be safely performed as a dual-modality imaging
28 biomarker for PET/phMRI studies. This strategy may be useful to explore variability in
29 neurovascular coupling associated with the acute response to buprenorphine in future
30 multimodal pharmacological studies.

31

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5

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10 **Author contribution**

11 SA, MB, DV and NT contributed to conception of the work, data analysis and manuscript
12 writing. SA, SG and AP contributed to data acquisition. CW, MT and CL helped for PET and
13 MR imaging analysis. FC performed radiochemistry.

14

15 **Supplementary information**

16 Supplemental material accompanies this paper at (<https://doi.org/>).

17

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8
9

1 **Figures legends**

2

3 **Fig. 1. Impact of selected opioid antagonists on the regional binding of ¹¹C-**
4 **buprenorphine *in vivo* in rats.** PET acquisitions were performed without (baseline) or after
5 pharmacological blocking conditions using the non-selective OR antagonist naloxone (1
6 mg/kg i.v., 5 min before PET), the selective μ -OR antagonist naloxonazine (10 mg/kg i.v., 5
7 min before PET), the selective κ -OR antagonist norbinaltorphimine (10 mg/kg i.v., 30 min
8 before PET) and the selective δ -OR antagonist natriindole (3 mg/kg i.v., 5 min before PET).
9 Representative summed PET images (40-60min) obtained in each condition and
10 coregistered to a rat brain template are shown in A. Uptake ratios (region/cerebellum,
11 mean \pm S.D, n=4) are shown in B. ***p<0.001 compared with baseline, *ns* = non-significant.

12

13 **Fig. 2. Parametric PET data of ¹¹C-buprenorphine obtained from the co-injection study**
14 **in macaques.** Representative parametric images expressed in V_T (A). Regional V_T measured
15 using the Logan plot analysis for each investigated brain region and each co-injected dose of
16 unlabeled buprenorphine (B). Data are shown as mean \pm SD, n=4). ***p<0.001 compared with
17 microdose, *ns* = non-significant.

18

19 **Fig. 3. Correlation between ¹¹C-buprenorphine V_T measured during the co-injection**
20 **study and the corresponding microdose ¹¹C-buprenorphine V_T in macaques.** Data are
21 represented as mean \pm SD. The slope of each correlation is indicated in the right panel.

22

23 **Fig. 4. Correlation of outcome parameters derived from the kinetic modeling of ¹¹C-**
24 **buprenorphine PET data obtained in macaques using microdose or therapeutic dose**
25 **of buprenorphine.** Correlation between the binding potential ($BP_{p,microdose}$) and the total
26 volume of distribution ($V_{T,microdose}$) of microdose ¹¹C-buprenorphine is shown in A. Correlation
27 of ¹¹C-buprenorphine distribution volume ratio (DVR, Logan reference method) estimated in
28 microdose experiments ($DVR_{microdose}$) and DVR obtained after co-injection with unlabeled
29 buprenorphine ($DVR_{0.003mg/kg}$) is reported in B. Difference between $DVR_{microdose}$ and
30 $DVR_{0.003mg/kg}$ was not significant (paired *t*-test). Correlation of either $DVR_{microdose}$ or
31 $DVR_{0.003mg/kg}$ with $BP_{p,microdose}$ are shown in C and D, respectively. The coefficient of
32 determination (R^2) is reported for the correlation of outcome parameters estimated in brain
33 regions of each individual.

Tables

Table 1. Outcome parameters obtained with PET pharmacokinetic modeling and *in vivo* binding experiments in macaques.

Brain region	$V_{ND,saturation}$ (= $V_{T, 0.11}$ mg/kg)	$V_{ND,graphical}$	$BP_p, microdose$	$DVR_{microdose}$	$DVR_{0.003 \text{ mg/kg}}$	Estimated receptor occupancy (%) associated with plasma levels of buprenorphine						EC_{50} ($\mu\text{g/L}$)
						0.3 $\mu\text{g/L}$	0.6 $\mu\text{g/L}$	1 $\mu\text{g/L}$	3 $\mu\text{g/L}$	6 $\mu\text{g/L}$	9 $\mu\text{g/L}$	
Frontal cortex	3.62 ± 0.68	3.44 ± 1.70	4.80 ± 0.75	1.40 ± 0.19	1.47 ± 0.11	33.2	49.7	62.1	82.7	90.2	93.1	0.60 (0.07 to 1.12)
Orbital cortex	3.56 ± 0.78	3.42 ± 1.33	4.01 ± 0.66	1.22 ± 0.15	1.39 ± 0.09	27.4	43.1	55.8	79.1	88.3	91.9	0.79 (0.30 to 1.30)
Cingulate cortex	3.77 ± 0.70	3.60 ± 1.78	5.48 ± 1.03	1.50 ± 0.28	1.67 ± 0.19	29.4	45.5	58.1	80.6	89.3	92.6	0.72 (0.33 to 1.11)
Temporal cortex	3.67 ± 0.75	3.46 ± 2.13	3.88 ± 0.83	1.26 ± 0.15	1.41 ± 0.05	31.0	47.4	60.0	81.8	90	93.1	0.67 (0.18 to 1.15)
Parietal cortex	3.57 ± 0.63	3.41 ± 1.80	4.48 ± 1.05	1.37 ± 0.13	1.40 ± 0.10	34.3	51.1	63.5	83.9	91.3	94	0.58 (0.20 to 0.95)
Occipital cortex	2.93 ± 0.62	2.84 ± 2.29	2.42 ± 1.05	NA	NA	†	†	†	†	†	†	†
Caudate	4.11 ± 0.56	3.91 ± 1.80	5.82 ± 1.39	1.57 ± 0.31	1.8 ± 0.25	23.6	38.3	50.8	75.6	86.1	90.3	0.99 (0.43 to 1.50)
Putamen	4.42 ± 0.79	4.21 ± 1.83	6.41 ± 1.44	1.71 ± 0.31	1.9 ± 0.21	28.0	43.8	56.5	79.6	88.6	92.1	0.77 (0.34 to 1.20)
Amygdala	3.92 ± 0.59	†	4.90 ± 1.05	1.39 ± 0.24	1.62 ± 0.19	14.5	25.3	36.1	62.9	77.2	83.6	1.77 (0.60 to 2.94)
Thalamus	4.61 ± 0.76	4.33 ± 1.99	5.17 ± 0.93	1.54 ± 0.27	1.77 ± 0.19	23.4	37.9	50.4	75.3	85.9	90.1	0.98 (0.33 to 1.64)
Hypothalamus	3.85 ± 0.70	†	4.38 ± 0.79	1.27 ± 0.17	1.55 ± 0.08	23.4	37.9	50.4	75.3	85.9	90.1	0.98 (0.22 to 1.74)
Cerebellum	3.55 ± 0.70	3.43 ± 2.13	2.21 ± 0.93	1.06 ± 0.12	1.11 ± 0.05	†	†	†	†	†	†	†

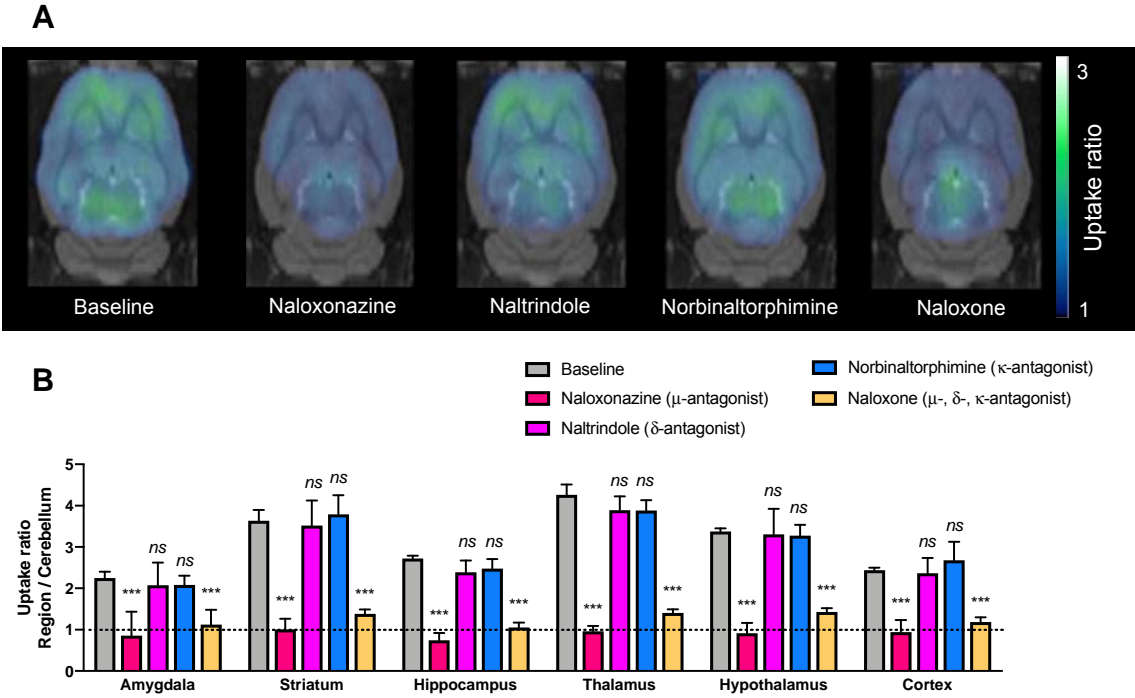
V_{ND} is the non-displaceable volume of distribution. $V_{ND,saturation}$ has been estimated using the Logan plot method and the maximum co-injected dose of unlabeled buprenorphine ($V_{T, 0.11}$ mg/kg). $V_{ND,graphical}$ has been graphically estimated (see supplemental material, Fig. S3). BP_p is the binding potential relative to the plasma kinetic of ^{11}C -buprenorphine. DVR is the distribution volume ratio estimated with the Logan reference method using the occipital cortex as the pseudo-reference region. EC_{50} is the estimated plasma concentration of buprenorphine associated to

50% of buprenorphine brain receptor occupancy. *NA* = non-applicable, † = poorly estimated. Data are expressed as mean (receptor occupancy) or mean ± SD. Estimated EC₅₀ are reported as mean (confidence interval 95%).

1 **Figures**

2

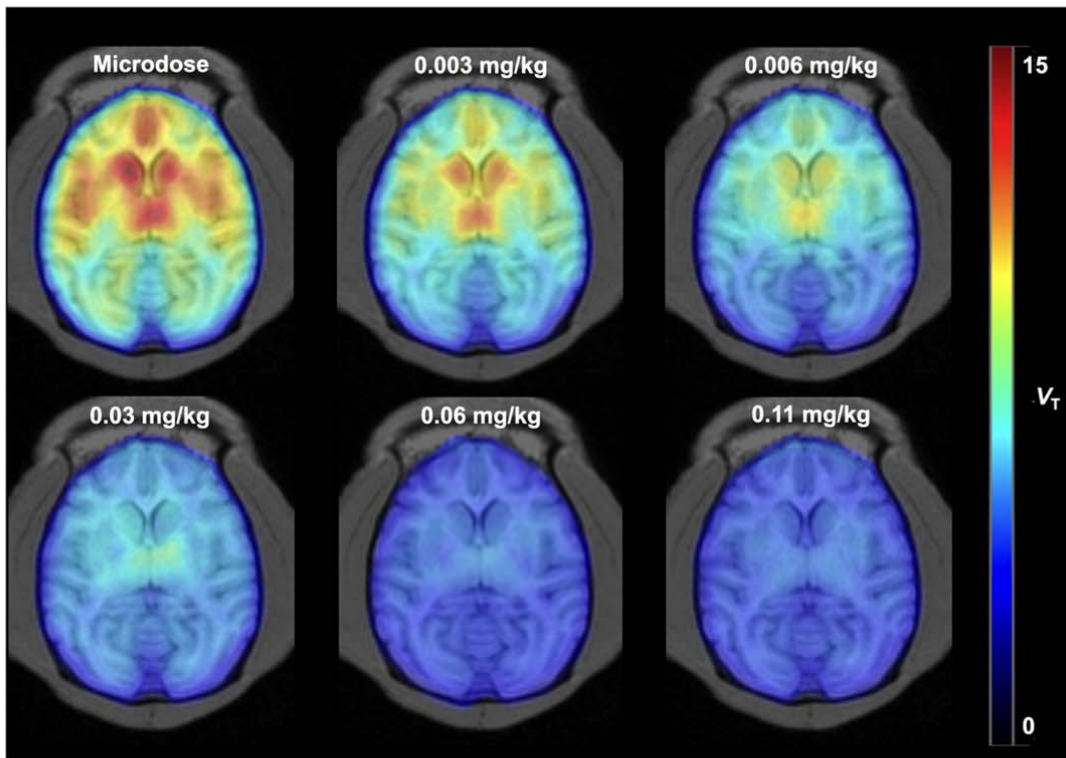
3 **Figure. 1**



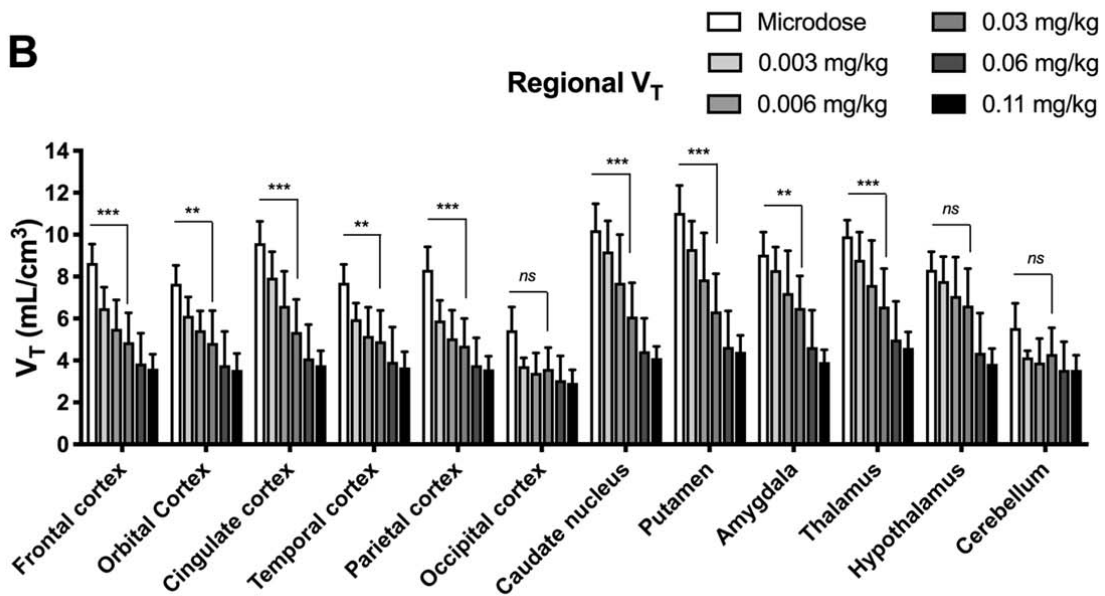
4
5

1 **Figure 2**

A



B



2

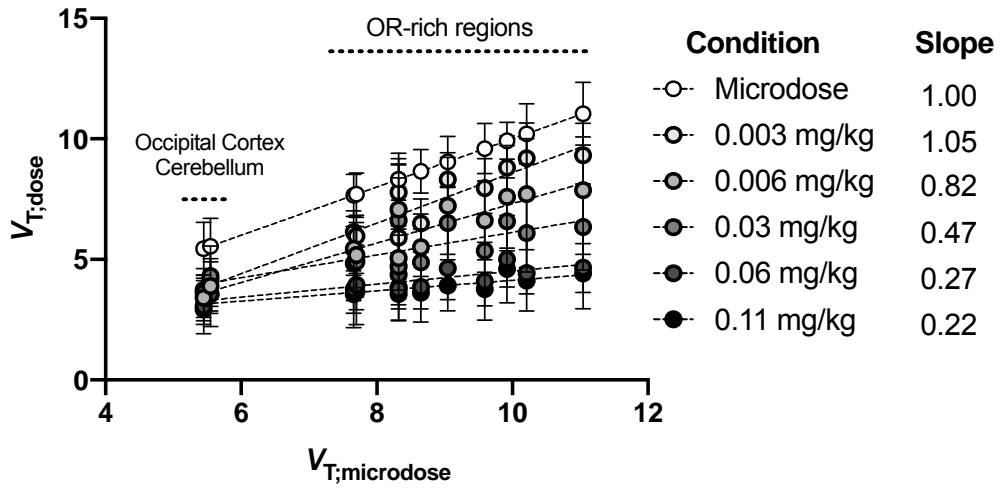
3

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1 **Figure 3**

2

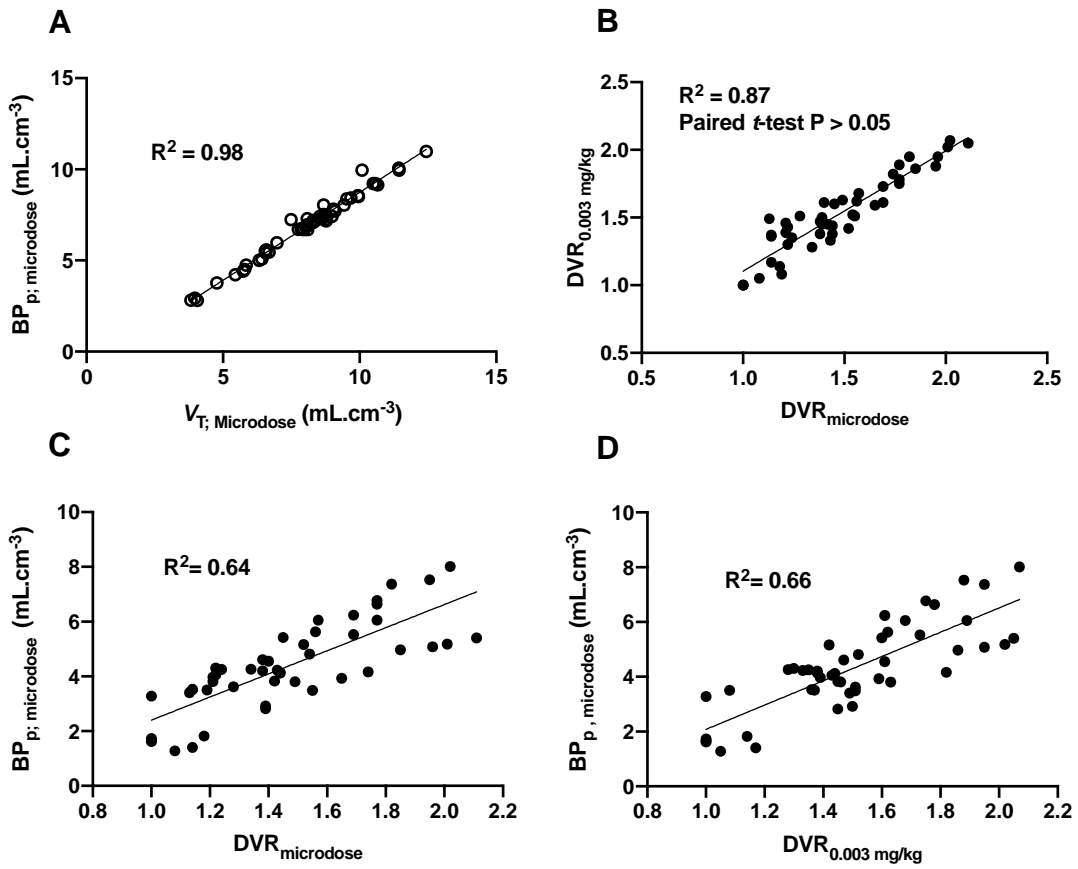


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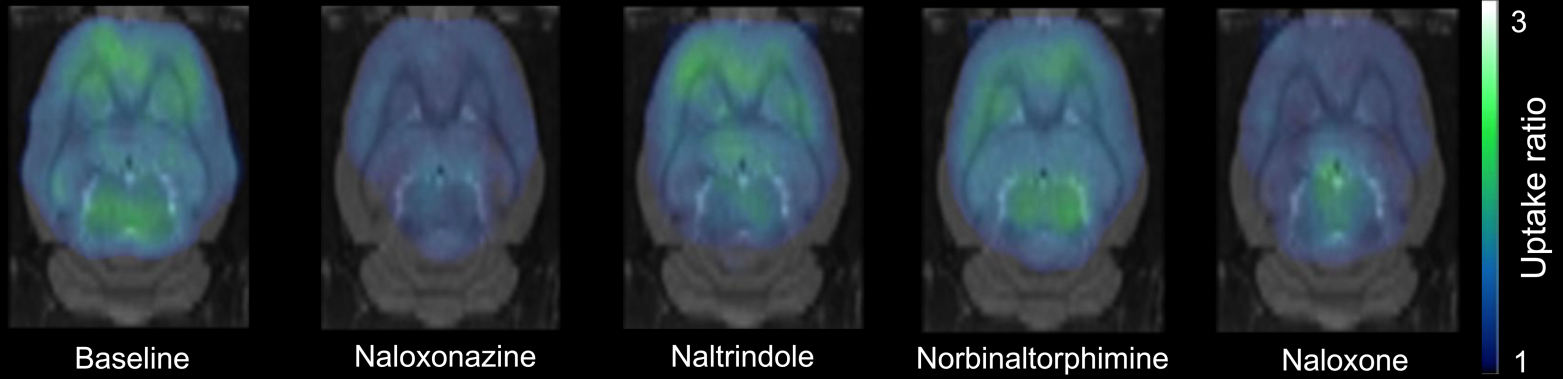
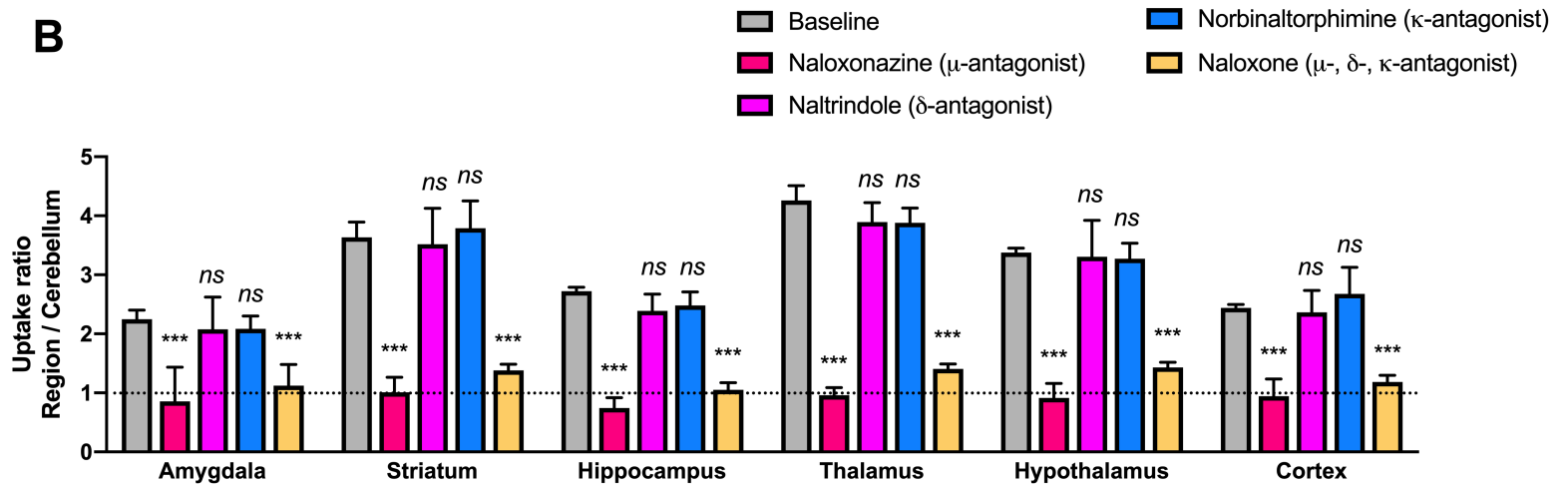
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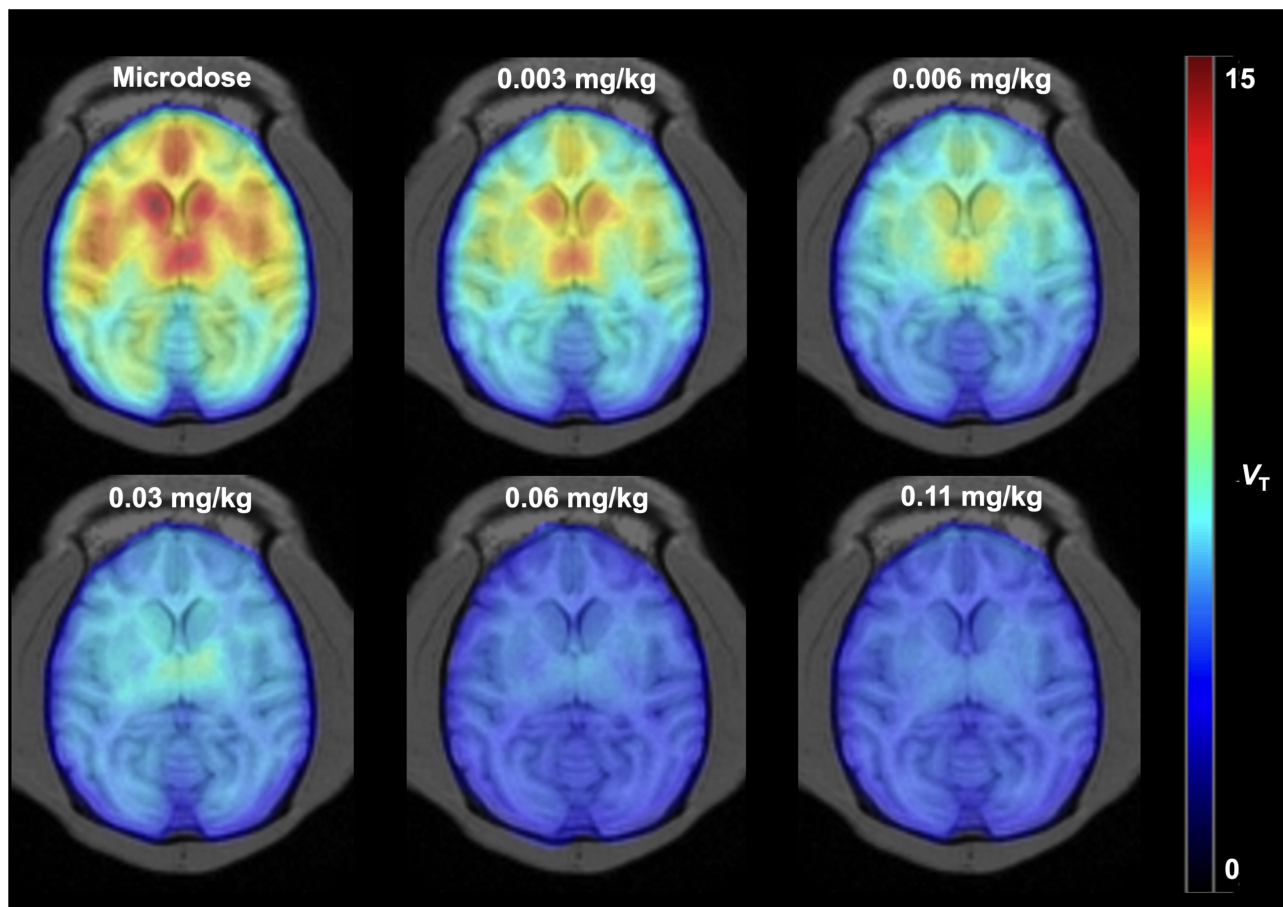
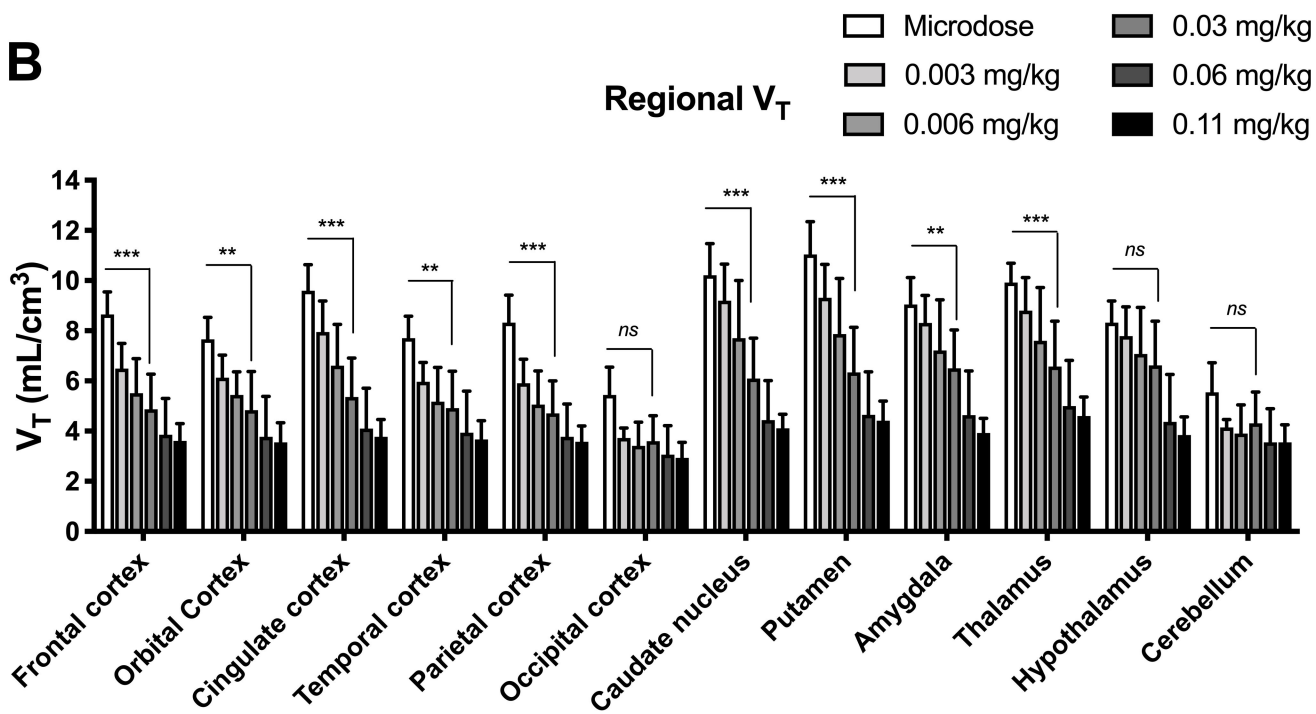
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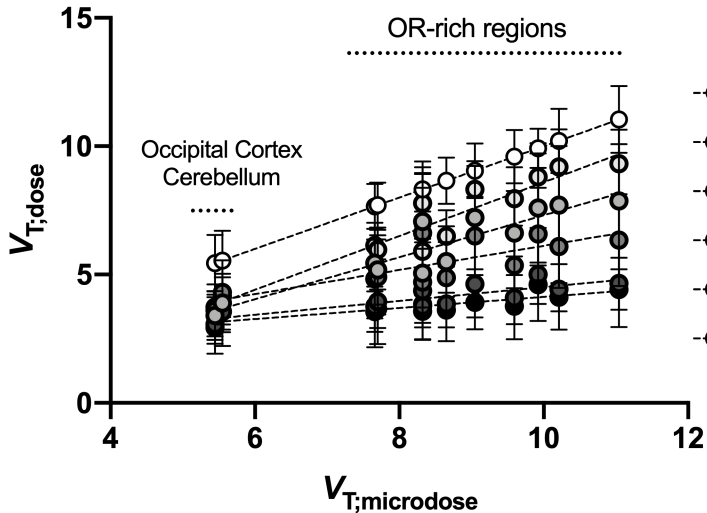
1 **Figure 4**



2

A**B**

A**B**



Condition	Slope
○ Microdose	1.00
○ 0.003 mg/kg	1.05
● 0.006 mg/kg	0.82
● 0.03 mg/kg	0.47
● 0.06 mg/kg	0.27
● 0.11 mg/kg	0.22

