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THE ALPHA$_{2C}$-ADRENOCEPTOR AS A NEUROPSYCHIATRIC DRUG TARGET – PET STUDIES IN HUMAN SUBJECTS

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ACADEMIC DISSERTATION

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ABSTRACT

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The alpha$_{2C}$-adrenoceptor as a neuropsychiatric drug target – PET studies in human subjects

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Positron emission tomography imaging has both academic and applied uses in revealing the distribution and density of different molecular targets in the central nervous system. Following the significant progress made with the dopamine D_2 receptor, advances have been made in developing PET tracers to allow analysis of receptor occupancy of many other receptor types as well as evaluating changes in endogenous synaptic transmitter concentrations of transmitters e.g. serotonin and noradrenaline.

Noradrenergic receptors are divided into α_1-, α_2- and β-adrenoceptor subfamilies, in humans each of which is composed of three receptor subtypes. The α_2-adrenoceptors have an important presynaptic auto-inhibitory function on noradrenaline release but they also have postsynaptic roles in modulating the release of other neurotransmitters, such as serotonin and dopamine. One of the subtypes, the α_{2C}-adrenoceptor, has been detected at distinct locations in the central nervous system, most notably the dorsal striatum. Several serious neurological conditions causing dementia, Alzheimer's disease and Parkinson's disease have been linked to disturbed noradrenergic signaling. Furthermore, altered noradrenergic signaling has also been implicated in conditions like ADHD, depression, anxiety and schizophrenia.

In order to benefit future research into these central nervous system disorders as well as being useful in the clinical development of drugs affecting brain noradrenergic neurotransmission, validation work of a novel tracer for positron emission tomography studies in humans was performed. Altogether 85 PET imaging experiments were performed during four separate clinical trials. The repeatability of [^{11}C]ORM-13070 binding was tested in healthy individuals, followed by a study to evaluate the dose-dependent displacement of [^{11}C]ORM-13070 from α_{2C}-adrenoceptors by a competing ligand, and the final two studies examined the sensitivity of [^{11}C]ORM-13070 binding to reflect changes in endogenous noradrenaline levels.

The repeatability of [^{11}C]ORM-13070 binding was very high. The binding properties of the tracer allowed for a reliable estimation of α_{2C}-AR occupancy by using the reference tissue ratio method with low test-retest variability. [^{11}C]ORM-13070 was dose-dependently displaced from its specific binding sites by the subtype-nonselective α_2-adrenoceptor antagonist atipamezole, and thus it proved suitable for use in clinical drug development of novel α_{2C}-adrenoceptor ligands e.g. to determine the best doses and dosing intervals for clinical trials. Convincing experimental evidence was gained to support the suitability of [^{11}C]ORM-13070 for detecting an increase in endogenous synaptic noradrenaline in the human brain. Tracer binding in the thalamus tended to increase in accordance with reduced activity of noradrenergic projections from the locus coeruleus, although statistical significance was not reached. Thus, the investigation was unable to fully validate [^{11}C]ORM-13070 for the detection of pharmacologically evoked reductions in noradrenaline levels.

Key words: PET, noradrenaline, ketamine, atipamezole, dexmedetomidine

TIIVISTELMÄ

Jussi Lehto

Alfa$_{2C}$-adrenoseptori neuropsykiatristen lääkkeiden kohdemolekyylinä – PET-tutkimuksia terveillä vapaaehtoisilla

Turun yliopisto, Biolääketieteen laitos, Farmakologia, lääkekehitys ja lääkehoito

Positroniemissiotomografiaa käytetään yleisesti akateemisten tutkijoiden ja teollisuuden tarpeisiin, kun halutaan selvittää keskushermoston reseptorimolekyylien esiintymistiheyttä eri aivoalueilla. Dopamiinin D_2-reseptorien PET-kuvantamiseen liittyneen tieteellisen edistyksen myötä metodia on alettu soveltaa myös muihin reseptoreihin ja välittäjäaineisiin. PET:llä voidaan havainnoida eri reseptoreihin vaikuttavien lääkeaineiden sitoutumisasteen lisäksi ihmisen omien aivovälittäjäainepitoisuuksien muutoksia, eli hermovälitystä esimerkiksi serotoniini– ja dopamiini-välittäjäainejärjestelmissä.

Noradrenaliinin reseptorit jaetaan α_1-, α_2- ja β-reseptoreihin, ja ihmisillä nämä jaetaan edelleen kolmeen alatyyppiin. Noradrenaliinin α_2-reseptorit vaikuttavat pääasiallisesti noradrenergisten hermosolujen hermopäätteissä hilliten itsesäätelyn kautta noradrenaliinin vapautumista synapsirakoon, mutta niillä on havaittu olevan myös säätelevää vaikutusta muihin välittäjäainejärjestelmiin, esimerkiksi serotoniinin ja dopamiinin vapautumiseen. Tätä vaikutusta välittävät ko. hermopäätteissä olevat α_2-reseptorit. Reseptoreiden α_{2C}-alatyyppiä on ihmisillä merkittäviä määriä vain tietyillä aivoalueilla, ennen kaikkea tyvitumakkeissa. Dementoivien neurologisten sairauksien osalta mm. Alzheimerin tauti ja Parkinsonin tauti on liitetty häiriintyneeseen noradrenaliinihermovälitykseen, ja tämän välittäjäainejärjestelmän häiriöihin on liitetty myös psykiatrisia sairauksia, kuten masennus, ahdistuneisuus ja skitsofrenia.

Väitöskirjatyön aikana validoitiin uusi noradrenaliinin α_{2C}-PET-merkkiaine ihmiskäyttöön. Merkkiaineen toivotaan edistävän sekä lääkekehitystä että neurologisten ja psykiatristen sairauksien tutkimusta. Väitöskirjatyön aineisto sisältää yhteensä 85 PET-kuvausta. Ensin terveillä vapaaehtoisilla tutkittiin [^{11}C]ORM-13070-merkkiaineen sitoutumisen toistettavuutta, jonka jälkeen testattiin merkkiaineen annosriippuvaista sitoutumisen vähenemistä samoihin α_{2C}-reseptoreihin sitoutuvan kilpailevan lääkeaineen vaikutuksesta. Lopuksi [^{11}C]ORM-13070:n herkkyyttä ilmentää noradrenaliinihermovälityksen muutoksia testattiin kahdessa eri tutkimuksessa.

[^{11}C]ORM-13070:n sitoutumisen toistettavuus oli erittäin hyvä, ja sitoutumisominaisuuksien katsottiin mahdollistavan α_{2C}-reseptoreiden miehitysasteen luotettavan arvioinnin vertaamalla vertailukudoksen ja kohdekudoksen radioaktiivisuutta. Samaan reseptoriin sitoutuva salpaajamolekyyli, atipametsoli, kykeni syrjäyttämään [^{11}C]ORM-13070:n kohdereseptoristaan annosriippuvaisella tavalla. Täten merkkiaine todettiin käyttökelpoiseksi työkaluksi uusien α_{2C}-reseptoreihin sitoutuvien lääkkeiden kliinisessä kehitystyössä. [^{11}C]ORM-13070:lla on mm. mahdollista määrittää uusien lääkkeiden sopiva annostus ja annosväli. Lisäksi saatiin vahvaa näyttöä merkkiaineen käyttökelpoisuudesta noradrenaliinihermovälityksen lisääntymisen havaitsemiseen ihmisillä. Viimeisessä osatyössä saatiin viitteitä nukutusaine deksmedetomidiinin aiheuttamasta noradrenaliini-hermovälityksen vähenemisestä talamuksen alueella sopien locus coeruleuksesta nousevan hermovälityksen vähenemiseen tällä alueella, mutta tilastollista merkitsevyyttä ei saavutettu, ja tieteellisesti vakuuttava näyttö merkkiaineen käyttökelpoisuudesta vähentyneen noradrenaliinin havaitsemisessa jäi saamatta.

Avainsanat: PET, noradrenaliini, ketamiini, atipametsoli, deksmedetomidiini

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ABBREVIATIONS

[^{11}C]MNPA	(R)-2-CHO-N-n-propylnorapomorphine
[^{11}C]PiB	^{11}C-labelled Pittsburgh compound B
[^{18}F]FPTC	^{18}F-fluorinated 1-((9H-carbazol-4-yl)oxy)-3-4(4-((2-(2-(fluoromethoxy)-ethoxy)methyl)-1H-1,2,3-triazol-1-yl)propan-2-ol
[B]	Concentration of bound ligand
5-HT	5-Hydroxytryptamine (serotonin)
ACh	Acetylcholine
AD	Alzheimer's disease
ADHD	Attention deficit hyperactivity disorder
Aβ	Amyloid beta
AMPT	Alpha-methyl-p-tyrosine
AR	Adrenoceptor
BBB	Blood-brain barrier
B/F	Bound per free
B_{max}	Receptor density
BP	Binding potential
BL	Baseline
ICC	Intra-class correlation coefficient
CBF	Cerebral blood flow
CSF	Cerebrospinal fluid
CT	Computed tomography
DAT	Dopamine transporter
DSTR	Dorsal striatum
EC	Ethics Committee
FDG	2-deoxy-2-[^{18}F]fluoro-D-glucose
GABA	Gamma-aminobutyric acid
GCP	Good Clinical Practice
GCPR	G-protein-coupled receptor
HD	High dexmedetomidine
IC50	Half maximal inhibitory concentration
ICU	Intensive care unit
I.v.	Intravenous
K_D	Dissociation constant
LC	Locus coeruleus
LD	Low dexmedetomidine
MHPG	3-Methoxy-4-hydroxyphenylglycol
MRI	Magnetic resonance imaging
nbM	Nucleus basalis of Meynert
NET	Noradrenaline transporter
MOR	μ-opioid receptors
ORM-13070	1-[(*S*)-1-(2,3-dihydrobenzo[1,4]dioxin-2-yl)methyl]-4-(3-^{11}C-methoxymethylpyridin-2-yl)-piperazine
PD	Parkinson's disease

PET	Positron emission tomography
PFC	Pre-frontal cortex
PK	Pharmacokinetic
PPI	Pre-pulse inhibition
ROI	Region of interest
SERT	Serotonin transporter
SNRI	Serotonin-noradrenaline re-uptake inhibitor
SPM	Statistical parametric mapping
SRTM	Simplified reference tissue model
$t_{1/2}$	Half-life
TAC	Time-activity curve
TCA	Tricyclic antidepressant
TCI	Target-controlled infusion
VAS	Visual analog scale
VSTR	Ventral striatum
VTA	Ventral tegmental area

LIST OF ORIGINAL COMMUNICATIONS

I. Lehto J, Virta JR, Oikonen V, Roivainen A, Luoto P, Arponen E, Helin S, Hietamaki J, Holopainen A, Kailajärvi M, Peltonen J, Rouru J, Sallinen J, Virtanen K, Volanen I, Scheinin M and Rinne, J (2015). Test-retest reliability of [^{11}C]-ORM-13070 in PET imaging of alpha2C-adrenoceptors in vivo in the human brain. *Eur J Nucl Med Mol Imaging* **42:** 120-127.

II. Lehto J, Hirvonen MM, Johansson J, Kemppainen J, Luoto P, Naukkarinen T, Oikonen V, Arponen E, Rouru J, Sallinen J, Scheinin H, Vuorilehto L, Finnema S, Halldin C, Rinne J and Scheinin M (2015). Validation of [^{11}C]ORM-13070 as a PET tracer for alpha2c -adrenoceptors in the human brain. *Synapse* **69:** 172-181.

III. Lehto J, Johansson J, Vuorilehto L, Luoto P, Arponen E, Scheinin H, Rouru J and Scheinin M (2015). Sensitivity of [^{11}C]ORM-13070 to increased extracellular noradrenaline in the CNS - a PET study in human subjects. *Psychopharmacology (Berl)* **232:** 4169-4178.

IV. Lehto J, Scheinin A, Johansson J, Marjamäki P, Arponen E, Scheinin H and Scheinin M. Detecting a dexmedetomidine-evoked reduction of noradrenaline release in the human brain with the alpha$_{2C}$-adrenoceptor PET ligand [^{11}C]ORM-13070. *Synapse. Accepted for publication.*

1. INTRODUCTION

One of the consequences of *in vivo* binding studies of antipsychotic drugs in areas with abundant dopaminergic innervation, was the concept of assessing endogenous transmitter levels with positron emission tomography (PET) as first postulated by Friedman *et al.* in 1984. During the following decades, a large number of PET tracers have been developed which target many types of neurotransmitter receptors in the human central nervous system (CNS). Researchers operating in both academia and the pharmaceutical industry have obtained novel opportunities to investigate many types of CNS drug targets such as G-protein coupled receptors (GPCRs) and monoamine transporters *in vivo.* For example, this had made it possible to achieve a smoother translation of results from *in vitro* cell and tissue culture models and animal experiments into a better understanding of receptor binding profiles and pharmacodynamic effects in humans. PET has often been able to provide crucial information to assist in the clinical development of new pharmaceuticals. As an example, nalmefene, an opioid receptor antagonist used to treat alcohol addiction, was found to exhibit significant occupancy of the target receptors in the brain still 24 h after oral drug administration (Ingman et al., 2005), even if at that time, the drug concentration in blood was only a fraction of the peak concentration. This result was used to provide a justification for a once daily administration schedule, which was ideal in terms of treatment compliance.

Academic research has also benefited from the development of PET methods since it is now possible to study altered binding profiles of receptor subtype-selective PET tracers, and thereby to draw conclusions about the location and nature of CNS pathology associated with many psychiatric and neurological diseases. In particular, this has advanced the study of schizophrenia (Breier et al., 1997; Laruelle et al., 2000; Howes et al., 2012), largely attributable to the existence of a relatively long-standing, validated method to investigate dopamine D_2-receptors, i.e. the PET tracer [^{11}C]raclopride.

Disturbed noradrenaline neurotransmission has been implicated in many neuropsychiatric and neurodegenerative disorders (Scheinin et al., 2001; Marien et al., 2004, Alsene et al., 2011) and many of the currently used treatments for depression and attention deficit/hyperactivity disorder (ADHD), i.e. tricyclic antidepressants and selective noradrenaline transporter inhibitors such as atomoxetine, exert their pharmacodynamic effects by modulating the synaptic availability of noradrenaline.

The goal of this thesis project was to validate a novel PET tracer which could target noradrenergic α_{2C}-adrenoceptors (ARs) to be used in clinical trials aiming to develop new treatments for CNS disorders involving noradrenergic disturbances, and also to develop a sensitive tool for monitoring fluctuations in synaptic concentrations of noradrenaline, which could be used to investigate the pharmacodynamic effects of CNS drugs on synaptic noradrenaline levels, and also various diseases affecting synaptic noradrenaline concentrations and/or the availability of α_{2C}-AR binding sites in humans.

2. REVIEW OF THE LITERATURE

2.1. POSITRON EMISSION TOMOGRAPHY (PET)

2.1.1. BASIC PRINCIPLES OF PET

Positron emission tomography (PET) imaging uses small quantities of radioactive substances, referred to as tracers, which accumulate in different organs and regions of the body according to each tracer's individual properties. A tracer molecule has two functional components: its molecular nature determines its distribution in the living organism, and a short-lived positron-emitting radionuclide allows its quantitation within the investigated tissues *in vivo*.

Radionuclides most commonly utilized in PET tracers are ^{11}C, ^{13}N, ^{18}F and ^{15}O. The most important difference between these radiolabels is their radiochemical half-life ($t_{1/2}$), which represents an important practical difference between these positron emitters, in part determining their usefulness for different imaging protocols. The radiochemical $t_{1/2}$ of the four labels are approximately 20 min, 10 min, 110 min and 2 min, respectively. As the radionuclide decays, it emits a positron (a positively charged subatomic particle with the same mass as the electron), which then travels in the surrounding tissue until it collides with an electron. An annihilation reaction (Fig. 1) then ensues, which produces two 511 keV ɣ-rays, i.e. photons, which travel in opposite directions.

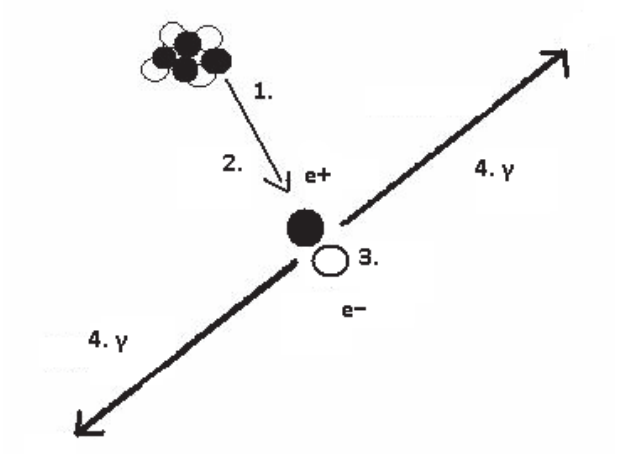

Figure 1. Annihilation reaction: 1. A positron is emitted from the decaying radionuclide contained in the tracer molecule 2. The positron travels in tissue 3. An annihilation reaction with an electron in the tissue 4. Emission of two 511 keV gamma rays in opposite directions.

As the two emitted photons simultaneously hit two detector crystals at opposite sides of the PET camera, a coincidence event is registered. As multiple coincidence events are registered over a period of time, a dynamic 4D PET image with spatial modalities as well as a time modality can be computationally reconstructed. The accuracy of the reconstructed image is affected by the travel

distance of the positron before it collides with an electron, i.e. the positron range, which is a limiting factor in the spatial resolution of a PET image, and which depends on the radionuclide itself. Other important factors determining the spatial resolution include the annihilation photon non-collinearity, off-axis detector penetration, detector Compton scatter, and under-sampling of the signal for the image reconstruction process. Reducing the size of the detector crystals has improved spatial resolution up to a point where other factors start to limit further advances achievable by this method (Levin and Hoffman, 1999).

Another important way of increasing PET image quality is to restrict the movement of the target (the subject). In brain imaging, this is typically achieved by applying an individually molded thermoplastic mask on the subject's face. This mask is then fastened to the examining table, effectively preventing any large-scale movement by the subject. Additional movement correction can be performed during the pre-processing of PET data and additionally, subject movement is also typically registered by an infrared camera.

PET tracers are usually administered in low microgram quantities that are devoid of pharmacological effects of their own, because of the very low target occupancy that is achieved. This is important because the tracer should not perturb the system under investigation. Low doses are enabled by advanced radiosynthetic methods providing high specific radioactivity, which means that the proportion of the tracer molecules containing the desired radiolabel is relatively high. Nonetheless, the best methods are only able to achieve a small fraction of the theoretical maximum, which would be one mole of radionuclide per mole of tracer. With ^{11}C, specific radioactivity in excess of approximately 200 GBq/µmol is typically considered "high", i.e. sufficient, even though less than 1 out of every 10,000 tracer molecules are radiolabeled while the rest contain ^{12}C. For example, In the case of the ^{11}C-labeled tracer raclopride, ultra-high specific activity in the 5000 GBq/µmol range was not found to affect binding estimates in comparison to the standard radiosynthetic method yielding compounds with high specific activity (Fujimura et al., 2010).

These above factors apply to PET tracers that bind to specific low-abundance targets in the body, such as neurotransmitter receptors and transporters, but it does not apply to all PET tracers. For example, 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) until now has been the most successful PET tracer in terms of volume of studies. This radiolabelled glucose analog has a wide array of indications in diagnostics and also in studies on tissue metabolism. As the tracer does not bind to any specific receptor-type binding site, there is no similar stringent demand for high specific activity. ^{18}F-FDG is taken up into tissues as a glucose analog and small quantities of this tracer do not affect the processes involved in glucose uptake, storage and metabolism. Thanks to its widespread use, the quality system of ^{18}F-FDG production is at a very high level and much effort has been put into developing efficient radiosynthetic methods. As a result, ^{18}F-FDG radiosynthesis is among the most repeatable of all PET tracers, and radiochemical yields of approximately 50-60% are routinely achieved (Yu, 2006).

2.1.2. DIAGNOSTIC APPLICATIONS OF PET

PET combined with computed X-ray tomography (CT), i.e. PET-CT scanning, is an imaging tool capable of gathering functional (PET) and anatomical (CT) information. The ability to detect small metabolically active targets otherwise undetectable by traditional imaging methods is especially valuable in oncology. The most common diagnostically used radiotracer is ^{18}F-FDG (Fig. 3). Its uptake is increased in all metabolically active sites in which there is increased glucose metabolism, including rapidly dividing cancer cells or sites of active inflammation. The latest application of PET imaging is PET-MRI, which combines functional imaging with the superior soft tissue resolution of magnetic resonance imaging (MRI) and exposes the subject to a smaller total dose of ionizing radiation than PET-CT. The disadvantages of PET-MRI include the higher cost and longer scanning times compared to PET-CT.

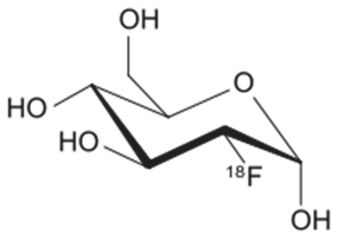

Figure 2. The chemical structure of 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG).

^{18}F-FDG-PET was introduced as a clinical tool in oncology in the 1980s to assist in diagnosing and staging of cancers, but it was not until 1997 when ^{18}F-FDG-PET became an established imaging tool in oncology after approval for its commercial manufacturing and distribution was granted by the US Food & Drug Administration (FDA). The usefulness of ^{18}F-FDG-PET in oncology derives from its high sensitivity, as functional alterations precede detectable structural changes, thus allowing earlier and more sensitive diagnosis. Even although conventional CT and MRI still remain the primary tools in first-line tumor imaging, PET has a confirmed place in cancer staging and follow-up, when the initial diagnosis has already been established. PET imaging, if used alone, results in low diagnostic specificity, and PET should mainly be used in conjunction with CT or MRI (Kitson et al., 2009). ^{18}F-FDG-PET improves the diagnostic accuracy over traditional imaging methods in many malignancies including lung cancer, colorectal cancer, cancer of the esophagus, breast cancer, head and neck cancers, lymphomas, sarcomas and melanoma (Czernin, 2002). The isotope-labelled amino acid [^{11}C]methionine has been used to detect hormonally active parathyroid adenomas with high sensitivity (Oksüz et al., 2011). [^{18}F]FDOPA is also used to image neuroendocrine tumors and it has been recommended that the tracer should be the first choice in PET/CT imaging of medullary thyroid carcinomas (Slavikova et al., 2013).

The increased ^{18}F-FDG uptake in sites of inflammation may lead to difficulties in the differential diagnosis in oncology. However, PET may also have useful clinical applications in the diagnosis and

treatment of infectious diseases such as tuberculosis or fever of unknown origin, but further research in this field is required (Kitson et al., 2009). ^{18}F-FDG-PET was estimated to exhibit an accuracy of >90% in evaluating chronic osteomyelitis and infections surrounding orthopedic hip prostheses, as well as in detecting or excluding underlying osteomyelitis related to soft tissue infections (Chacko et al., 2003).

Other more recent applications of ^{18}F-FDG-PET include imaging of the brain affected by neurodegenerative diseases, such as Alzheimer's disease (AD) and the heart after myocardial ischemia. ^{18}F-FDG-PET is the most sensitive method to perform imaging of a viable myocardium, but it lacks specificity compared to the more commonly employed echocardiography methods (Di Carli, 2002). The differential uptake of glucose in normal and in ischemic myocardium has also led to the experimental use of ^{18}F-FDG as an imaging agent for exercise-induced myocardial ischemia (Jain and He, 2008). Since the uptake of [^{11}C]acetate into the tricarboxylic acid cycle is directly correlated with oxygen consumption in myocardial cells, this tracer can be used to quantify oxygen consumption in the myocardium (Visser, 2001). Cardiac output and myocardial mass can also be quantified with PET or with a simultaneous ultrasound or MRI assessment, and together with oxygen consumption, these variables can be used to calculate the mechanical efficiency of the heart, e.g. the extent to which it has been damaged in pathological states like heart failure. It has been hypothesized that inefficient energy expenditure is involved in disease progression (Knaapen et al., 2007).

The diagnosis of Parkinson's disease (PD) is traditionally made based on clinical symptoms and typical disease progression, but differentiating early-stage PD from other diseases that cause Parkinsonian symptoms, including essential tremor, Lewy body dementia and supranuclear palsy, is not always possible by conventional methods. PET tracers, such as [^{18}F]DOPA, which are taken up into dopaminergic brain cells, and [^{18}F]PE2I and [^{11}C]PE2I, which bind to dopamine transporters (DAT), can be employed in the differential diagnosis. Decreased [^{18}F]DOPA uptake in the putamen is a typical characteristic of PD (Kitson et al., 2009).

In AD, asymmetric disturbances affecting the association neocortices but sparing the primary sensory and motor neocortices precede and predict deficits in cognitive functions in the early stages of this disease, indicating that PET could be used for its early diagnosis. In late-stage AD, disturbances in glucose metabolism correlate with the regional densities of neurofibrillary tangles but not of senile plaques (Rapoport et al., 1991). ^{11}C-labelled Pittsburgh compound B ([^{11}C]PiB) PET can be used to image cortical amyloid beta (Aβ) deposition *in vivo*. [^{11}C]PiB has been successfully used for the imaging of possible treatment-related reductions in the Aβ load in AD patients who had received the anti-Aβ antibody bapineuzumab or a placebo for 78 weeks (Rinne et al., 2010). In subjects with mild cognitive impairment (MCI), increased [^{11}C]PIB retention in the frontotemporal regions and anterior and posterior cingulate predicted conversion to AD (Brück et al., 2013). Unfortunately, the clinical significance of early detection methods for AD has been reduced by the non-availability of disease-modifying treatments i.e. the present therapies have only limited value in delaying the need for institutional care and cannot significantly decelerate disease progression. Further research is needed to develop better tools for early detection of AD but especially to devise effective disease-modifying medications.

2.1.3. PET PHARMACOKINETICS AND MODELLING OF RADIOLIGAND UPTAKE

The raw data in PET imaging consist of data points containing information about location, time and radioactivity; this is usually converted to regional time-activity curves (TACs, Fig. 3) and expressed as units of radioactivity in a given tissue volume over a defined time window (e.g. as Bq × cm^{-3} × min^{-1}). This data registered by the PET camera is sometimes related to plasma TACs (e.g. expressed as Bq × ml^{-1} × min^{-1}) produced by registering the radioactivity present in arterial blood that is collected with a blood pump and/or manual sampling from an arterial cannula during the scan. The behaviour of a radiotracer in these compartments, i.e. the tissue compartments of interest and the plasma compartment can be modelled with varying degrees of complexity and the method required is dependent on the characteristics of each tracer. Many of these tracers' kinetics can be modelled in several different methods that yield rather similar outcomes.

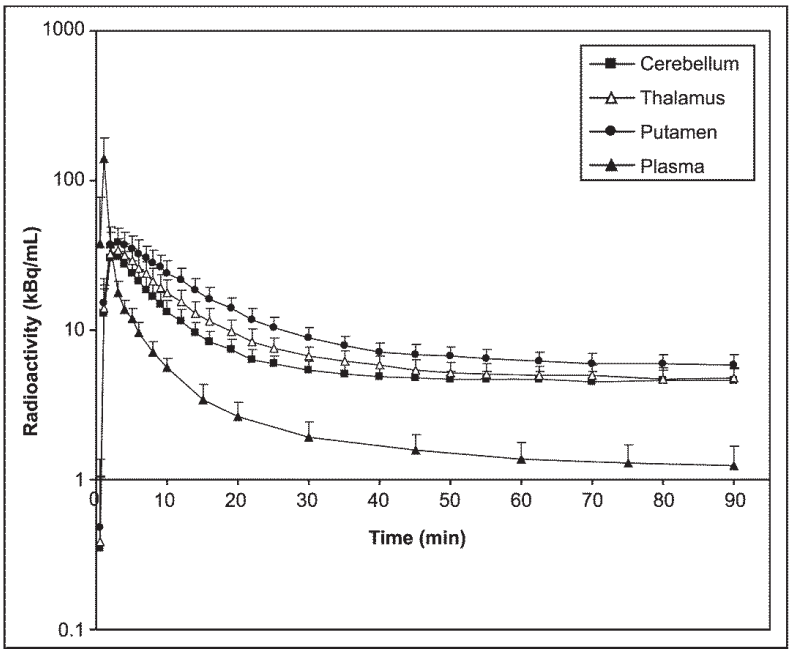

Figure 3. Time radioactivity curves (TACs) measured in arterial plasma, putamen, thalamus and cerebellum after an intravenous bolus injection of a carbon-11 labeled tracer (Original communication I).

The binding of a receptor ligand to its cognate receptor under equilibrium conditions can be expressed with equation 1 (Eq. 1), where the amount of bound ligand [B] is determined by the amount of available binding sites, i.e. receptor density [B_{max}], the dissociation constant K_D and the concentration of the free radioligand [F].

Eq. 1 $$[\mathrm{B}] = \frac{[Bmax]\times[F]}{KD+[F]}$$

In the evaluation of tracer pharmacokinetics, [F] is typically neglected. This is permissible when the tracer has high specific activity, i.e. the proportion of the radioactively labeled ligand is high compared to the total free ligand concentration (sum of labeled and non-labeled or "cold" ligand); this allows for a very low overall ligand concentration without sacrificing the required amount of bound radioactivity. Very low levels of bound radioactivity would inevitably reduce the ratio of true signal-to-noise. In PET imaging, Eq. 1 is therefore reduced to Eq. 2, where the hyperbolic binding curve is converted into a linear relationship.

Eq. 2 $$\frac{B}{F} = \frac{Bmax}{KD} = \mathrm{BP}$$

The term "binding potential" (BP, Eq. 2) was first defined in PET imaging by Mintun et al. (1984) as the ratio B_{max}/K_D. As explained above, with tracer doses of a receptor-binding ligand, this ratio is equal to the ratio of bound to free tracer concentrations at equilibrium between the tissue compartments. When BP is designated without a subscript, this generally represents an *in vitro* measurement of the B_{max}/K_D ratio, whereas BPs with subscripts (BP_P, BP_F and BP_{ND}) usually refer to results of *in vivo* PET measurements obtained with different modeling techniques, i.e. techniques that use the total tracer-associated (metabolite-corrected) radioactivity concentration in arterial plasma, the protein-unbound (free) concentration in plasma and the non-displaceable radioactivity uptake in a reference tissue, respectively, as reference; they reflect but never completely equal the *in vitro* measured BP (Innis et al., 2007).

In traditional pharmacological terminology, the model displayed in Figure 4 would be called a four compartment model.

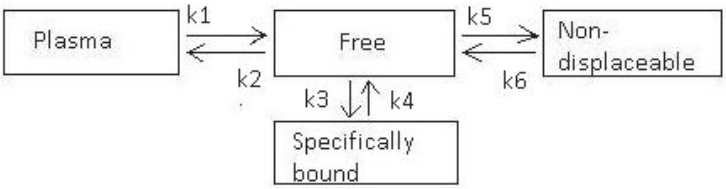

Figure 4. The four compartments of PET tracer pharmacokinetics. In situations where the non-displaceable and free compartments can be combined, only rate constants k_1, k_2, k_3 and k_4 are used.

In these models, there are four compartments; 1) the plasma compartment, 2) a compartment of free radioligand in tissue (assumed to be in equilibrium with the protein-unbound or free radioligand in plasma), 3) a compartment of non-displaceable (or non-specific) binding in the tissue of interest and 4) the compartment of specific binding, where the tracer binding to its target is thought to take place according to normal receptor kinetics (Eq. 1). In PET modeling, the nomenclature usually follows the number of tissue compartments, and the plasma compartment is often omitted even though with some PET tracers, this compartment has to be included in the model.

Therefore, a two-tissue compartment model in PET in which the free and non-displaceable compartments are combined translates to [Plasma] ↔ [Free + Non-displaceable] ↔ [Specifically bound] and a one-tissue compartment model translates to [Plasma] ↔ [Free + Non-displaceable +

Specifically bound] (Innis et al., 2007). This last model is employed in conjunction with different reference tissue models, where tracer uptake is quantified using the total amount of radioactivity in the target tissue in reference to a tissue known or assumed to display no specific binding of the tracer.

The two main modeling approaches in the analysis of raw PET data either include the use of an arterial input function or circumvent the need for arterial cannulation via the use of a reference tissue. An arterial input function enables the use of a direct kinetic approach, where the rate constants between the compartments (k_1, k_2, k_3, k_4 if two tissue compartments are used) are acquired by nonlinear least squares fitting to regional time activity curves using a model of 1-3 tissue compartments and a plasma compartment which, depending on the properties of the tracer, can be metabolite-corrected, as well as subdivided into free and protein-bound compartments.

There are several techniques of using a reference tissue devoid of specific binding in PET studies. The cerebellum has been found to be suitable for this purpose and this brain area is used with many brain receptor tracers, such as the dopamine D_2 receptor tracer [^{11}C]raclopride and the μ-opioid receptor tracer [^{11}C]carfentanil. The advantages of reference tissue models include their minimal invasiveness and the rather straightforward modeling, i.e. there is no need for a metabolite-corrected arterial plasma function. Their possible disadvantages include the assumption that non-specific, non-displaceable binding is similar in the reference tissue as it is in the investigated brain region, which cannot always be fully verified, and that there are no treatment- or condition-related alterations in this non-specific tracer uptake.

The simplified reference tissue model (SRTM) emerged from the experiments conducted by Lammertsma and Hume (1996). The model is based on the following equations, where k'_1 and k'_2 are the rate constants for transfer of tracer from plasma to the reference tissue and *vice versa*, k_1 and k_2 are the rate constants for transfer from plasma to the free tissue compartment of the region of interest and *vice versa*, and k_3 and k_4 are the rate constants for transfer from the free compartment to the specifically bound compartment and *vice versa*. Subscripts p, r, f and b designate plasma, reference, free and specifically bound, respectively:

Eq. 3 $$dC_r(t)/dt = k'_1C_p(t) - k'_2C_r(t)$$

Eq. 4 $$dC_f(t)/dt = k_1C_p(t) - k_2C_f(t) - k_3C_f(t) + k_4C_b(t)$$

Eq. 5 $$dC_b(t)/dt = k_3C_f(t) - k_4C_b(t)$$

C_f and C_b cannot be directly determined and C_p can only be measured from arterial blood. The distribution volumes of the non-displaceable compartments in the target and reference regions are assumed to be equal, which results in k'_1/k'_2 being equal with k_1/k_2. If the free and specific compartments can be satisfactorily fitted into a single tissue compartment, Equations 4 and 5 can be replaced by Eq. 6, where k_{2t} represents the total rate constant from the specific compartment, i.e. [bound + free + non-displaceable], to plasma:

Eq. 6 $$dC_t(t)/dt = k_1C_p(t) - k_{2t}C_t(t)$$

BP_{ND} can be determined from the solved differential function (Eq. 7) derived from Eq. 3, Eq. 6 and the assumptions described above and require knowledge of only the following parameters: the ratio

(R) of k_1'/k_1 , K_2, C_t and C_r, where C_t is the total measured activity in the target region. K_1 and k_2 can be derived by non-linear regression from the TACs obtained from the reference region and the target region.

Eq. 7 $$C_t(t) = RC_t(t) + [k_2 - Rk_2/(1+BP_{ND})]C_r(t) \times e^{[-k2\ t/(1+BP)]}$$

Other reference tissue methods include the model-independent graphical approach, i.e. the Logan plot (Logan 1996) and the tissue ratio method, where the areas under the curve (AUCs) of radioactivity are compared between the region of interest (ROI) and the reference region according to Eq. 8, where $C_{specifically\ bound} = AUC_{ROI} - AUC_{ref}$.

Eq. 8 $$BP = \frac{\int_t^t C_{specifically\ bound}(t)dt}{\int_t^t C_{cerebellum}(t)dt}$$

There are several different ways for determining (t) in Eq. 8: the transient equilibrium (Farde et al., 1989) method uses the time point where the derivative for specific binding is zero. The interval method uses a pre-defined time interval, which commonly contains parts of the ascending and descending parts of the TACs and consequently also the point of transient equilibrium, and the late time method only utilizes the late part of the curve (Ito et al., 1998). The aforementioned approaches are all viable when the PET tracer is given as a rapid bolus injection; in the case of [^{11}C]raclopride, their results have been shown to agree relatively well with true equilibrium conditions (Ito et al., 1998). The labour-intensive process of defining the exact point of transient equilibrium is often omitted as this method has been found to introduce bias into the binding potential estimates (Ito et al., 1998). True equilibrium can be achieved with the bolus-infusion method, where a rapid tracer bolus is followed by a continuous intravenous infusion (Ito et al., 1998). The fraction of the total radioactivity dose given in the initial bolus is dependent on the kinetics of the tracer. The examined time interval is determined by the time it takes to reach equilibrium, which can take more than 60 minutes. In contrast, a typical total scanning time with a bolus approach is only 30-60 minutes, which explains why the bolus approach is often preferred in the clinical setting with human study subjects. Additionally, with ^{11}C-labelled receptor tracers, the amount of radioactivity required for long infusion protocols often exceeds the radioactive yields attainable. In addition, long infusions may increase the total administered tracer dose to unacceptable levels, both in terms of radioactivity and tracer mass.

When a reference tissue method is employed, there is no need to correct for the metabolism of the tracer or its binding to plasma proteins – as long as there are no treatment- or condition-related alterations in its metabolism. This can be ascertained by collecting venous plasma samples during the scans and requires the insertion of an additional venous cannula, but the invasiveness of this procedure is clearly less than that of arterial cannulation. However, in cases when a tracer is extensively metabolised, it is not always clear how the radioactive metabolites behave with regards to binding in the specific and non-specific compartments. This problem can be at least partly circumvented with the use of a robust ratio method, which cancels out any possible bias caused by radioactive metabolites as long as non-displaceable binding is equal in the ROI and in the reference tissue (Passchier et al., 2002). However, should the tracer or a radioactive metabolite bind to a receptor other than the target receptor, the ratio method would not be able to compensate for this property and this would introduce an error into the final result. Additionally, some radioactive

metabolites may accumulate in the non-displaceable compartment in the brain, which can result in reduced signal-to-noise ratios with longer scanning times.

2.1.4. THE OCCUPANCY MODEL AND ITS APPLICATION TO NEUROTRANSMITTER SYSTEMS

The theoretical basis for using PET to examine synaptic neurotransmission *in vivo* is referred to as the occupancy model, where changes in tracer BP are assumed to be a direct result of a changing concentration of an endogenous transmitter occupying the receptor binding sites (Laruelle 2000). The model predicts that as the endogenous transmitter concentration increases, tracer binding should decrease accordingly and *vice versa*. In this technique, researchers are able to monitor acute and chronic fluctuations in neurotransmitter concentrations, for example in order to understand better the pathophysiology behind different psychiatric and neurological disorders affecting the central nervous system (CNS) in humans.

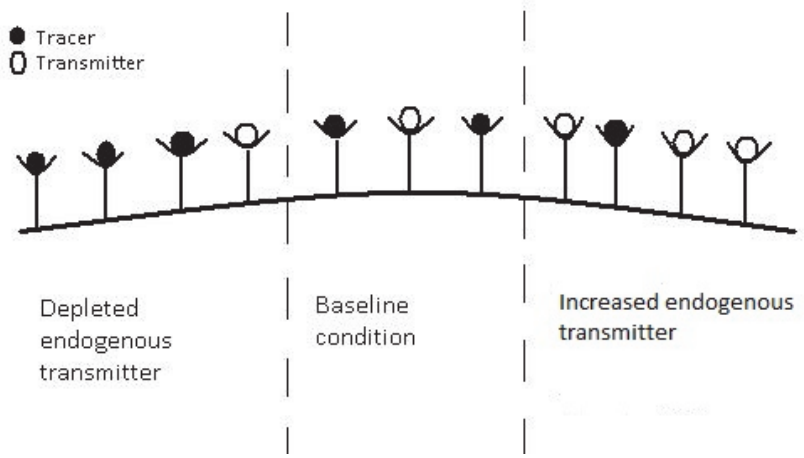

Figure 5. A schematic representation of the occupancy model. In the *in vivo* condition, receptor occupancy by the tracer is negligible but the proportional changes in tissue radioactivity and binding potential are comparable to the situation in the schematic presentation. Adopted from: Laruelle. J Cereb Blood Flow Metab. 2000.

The adoption of the occupancy model has also enabled monitoring of drug-induced changes in endogenous transmitter levels, which has provided information on CNS drug pharmacodynamics beyond target occupancy analysis. Following the initial success with dopamine (discussed below), increased effort has been put into conducting assessment of changes in extracellular concentrations of other neurotransmitters e.g. serotonin, noradrenaline (see original communications II-IV), γ-aminobutyric acid (GABA), acetylcholine (ACh), and opioid peptides. Here, as a supplement to the PET results, an additional exploration will be provided of the interaction of ketamine with dopaminergic neurotransmission (see below), as ketamine is a central drug in the context of this thesis work, while the other transmitter systems will be discussed more briefly.

Dopamine and its D_2-type receptor is the receptor-ligand pair which has been investigated most extensively with the occupancy model. While some results have been inconclusive, most studies

performed in non-human primates and human study subjects, where increased endogenous dopamine levels have been most commonly evoked by administration of either amphetamine or methylphenidate, comply with the occupancy model (Laruelle, 2000). Amphetamine, a drug with strong effects on synaptic monoamine concentrations including dopamine, has been reported to reduce the binding of [^{11}C]raclopride (Breier et al., 1997) and [^{123}I]IBZM (a SPECT ligand; Abi-Dargahan et al., 1998) significantly more in patients with schizophrenia in comparison with healthy controls. Studies have also linked decreased tracer uptake to transient increases in the positive symptoms of schizophrenia (Laruelle et al., 1996; Laruelle, 2000). SPECT experiments with the D_2 tracer [^{11}C]IBZM have demonstrated that radioligand binding can only be reduced when endogenous dopamine is present: administration of monoamine-depleting agents alpha-methyl-*p*-tyrosine (AMPT) and reserpine inhibited the amphetamine-induced reduction in specific radioligand binding (Innis et al., 1992; Laruelle et al., 1996). When measured with PET, AMPT-induced dopamine depletion caused a larger increase of [^{11}C]raclopride binding in the pre-commissural dorsal caudate nucleus in untreated schizophrenics compared to healthy controls, a finding which indicates that there is increased synaptic dopaminergic activity in the associative striatum in schizophrenia, but raises questions about the therapeutic relevance of the mesolimbic selectivity of 2nd generation antipsychotics (Kegeles et al., 2010).

Ketamine did not reduce the extent of striatal [^{11}C]raclopride binding in healthy human subjects in a parallel-group study design with ketamine and placebo groups, and there was no relationship between the hallucinogenic effects of ketamine and the amount of [^{11}C]raclopride BP (Aalto et al., 2002), even though ketamine is generally accepted as a clinical model for studying schizophrenia (Murray et al., 2013), a disorder associated with disturbed dopamine neurotransmission where positive symptoms correlate with increases in D_2-agonism; furthermore neuroleptics, which primarily function as D_2-antagonists, are efficacious in treating positive symptoms. Negligible effects on striatal dopamine release by ketamine have been also been reported in microdialysis experiments performed in non-human primates (Adams et al., 2002), and in rodents, NMDA antagonism has been found to mainly affect cortico-limbic dopamine release (Hertel et al., 1995; Adams and Moghaddam, 1998). Ketamine has been demonstrated to evoke the release of dopamine levels in the rat prefrontal cortex (PFC) (Lindefors et al., 1997), and chronic ketamine exposure has also been reported to increase D_1-receptor availability in the PFC (Narendran et al., 2005). These results suggest that while the dorsal striatum receives an extensive dopaminergic innervation, ketamine seems to affect primarily dopamine neurotransmission in (prefrontal) cortical areas. This conclusion is also supported by the aforementioned finding of elevated synaptic dopamine in the precommissural caudate in schizophrenia, as this area has functional connections with the PFC (Kegeles et al., 2010), thus providing a possible mechanism to explain the similarity in clinical symptoms. Baseline binding of the high-affinity antagonist $D_{2/3}$ radioligand [^{18}F]fallypride in both the cortical areas and the caudate nucleus was predictive of the severity of ketamine-induced psychotic symptoms in human study subjects, even though ketamine administration did not significantly affect tracer uptake (Vernaleken et al., 2013). Another D_2 radioligand, the agonist tracer [^{11}C]MNPA, was found to be more sensitive to stimulant-induced dopamine release in the striatum of non-human primates than the antagonist ligand [^{11}C]raclopride (Seneca et al., 2006).

While some subtypes of 5-HT receptors have also been targeted similarly to dopamine receptors, the main focus has been centered around the serotonin transporter (SERT), which is currently the most prevalent drug target in this system. [^{11}C]McN5652 was the first radiotracer used to image SERT

density in brains of nonhuman primates (Laruelle, 2002). Subsequent experiments have focused on differences in receptor density associated with psychiatric disorders and displacement studies with SERT ligands, rather than the occupancy by the endogenous transmitter itself, although the binding differences measured in reality are a combination of receptor density and ligand affinity, as is always the case with binding potentials measured with PET. The increased availability of SERT which has been observed in certain psychiatric conditions such as depression (Reivich et al., 2004), when imaged with tracers like [^{11}C]McN5652, may not be entirely due to adaptive changes taking place in the expression of the target (i.e. SERT) secondary to decreased basal serotonin levels, but in part attributable to a higher concentration of the endogenous ligand serotonin competing with the tracer in healthy controls. Many of the commonly used drugs which elevate synaptic serotonin levels also bind to SERT, which complicates pharmacological challenge protocols.

A more recently developed diarylsulfide tracer [^{11}C]MADAM has also been used to investigate SERT occupancy of drugs such as the novel multi-modal antidepressant vortioxetine (Stenkrona et al., 2013). Imaging of fluctuations in endogenous transmitter levels with a SERT tracer has been attempted by dietary tryptophan depletion, a procedure postulated to deplete synaptic serotonin, and by administering a monoamine oxidase (MAO) inhibitor to increase synaptic serotonin concentrations. Two PET studies carried out in monkeys with [^{11}C]DASB were successful in decreasing tracer binding after treatment with an MAO inhibitor (Lundquist et al., 2007) and with the 5-HT precursor, 5-hydroxy-L-tryptophan (Yamamoto et al., 2007), but human studies utilizing tryptophan depletion have yielded negative results (Talbot et al., 2005).

In addition to SERT, also most 5-HT receptor subtypes have been examined with PET methods: antagonist tracers have been characterized for the 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2A}$, and 5-HT$_4$ receptors (Paterson et al., 2013). Nonetheless, displacement protocols with endogenous serotonin utilizing the 5-HT$_{1A}$ ligand [^{11}C]WAY-100635 have been unsuccessful in healthy human subjects (Rabiner et al., 2002), and rodent studies have yielded mixed (Hume et al., 2001) or negative (Maeda et al., 2001) results. The newly developed 5-HT$_{1B}$ tracer [^{11}C]AZ10419369 was reported to be sensitive to fenfluramine-induced serotonin release in cynomolgus monkeys (Finnema et al., 2010). Further studies will be needed to confirm whether the occupancy model can be applied to the serotonin system with this tracer, but thus far there has been no success in cross-species validation of 5-HT receptor PET tracers for studying endogenous serotonin levels (Finnema et al., 2015).

There has also been academic and pharmaceutical interest to develop radioligands for the purpose of investigating ACh neurotransmission, as boosting of ACh neurotransmission in the brain is the current principal mechanism of action of the clinically approved treatments for AD, even although their efficacy is far from satisfactory. There are imaging agents which target nicotine-type α4β2 ACh receptors e.g. 2-[^{18}F]FA-85380 and [^{18}F]nifene, the latter compound has rapid kinetics suitable for clinical trials in humans. [^{18}F]nifene binding was found to be significantly reduced in response to treatment with the ACh esterase inhibitors physostigmine and galantamine in an *in vitro* binding assay with 100 nM ACh (Easwaramoorthy et al., 2007) and that positive result could be duplicated *in vivo* in a PET study in anesthetized Sprague-Dawley rats (Hillmer et al., 2013).

The μ-opioid receptors (MOR) are expressed in brain regions associated with circuits of addiction. MORs are connected to the dopamine system via inhibiting GABA neurons; the activation of μ-opioid receptors disinhibits the mesolimbic dopaminergic neurons and thus increases dopamine

neurotransmission (Nutt, 2014). [^{11}C]Carfentanil is a widely used selective MOR tracer which has been exploited to investigate receptor occupancy by drugs like nalmefene (Ingman et al., 2005) that are used to treat substance abuses, as well as occupancy by endogenous opioid peptide ligands (see below).

Successful competition protocols include a study involving an assessment of the levels of endogenous transmitters in a single chronic pain patient where the extent of [^{11}C]carfentanil BP_{ND} was considerably reduced after treatment with transcranial direct current stimulation (tDCS), and this change correlated with the degree of alleviated symptoms (DosSantos et al., 2012). Another study linked the endogenous reward system to d-amphetamine administration in healthy volunteers. [^{11}C]Carfentanil BP_{ND} was significantly reduced after d-amphetamine (5 mg/kg) administration (Colasanti et al., 2012). The alcohol-induced release of opioid peptide could be detected with [^{11}C]carfentanil in both the nucleus accumbens and the orbitofrontal cortex and tracer uptake in the orbitofrontal cortex was linearly associated with excessive alcohol use (Mitchell, 2012).

2.1.5. REQUIREMENTS FOR CNS PET TRACERS AND CURRENTLY AVAILABLE TRACERS FOR THE NORADRENERGIC SYSTEM

PET tracers with specific receptor binding properties can be used in drug development to demonstrate target engagement, to determine whether novel drug candidates can gain access to the CNS or other target tissues, and if so, what doses and dosing intervals should be used in efficacy trials (clinical phases 2 and 3). This can be achieved by quantifying reductions in PET tracer binding to the target receptor due to the dose-dependent competition binding of the novel drug candidate. This information can help to estimate the doses required to reach significant target occupancy and clinical efficacy. In addition to their significant potential in clinical and preclinical drug development, specific receptor tracers can also be used to obtain a dynamic perspective into the activities of endogenous transmitter systems, which can also benefit the clinical development of drugs affecting neurotransmission in the brain, as well as in the exploration of various neurological and psychiatric disorders.

In order to be useful for brain imaging, a PET tracer must meet several criteria; i.e. relatively high lipophilicity and an ability to cross the blood-brain barrier in order to enter the brain in detectable amounts, and if possible without producing confounding radioactive tracer metabolites. It is desirable that peripheral metabolism is not extensive, and if metabolites are formed, they should preferably be hydrophilic to preclude their access into the CNS. If the lipophilicity of the parent tracer is excessively high, nonspecific binding can become an issue and low signal-to-noise ratios may ensue. While reversible brain uptake is preferred, a prospective PET tracer should not be a substrate for efflux transporters, and clearance from the CNS should not be too rapid. Additionally, rapid receptor binding is necessary to allow the binding equilibrium to develop during the scan time window that is determined by several factors associated with the employed radiolabel (Laakso and Hietala, 2000; Pike, 2009). The prospective tracer's affinity to the target receptor should be in the low nanomolar range (approximately 0.5-10 nM). The use of a low affinity tracer will be reflected in a low B_{max}/K_d ratio, which leads to a poor capacity to detect drug interactions in PET imaging (Passchier et al., 2002).

Most experience of selective radioligands for PET imaging of CNS adrenoceptors has originated from attempts to develop PET radioligands for α_2-ARs, although efforts have also been expended in developing ligands for α_1-ARs and β-ARs. [^{11}C]-labeled sertindole analogues exhibited high *in vitro* selectivity for α_1-ARs, but a high degree of non-displaceable binding combined with poor CNS uptake makes these ligands unsuitable for *in vivo* experiments (Airaksinen et al., 2013). [^{18}F]FPTC exhibited specific binding to β-ARs *in vitro*, but the standardized uptake value (SUV) increased rather than decreased in response administration of the non-selective β-AR antagonist propranolol (expected to displace [^{18}F]FPTC from its specific binding sites), which was interpreted as evidence of non-displaceable CNS binding *in vivo* in rodents, even though propranolol had dose-dependently inhibited [^{18}F]FPTC binding *in vitro* (Mirfeizi et al., 2014)

Until recently, no radioligand for PET imaging of brain α_{2C}-ARs has met the aforementioned criteria. Two recently developed PET tracer candidates, [^{11}C]MBF and [^{11}C]JP-1302, are selective for the α_{2C}-AR subtype, but are unsatisfactory since they are substrates for efflux transporters, which became evident in a study performed with p-glycoprotein/breast cancer resistance protein knockout mice compared to wild-type mice (Kawamura et al., 2010). Other tracers like [^{11}C]MK-912 (Shiue et al., 1998), [*O*-methyl-^{11}C]RS-15385-197, [^{11}C]R107474 (Van der Mey et al., 2006), [^{11}C]yohimbine (Jakobsen et al., 2006; Landau et al., 2012) and [^{11}C]mirtazapine do not differentiate between the α_2-AR subtypes. Additionally, [*O*-methyl-^{11}C]RS-15385-197 exhibits minimal brain extraction in humans (Hume et al., 2000).

In addition, with the exception of [^{11}C]mirtazapine (Munk et al., 2011), none of these tracer candidates has been validated in humans. [^{11}C]Mirtazapine binding was significantly reduced in antidepressant non-responders compared to healthy controls (Smith et al., 2009), but the receptor level implications of this finding are speculative at best, i.e. in addition to subtype-nonselective binding to α_2-ARs, mirtazapine also binds with high affinity to 5-HT$_2$, 5-HT$_3$ and histamine H$_1$ receptors (Kast, 2001; Van der Mey, 2006).

2.2. NORADRENALINE

2.2.1. NORADRENALINE AND ITS RECEPTORS

Noradrenaline is a monoamine neurotransmitter that shares its synthesis pathway and its main structural features with the two other catecholamine neurotransmitters, adrenaline and dopamine. The catecholamines are composed of a benzene ring substituted with two hydroxyl groups and an amine side chain. Their synthetic route from the precursor L-tyrosine is shown in Fig. 6.

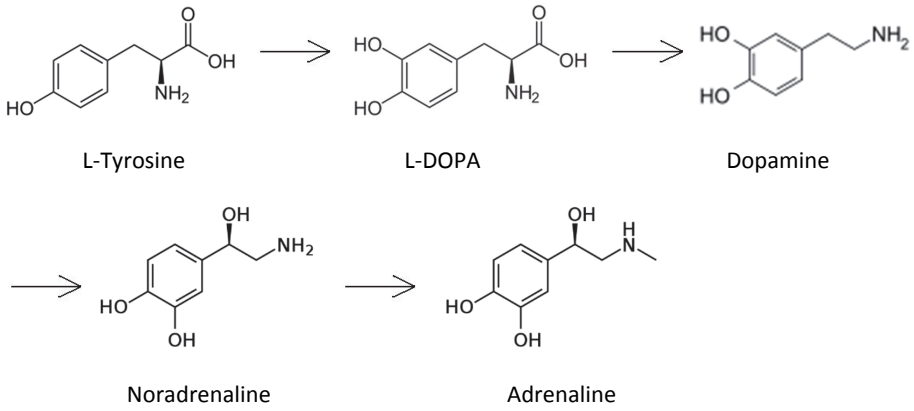

Figure 6. The synthesis pathway of the catecholamine neurotransmitters.

Adrenaline is a fight-or-flight hormone with mainly peripheral actions mediated via the bloodstream, whereas noradrenaline mainly functions as a local neurotransmitter in the CNS and in the peripheral sympathetic nervous system. Upon intravenous administration, the primary peripheral effect of noradrenaline is an elevation in blood pressure, and the immediate effect is mostly due to the increased vascular resistance caused by activation of vascular α_1-adrenoceptors (ARs). Additionally, systemic administration of noradrenaline increases heart rate and cardiac contractility via activation of cardiac β_1-adrenoceptors, which also acts to elevate the blood pressure. Vascular α_{2B}-ARs contribute by exerting direct vasoconstriction, but their effect is weaker than that of the α_1-ARs. α_{2A}-ARs in the CNS have an opposite effect as they reduce sympathetic outflow, which results in a delayed decrease in blood pressure.

With respect to noradrenaline's neurotransmitter function in the CNS, normal cognitive functioning is associated with moderate transmitter levels, whereas cognitive dysfunction is typically related to decreased or increased levels of one or several monoamine neurotransmitters, resulting in an inverted U -shaped dose-response curve (Devoto and Flore, 2006). According to observations of behavioral and cellular effects in experimental animals, a moderate level of noradrenaline strengthens working memory by engaging of post-synaptic α_2-ARs, whereas stress induces excessive levels of noradrenaline resulting in a decline in cognitive performance (Arnsten et al., 1997). The positive symptoms in schizophrenia, i.e. hallucinations, have been associated with increased dopaminergic activity (see sections 2.2.2-4. below), and thus atypical antipsychotics have been developed to act primarily as dopamine (D_2) antagonists, but they also simultaneously increase

dopamine and noradrenaline levels throughout the cortex (Li et al., 1998; Devoto et al., 2003), an effect which was reversed by the α_2-AR-agonist clonidine, and which is regarded as fundamental to their overall clinical efficacy (Devoto et al., 2003, Kalkman and Loetcher, 2003).

Table 1. The classification of adrenergic receptors. The relative abundance of the AR subtypes in the CNS is indicated by +/-. Typical peripheral tissues where each subtype is expressed are also listed, although the distributions overlap considerably.

α_{1A}	α_{1B}	α_{1D}	α_{2A}	α_{2B}	α_{2C}	β_1	β_2	β_3
+	++	++	++	-*	+	+	+	-
Vascular smooth muscle, prostate			large arteries, pancreas, kidney	Small arteries, veins, kidney	kidney	Heart, liver, muscle	Smooth muscle, heart, muscle	Adipose tissue

Modified from: Piascik and Perez, 2001; Fagerholm et al., 2008; MacDonald et al., 1997; Scheinin et al., 1994; Ferrer-Lorente et al., 2005; Faber et al., 2001; Nicholas et al., 1996. *Present in negligible amounts compared to the other subtypes, exclusively in the thalamus.

Noradrenergic receptors (Table 1) belong to the family of G-protein coupled receptors (GPCRs) with seven transmembrane domains. Although other drug targets like receptor tyrosine kinases and ion channels have emerged as focal points of pharmaceutical industry interest in recent years, GPCRs are still, by far, the most common target receptors for the currently marketed drugs (Flower, 1999).

Based on their signaling mechanisms, structure and function, the noradrenergic GPCRs are divided into three main classes, i.e. the α_1-, α_2- and β-adrenoceptors, each of which is further divided into three subtypes. Three α_2-adrenoceptor subtypes have been identified in humans and other mammalian species, i.e. the α_{2A}-, α_{2B}- and α_{2C}-ARs. The actions of the α_{2A}-subtype are predominantly pre-synaptic and auto-inhibitory, and this receptor subtype has the most widespread distribution both peripherally and in the CNS. The α_{2B}-subtype has a largely peripheral distribution and *in situ* hybridization studies performed in rats have revealed a CNS distribution limited to the thalamus (MacDonald et al., 1997). The α_{2C}-subtype is considered to have a predominantly modulatory function with a presynaptic heteroreceptor localization, but there is also evidence of a presynaptic auto-inhibitory role similar to the more widespread α_{2A}-subtype (Sallinen et al., 1997; Ihalainen and Tanila, 2002), and the CNS distributions and functions of these two subtypes are partly overlapping. In contrast to the α_{2A}-ARs, α_{2C}-ARs are predominantly found in the CNS, where their distribution is much more limited compared to α_{2A}-ARs. The highest densities of α_{2C}-ARs are found in the ventral and dorsal striatum (Scheinin et al., 1994; MacDonald et al., 1997; Fagerholm et al., 2008). The paucity of peripheral targets together with its neuro-modulatory heteroreceptor role make the α_{2C}-AR subtype an appealing CNS drug target (Scheinin et al., 2001).

At the cellular level, α_{2C}-ARs have been detected in intracellular compartments as well as on the cell surface, whereas the α_{2A}-AR subtype has been found to exist almost exclusively on cell membranes, predominantly in axonal terminals (Olli-Lähdesmäki et al., 1999), which is in line with the known pre-

synaptic autoreceptor role of this subtype (Ihalainen and Tanila, 2002). Agonist-induced intracellular sequestration of the α_{2A}-AR subtype has been observed in cell-based model systems, while the α_{2C}-AR subtype has proven challenging to investigate in this regard due to its predominantly intracellular localization in the employed cell lines, but some evidence of agonist-induced internalization does exist (Daunt et al., 1997; Olli-Lähdesmäki et al., 2003). It has also been demonstrated that while the α_{2C}-AR subtype is predominantly intracellular in non-neuronal cells, cell surface expression is significantly more pronounced in neuronal cells (Hurt et al., 2000). To summarize the current knowledge of the α_{2C}-AR distribution in the CNS, this subtype is thought to have a prominent cell surface distribution in striatal neurons and it is possible that this expression is significantly affected by agonist/antagonist exposure.

2.2.2. ALPHA$_2$-ADRENOCEPTORS IN THE NORADRENERGIC TRANSMITTER SYSTEM AND MODULATORY EFFECTS ON OTHER TRANSMITTER SYSTEMS

Noradrenaline is produced and stored in axonal nerve endings, where its release is regulated by autoreceptors, primarily of the α_{2A}-AR subtype (Fig. 7). α_2-ARs are the primary autoinhibitory mechanism in the noradrenergic system (Millan et al., 2000).

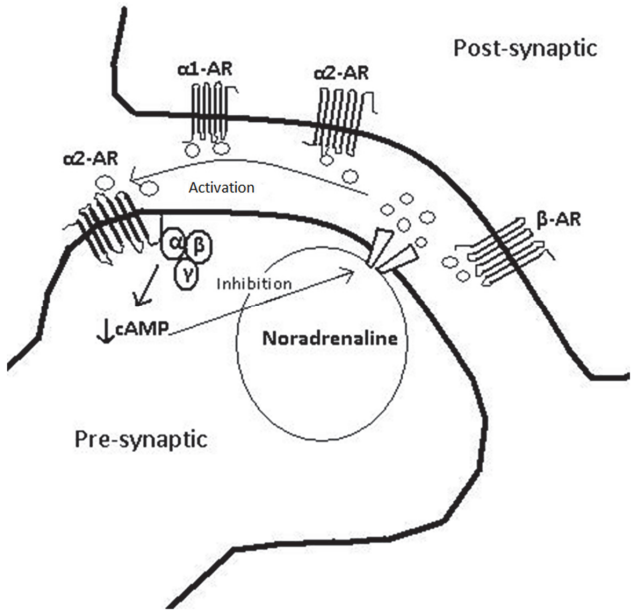

Figure 7. A schematic simplification of a noradrenergic synapse. Presynaptic α_2-AR autoreceptors regulate the stimulation-evoked release of noradrenaline and thereby the activation of postsynaptic adrenoceptors. Adapted from a figure by Prof. Lutz Hein (Südhof and Starke: Pharmacology of neurotransmitter release, Vol. 184, p. 279. Springer-Verlag Berlin Heidelberg. 2008).

While inhibiting the exocytosis of noradrenaline, α_2-ARs simultaneously inhibit the release of co-transmitters co-stored with noradrenaline in the same storage vesicles. For example, purinergic co-

transmission, in the form of ATP release, exerts a synergistic effect on oxytocin and vasopressin release (Burnstock, 2006). Furthermore, it appears that not all dopamine is converted to noradrenaline in noradrenergic nerve endings, and that dopamine is co-released with noradrenaline in the cerebral cortex (Devoto and Flore, 2006).

One way to assess the activity of a transmitter system in the brain is to measure the concentration ratio of a transmitter metabolite to its parent compound. The ratio of 3-methoxy-4-hydroxyphenylglycol (MHPG) to noradrenaline reflects noradrenaline turnover in the brain and is considered as an indicator of the activity of inhibitory pre-synaptic autoreceptors, i.e. the α_2-ARs. This can be demonstrated by injecting rats with atipamezole, a subtype non-selective α_2-AR antagonist which, by blocking inhibitory adrenoceptors, significantly increases the activity of the noradrenergic neurons in the rat brain, and thus also the MHPG/noradrenaline concentration ratio (Scheinin et al., 1988). The same effect has also been detected in human subjects in the peripheral blood, where noradrenaline concentrations are significantly increased after administration of atipamezole, accompanied by increases in blood pressure and heart rate (Karhuvaara et al., 1990).

There are seven (A1-A7) noradrenergic cell groups in the rat brain, of which the locus coeruleus (LC, or A6) is regarded as the primary noradrenergic nucleus; the cerebral cortex is exclusively innervated by the LC (Nicholas et al., 1996). The LC is located at the level of the roof of the fourth ventricle in the rostral pons and it sends projections to virtually the entire central neuraxis with cortical as well as subcortical targets (Keren et al., 2009). The cortical projections from the LC reach all lobes although with variable densities. Somatosensory areas receive an extensive innervation while innervation in the primary visual cortex is scarce. Axons project to all cortical depths, but layers II-V receive the most widespread innervation (Levitt et al., 1984). Other noradrenergic nuclei include the lateral tegmental (A1, A5, A7) and the dorsal medullary (A2) cell groups, which project to the lower brain stem and the spinal cord and form the ventral noradrenergic bundle projecting to the lower thalamic and hypothalamic regions (Ressler and Nemeroff, 2000; Marien et al., 2004).

Since the LC and other noradrenergic nuclei are anatomically small structures, functional *in vivo* imaging protocols investigating possible functional disturbances related to different neurological diseases have been hindered by the relatively low spatial resolution of currently available brain imaging methods (Keren et al., 2009). However, as the projection areas that these nuclei innervate are much larger and more readily defined, an indirect approach where changes in the activity of the LC and other noradrenergic nuclei can be assessed by examining changes in neurotransmitter release in the target areas; these approaches may be well suited for studying neurological and psychiatric disorders *in vivo* in humans.

Compared to DAT and SERT, the noradrenaline transporter (NET) has a relatively more limited distribution in the brain (Laakso and Hietala, 2000), and the anatomical knowledge of the distribution of noradrenergic axons is still incomplete. In non-human primates and other mammals, the highest concentrations of NET have been found in the LC and, at lesser levels, in other noradrenergic nuclei in the midbrain. NET is also found in the thalamus, hypothalamus, amygdala, raphe nuclei, ventral tegmental area (VTA) and parts of the neocortex (Charnay et all, 1995; Smith et al., 2006). Even though the α_{2C}-AR subtype is concentrated in the dorsal striatum (DSTR; Scheinin et al., 1994; MacDonald et al., 1997; Fagerholm et al., 2008), this region is almost completely devoid of NET. Compared to the DSTR, NET is relatively abundant in the ventral striatum (VSTR) and α_{2C}-ARs

are also found in this region of the brain, albeit in smaller quantities compared to the DSTR. It can be hypothesized that receptor expression is indicative of innervation, but the lack or presence of one adrenoceptor subtype cannot be considered to be evidence for or against the functionality of noradrenergic innervation in that brain region. There is some indirect evidence from a rodent study indicating that the nucleus accumbens, the region with the highest NET and α_{2C}-AR densities in the VSTR, does receive a noradrenergic innervation. Intraperitoneal administration of DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine), a neurotoxin selective to noradrenergic neurons, significantly decreased the amount of noradrenaline in the rostral part of the nucleus accumbens (Russell et al., 1989).

Noradrenergic heteroreceptors are involved in modulating the release of many neurotransmitters such as GABA, serotonin, ACh (Marien et al., 2004), and perhaps most notably, dopamine (Millan et al., 2000; Marien et al., 2004). The latter two are discussed further in conjunction with some associated neurological disturbances.

GABA is the primary inhibitory neurotransmitter in the CNS; GABA–containing interneurons are involved in inhibitory modulation of monoamine transmitters, including noradrenaline. GABA reduces noradrenergic cell firing via inhibitory GABAergic inputs from the nucleus prepositus hypoglossi to the LC (Ressler and Nemeroff, 2000). Experimental evidence also indicates that α_2-heteroreceptors reciprocally inhibit GABA release. Based on monitoring of synaptic events recorded using whole cell voltage clamp methodology *in vitro* in slice preparations of the rat brain stem, clonidine significantly inhibited GABAergic neurotransmission through its agonist effect on α_2-ARs (Philbin et al., 2010). The modulatory effect of α_2-ARs has also been demonstrated with patch-clamp methods in rat olfactory bulb slices, where inhibitory postsynaptic currents were reduced as a sign of attenuated GABAergic inhibition (Nai et al., 2009). GABAergic interneurons regulate many transmitter systems and conversely the GABA system is modulated by transmitters like glutamate (Gwak and Hulsebosch, 2010) and the endogenous opioid peptides (Nutt, 2014).

The μ-opioid receptors (MORs) are widely present in the mesolimbic and mesocortical dopamine pathways which are regarded as key components in the reward system linked to the development of addictive behaviours such as alcoholism. In the VTA, MORs are located on GABAergic inhibitory interneurons that inhibit dopaminergic neurotransmission to the striatal nucleus accumbens, the PFC and the amygdala (Nutt, 2014), which are also areas in which there is relatively abundant expression of α_2-ARs (Holmberg et al., 2003; Boyajian, 1987). Although the above receptor distributions had been assessed in rodents, PET studies in humans have not contradicted these results. The α_2AR subtype nonselective PET tracer [^{11}C]mirtazapine exhibited significant specific binding in the amygdala of healthy volunteers (n=18; Smith et al., 2009). The noradrenergic system of the brain is sensitive to opioid exposure and withdrawal symptoms display features which are attributable to noradrenergic hyperactivity. Rodent responses to clonidine administration in naloxone-induced withdrawal symptoms have been successfully potentiated by pre-treatment with the subtype non-selective α_2-AR antagonist yohimbine. It was concluded that α_2-AR-antagonists may be useful in the management of withdrawal by causing up-regulation of auto-inhibitory ARs (Streel et al., 2006). The effects of opioids on the noradrenergic system are possibly mediated through kappa-type opioid receptors, which have been localized in the somata and dendrites of LC neurons (Reyes et al., 2009).

The serotonin and noradrenaline transmitter systems share reciprocal connections, and α_2-heteroreceptors play an important role in the modulation of 5-HT release. The experimental evidence from test animals includes a study utilizing microelectrodes in the serotonergic dorsal raphe nucleus and the LC of sedated rats; milnacipran, a selective 5-HT/noradrenaline re-uptake inhibitor, administered alone, caused a 75 % reduction in the noradrenergic firing rate in the LC, and furthermore, the responsiveness to the agonist effect of clonidine following 14-day treatment with milnacipran was reduced by 60 %. The 5-HT neuron firing rate in the dorsal raphe nucleus was found to be significantly reduced after 2 days' administration of milnacipran but complete reversal of this effect was seen during a 14-day follow-up with continued administration. No significant desensitization to 5-HT in the dorsal raphe nuclei was observed and the result was interpreted as a modulatory effect of the noradrenaline transmitter system on 5-HT neurons (Mongeau et al., 1998). The α_2-AR-antagonist idazoxan was reported to enhance the release of cortical serotonin in rats when co-administered with citalopram (Maura et al., 1992). It is known that the dorsal raphe nuclei are extensively innervated by the LC (Mongeau et al., 1997), and there is evidence of significantly reduced 5-HT, noradrenaline and dopamine turnover as a response to α_2-AR-agonists like dexmedetomidine (MacDonald et al., 1997, Sallinen et al., 1997). In the mouse midbrain, intense α_{2A}-and α_{2C}-AR immunoreactivity has been observed in the LC, where the α_{2A}-subtype has a presynaptic axonal localization, while the α_{2C}-subtype has a predominantly postsynaptic dendritic localization (Lee et al., 1998). α_{2C}-AR immunoreactivity has also been detected in the substantia nigra, the VTA and the raphe nuclei (Homberg et al., 2003), and the LC is reciprocally innervated by the raphe nuclei. Taken together, experimental data suggest that noradrenergic α_2-heteroreceptor activation inhibits 5-HT release.

Considering the multi-layered nature and reciprocal connections only briefly described above, the influences of one transmitter system on another can never be simply accepted or dismissed as a simple causal relationship. A recent study linking the serotonin and opioid systems together with a parametric voxel-level approach represents one step in the right direction in an actual effort to connect functional inferences of two transmitter systems in a single study (Tuominen et al., 2013). However, progress in these types experiments highlight the need for receptor subtype-selective tracers to allow more detailed receptor mapping and better functional understanding in humans. As an example, although the connections of the dopamine system to the noradrenaline system described below are supported by solid experimental evidence, the neuronal patterns of the connections involved, or the possible multiple co-existing patterns and reciprocal connections behind this phenomenon in the human CNS are presently unclear.

2.2.3. DISORDERS LINKED WITH DISTURBANCES IN THE NORADRENERGIC TRANSMITTER SYSTEM

In recent years, Alzheimer's type dementia has been a major focal point of research in neuroscience and in the pharmaceutical industry, as this disease has been estimated to affect over 70 % of all individuals with progressive cognitive impairment. In an autopsy study of 675 demented individuals, 77 % fulfilled the histological criteria of AD, but only 60 % expressed "pure" AD neuropathology, while 8 % had additional features of PD and another 8 % had co-existing vascular lesions (Jellinger et al., 1990). As AD prevalence increases with age and as the mean age of the population of not only western societies but also those in the developing world continues to rise, the socioeconomic burden of AD is enormous and all avenues need to be explored to find effective treatments.

According to one theory supported by abundant experimental data, neurological diseases ranging from AD to PD are different manifestations of the same underlying pathology in the LC and in the related noradrenergic nuclei. These diseases initially manifest themselves with very different symptoms e.g. movement disorders (PD) and impaired cognition and working memory (AD), but as the diseases progress towards their end stage, the clinical manifestations begin to align remarkably, i.e. many PD patients will develop dementia and many AD patients will experience movement disorders. Patients with PD have a five-fold risk of developing dementia by the age of 80 and this risk is not related to accompanying movement disorders (Marien et al., 2004). Extrapyramidal Parkinsonian symptoms are prevalent in AD patients compared to age-matched control subjects, and autopsy results have confirmed that PD pathology is common in the brains of AD patients (Leverenz and Sumi, 1986).

Rodent models have linked abnormalities in several neurotransmitter systems including the serotonin and noradrenaline systems (Dringenberg, 2000) to AD, and immunocytochemistry has been used in non-human primates to visualize the anatomical interactions of monoaminergic axons and cholinergic cell bodies (Smiley et al., 1999). Nonetheless, based on post-mortem histopathological examinations of Alzheimer's patients, the main characteristic in AD remains the severe cholinergic disturbance, presenting with as much as 90 % loss in activity of the ACh synthesizing enzyme choline acetyltransferase and the associated decrease in cortical and hippocampal ACh levels, combined with the extensive cell loss in the basal forebrain's nucleus basalis of Meynert (nbM), the primary cholinergic nucleus which sends widespread projections to the affected brain areas (Coyle et al., 1983).

LC cell loss has been found to have an even stronger correlation to AD and the duration of AD symptoms compared to cell loss in the nbM (Zarow et al., 2003). LC cell loss is also a dominant feature in PD and has been found to be even greater in PD than in AD. In *post mortem* studies of PD patients, some evidence of cell loss in substantia nigra's pars compacta was also reported, but this was not as extensive as the loss in LC (Zarow et al., 2003). LC neuronal counts have also been shown to inversely correlate with the amount of Aβ plaques and neurofibrillary tangles (Bondareff et al., 1987), the two classical pathological hallmarks typically seen in AD brains.

The subtype non-selective α_2-AR agonist dexmedetomidine decreased electrically stimulated dopamine overflow, as measured with chronoamperometry, in the rat striatum, and atipamezole, a subtype non-selective antagonist, reversed this effect (Yavich et al., 1997). α_2-AR antagonists like atipamezole increase, while agonists decrease the release of ACh in the PFC of rodents (Tellez et al.,

1997). Thus, the noradrenaline transmitter system has been linked to both dopamine and ACh by pharmacological experiments in rodents as well as *post-mortem* studies in humans and disturbances in the two systems correlate with AD and PD pathology.

Based on experimental findings such as those listed above and the fact that dementia developing as a consequence of ischemic brain injury does not share this pattern of cell loss, it has been postulated that LC cell loss is not a retrograde process but in fact that it precedes dementia (Marien et al., 2004). In addition to elucidating the pathophysiology of AD, detecting this noradrenergic disturbance *in vivo* could prove to be a way of achieving early detection of AD as well as a tool for the differential diagnosis of dementia-like disorders. A validated adrenoceptor PET ligand could serve this purpose, although it seems unlikely that a PET imaging method would be feasible for mass screening of patients in the near future. However, earlier and more reliable AD biomarkers that precede neurological damage and the appearance of cognitive symptoms are constantly being explored for research purposes. It has been estimated that the Aβ depositions detectable by PET precede clinical symptoms of AD by at least 10-15 years (Bateman et al., 2012) and the cerebrospinal fluid (CSF) biomarker, Aβ42, reaches its pathologically low concentration approximately 10 years before conversion to AD (Bucchave et al., 2012). Clinical phase II-III trials aiming to discover new disease modulating treatments have tended to focus almost exclusively on MCI and prodromal AD (i.e. MCI with positive biomarkers for AD) in recent years. At present, it seems unlikely that treatments based on the amyloid hypothesis would possess significant therapeutic efficacy once moderate to severe cognitive impairment accompanied by neurodegeneration has developed. The amyloid load measured with PiB PET has been found to significantly correlate with impaired cognition in the elderly, but this correlation disappears in individuals with higher education (Roe et al., 2008). The gap between CNS pathology and symptoms has been explained by cognitive reserve and adaptive resistance to pathology, which have been hypothesized to be augmented by the noradrenergic stimulation induced by environmental enrichment and novelty. Possible mechanisms to explain this phenomenon include neurotrophic effects (i.e. cortical volume and connectivity) and anti-inflammatory effects, e.g. resistance to amyloid-induced cell death (Robertson et al., 2013).

Noradrenergic disturbances have also been implicated in mood disorders, and increased densities of α_2-ARs have been observed in the LC of patients with major depressive disorders (Ordway et al., 2003). This finding suggests that depression, one of the leading contributors to the disease-associated socioeconomic burden in western societies, is linked to increased noradrenergic neuronal activity in the LC. Since the α_2-ARs function primarily through presynaptic autoinhibition of noradrenaline release, the upregulation of α_2-ARs can be considered as an adaptive change. Stressful stimuli lead to activation of the LC through increased corticotrophin releasing factor (CRF) from the amygdala and hypothalamus, an effect which can be counteracted via an inhibitory serotonin input to these neuronal circuits. Together with the serotonergic system, dysregulation of the noradrenergic innervation to areas including the cerebral cortex, thalamus and hippocampus has been linked to the different clinical manifestations of depression, including impaired concentration, insomnia and loss of appetite, respectively (Ressler and Nemeroff, 2000).

Unipolar depression has been linked to increased MHPG levels in plasma and cerebrospinal fluid (Roy et al., 1988) as a sign of increased noradrenaline turnover. There have also been studies where no significant correlation was found (Oreland et al., 1981), but it is not reasonable to expect that there would be a clear correlation to synaptic noradrenaline levels which could be drawn from these

predominantly peripheral findings. Sewy et al. (1989) found no correlation between noradrenergic activity and major depressive disorder while increased plasma MHPG and noradrenaline levels were associated with generalized anxiety disorder, a condition which often accompanies depression. Nearly half of patients with bipolar depression fulfill the diagnostic criteria of an anxiety disorder at some point. Anxiety seems to be accompanied by depressive periods and this symptom is a predictor of an inadequate response to treatment (Vázquez et al., 2014).

As amphetamine is known to elevate synaptic dopamine levels (Laruelle et al., 1996), the dopamine hypothesis of schizophrenia has one of its origins in the observations that amphetamine was able to induce psychotic symptoms in healthy individuals that mimic schizophrenia as well as the property of the drug to exacerbate the symptoms in schizophrenics. Further support is derived from the known dopamine D2 receptor antagonist potency of all current antipsychotic drugs and the remarkable efficacy of these drugs in the treatment of psychotic disorders (Murray et al., 2013). Direct experimental support for this hypothesis was gained when amphetamine was found to affect [^{11}C]IBZM binding more in schizophrenics than in healthy controls, and the decrease in [^{11}C]IBZM binding, suggestive of increased dopaminergic activity, correlated to worsening of psychotic symptoms (Laruelle et al., 1996). Mouse strains over-expressing dopamine D2 receptors are used as animal models in schizophrenia research, and exhibit the same kinds of cognitive and motivational deficits which are associated with this condition in humans (Sumiyoshi et al., 2013).

Although dopamine-related drug targets dominate the current treatment regimen of schizophrenia and related psychotic disorders, the brain's dopamine systems are known to be modulated by noradrenergic innervation, and α_2-AR antagonism could potentially act as a co-treatment in reducing dopaminergic firing, as well as in alleviating the negative (cognitive) symptoms of schizophrenia. Receptor affinity results obtained with clinically used antipsychotic drugs and recombinant α_2-ARs have predicted antipsychotic efficacy for α_2-AR antagonists, as many important antipsychotic drugs have significant affinity for α_2-ARs, especially for the α_{2C}-AR subtype; in addition, clozapine, a drug which is among the most efficacious known antipsychotics, also had the highest α_{2C}/D_2 receptor affinity ratio (Kalkman and Loetscher, 2003). As mentioned above, there is indirect evidence of dopamine's co-transmitter role in noradrenergic nerve endings (Devoto and Flore, 2006), and while there is evidence of decreased turnover of all monoamine neurotransmitters in response to α_2-AR agonists (MacDonald et al., 1997; Sallinen et al., 1997), in particular increased prefrontal dopamine release has been detected in rodent experiments with α_{2C}-AR antagonists (Sallinen et al., 2013).

According to *post mortem* immunohistochemical findings in monkeys, brain areas which are involved in attentional processing, including the parietal cortexes, the superior colliculi and the pulvinar nuclei, receive a dense noradrenergic innervation from the LC (Morrison and Foote, 1986). Catecholamines seem to enhance inhibitory as well as excitatory responses of target neurons to other stimuli. It has been postulated that noradrenaline-mediated increases in the responsiveness of individual cells could be generalized into an enhanced signal-to-noise ratio at the systemic level and better performance in attentional tasks at the behavioural level (Servan-Schreiber et al., 1990). Phasic activation of LC neurons does not simply result from a sensory stimulus but has been shown to occur either in conjunction with stimulus processing or with the resulting decision-making process, or both (Aston-Jones et al., 2000). The common neuropsychiatric condition, attention deficit hyperactivity disorder (ADHD), has been speculated to be a result of inappropriate tonic LC

function and disrupted transition to the phasic mode needed in focused attention (Aston-Jones et al., 1999).

One study conducted in mice revealed that injections of a noradrenergic agonist into specific brain areas innervated by the LC, particularly the mediodorsal thalamus, disrupted pre-pulse inhibition (PPI) (Alsene et al., 2011). The authors had previously found that stimulating the LC evoked a deficit in PPI through increases in noradrenaline levels in innervated areas and this effect could be countered by prior administration of second generation antipsychotics (Alsene and Bakshi, 2011). Disrupted PPI as a sign of inadequate sensorimotor gating is present in many psychiatric disorders characterized by inadequate processing of different modalities of sensory information. These include attention disorders, anxiety disorders and psychotic disorders. The PFC is another important brain area with regard to ADHD; lesions in that region have been found to evoke many ADHD-associated symptoms including hyperactivity, impulsivity and distractibility. In monkeys, α_{2A}-AR antagonism in the PFC produced a clinical picture much like ADHD with hyperactivity and impaired working memory (Arnsten, 2006).

2.2.4. DRUGS THAT TARGET NORADRENERGIC NEUROTRANSMISSION IN THE CNS

Classical tricyclic antidepressants (TCAs) like doxepine, amitriptyline and its active metabolite nortriptyline inhibit the neuronal reuptake of serotonin and noradrenaline by blocking the functions of SERT and NET (Sekine et al., 2010). Their troublesome anticholinergic side effects and relatively narrow therapeutic window have meant that they have been largely replaced by selective serotonin reuptake inhibitors (SSRIs) and other second/third generation antidepressants, which are superior in terms of side effect profiles and safety margins. Several antidepressants used in today's clinical practice, i.e. the serotonin-noradrenaline re-uptake inhibitor (SNRI) class of antidepressants, reduce noradrenaline reuptake through NET inhibition. These drugs include duloxetine, venlafaxine and milnacipran (Takano et al., 2006; 2013). Agomelatine is a melatonin$_{1/2}$ -and 5-HT$_{2C}$ receptor antagonist which has no affinity for α-ARs or β-ARs and no affinity for monoamine transporters, but it also thought to increase extracellular noradrenaline and dopamine levels, especially in the PFC (Millan et al., 2003). Moclobemide selectively and reversibly inhibits monoamine oxidase A (MAO-A), which is anticipated to result in increased extracellular concentrations of serotonin, noradrenaline and dopamine (Fulton and Benfield, 1996).

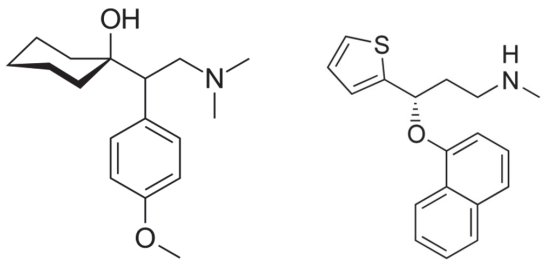

Figure 8. The molecular structures of venlafaxine (left) and duloxetine (right)

Mirtazapine's CNS effects are predominantly mediated through different 5-HT-receptors, but it also has significant α_2-AR antagonist properties and the result is an increase in all monoamine neurotransmitters including noradrenaline (Devoto et al., 2004; Masana et al., 2012; Kaminska et al., 2014). According to a large meta-analysis, mirtazapine is one of the most efficacious of the currently marketed antidepressants (Sipriani et al., 2009) although its side effects, especially sedation and weight gain, which are not shared by drugs in the SSRI class, reduce its acceptability and make it a second line choice for many individuals.

There is evidence of decreased 5-HT levels as well as a downregulated serotonergic input in depression but in addition, α_2-ARs are upregulated. This entire picture could be interpreted as a sign of attempted attenuation of noradrenergic signaling and the relative efficacy of SNRIs could be seen as simply a product of 5-HT re-uptake inhibition. Reboxetine, a selective NET inhibitor (Hajós al., 2004), is used for the treatment of major depressive disorder, even though meta-analyses indicate that SSRIs and mirtazapine are more efficacious than reboxetine in terms of both remission rate and cost-effectiveness (Ramsberg et al., 2012; Cipriani et al., 2012; Purgato et al., 2014). Depression is not an FDA-approved indication of reboxetine. The noradrenaline component of antidepressants has an established role in the treatment of chronic pain (Mika et al., 2013), a common comorbidity associated with depression, but at the cost of a less favourable adverse event profile compared to the SSRIs (Cipriani et al., 2009, 2012). TCAs have been reported to exert an alleviating effect on chronic pain which is independent of their anti-depressant efficacy (Mika et al., 2013). In much smaller doses than used to treat depression, TCAs such as amitriptyline are recommended to treat chronic pain (Mika et al., 2013). Conversely, duloxetine displayed efficacy in the treatment of chronic pain in patients with diabetic neuropathy, but a significant effect was only seen with typical anti-depressant doses and thus the recommended dosage is the same for both indications (Goldstein et al., 2005). It has been postulated that noradrenaline re-uptake inhibition has a stimulating effect on descending anti-nociceptive pathways (Mika et al., 2013) but the exact mechanism of this CNS gating effect is unclear. It is known that α_2-ARs are abundant in the dorsal horn of the spinal cord and it has been postulated that α_2-ARs inhibit dorsal horn nociceptive responses together with the μ-opioid receptors through a relay in the rostral medulla (Budai et al., 1998).

While NET is also found in the thalamus, hypothalamus, amygdala, raphe nuclei, VTA and parts of the neocortex, the largest concentrations of NET are present in the LC and other noradrenergic nuclei in the midbrain and an elevated concentration of α_2-ARs in the LC has been linked to depression (Ordway et al., 2003). Thus, re-uptake inhibition in this key area, resulting in locally

increased synaptic noradrenaline levels, which in turn would activate autoinhibitory α_2-ARs, could act to readjust the dysregulated firing of neurons in the vast projection areas originating from the LC. The clinical relevance of such an effect is, however, currently not backed up by efficacy trials in humans. Additionally, the commonly prescribed SNRI duloxetine, the only SSRI/SNRI class drug still on patent, has been found to have significantly lower tolerability than the most extensively prescribed SSRIs (Cipriani et al., 2012).

The most commonly prescribed medicine for ADHD in Finnish clinical neuropsychiatric practice is methylphenidate, a stimulant drug which is thought to affect extracellular dopamine and noradrenaline levels through re-uptake inhibition (Umehara et al., 2013; Takamatsu et al., 2015). One PET study has confirmed methylphenidate's high affinity for NET. The BP_{ND} of [^{11}C]MRB was reduced in a dose-dependent manner when methylphenidate was administered at clinically relevant doses and the estimated ED_{50} was lower than that of DAT, which would imply that noradrenaline re-uptake inhibition is an important part of methylphenidate's mechanism of action (Hannestad et al., 2010). It has been speculated that stimulant medications exert at least part of their actions through increased α_{2A}-AR activation in the PFC (Arnsten et al., 2006).

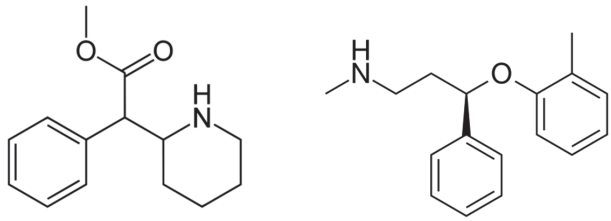

Figure 9. The molecular structures of methylphenidate (left) and atomoxetine (right).

Atomoxetine has previously been considered a highly selective inhibitor of NET and it was the first non-stimulant to be approved for the treatment of ADHD (Corman et al., 2004). Atomoxetine also exerts many peripheral noradrenergic effects, including increases in blood pressure and heart rate (Wernicke et al., 2003). In a PET study with non-human primates, atomoxetine administered as an i.v. infusion was estimated to result in complete NET occupancy at plasma concentrations corresponding to those achieved in humans after approximately 1.2 mg/kg repeated doses of the drug, i.e. clinically relevant oral doses (Takano et al., 2009). A recent PET study, however, revealed that atomoxetine also significantly occupied SERT at clinically relevant doses, thus it is not entirely NET-selective. The authors suggested that while the noradrenaline component was crucial in the treatment of ADHD, SERT blockade could mediate additional beneficial antidepressive effects (Ding et al., 2014). In rats, systemic administration of atomoxetine (3 mg/kg) has been found to elevate prefrontal extracellular dopamine and noradrenaline levels, but not those of serotonin (Ago et al., 2014).

Dexmedetomidine is used for sedation in intensive care units (ICUs) where it may offer advantages over other sedation regimens, e.g. propofol infusions, in terms of decreased length of ICU stay and reduced risk of delirium (Xia et al., 2013). Dexmedetomidine has also been used off-label in the

treatment of acute alcohol withdrawal symptoms, where increased sympathetic tone plays an important role (Muzyk et al., 2013). The major advantage of dexmedetomidine compared to other sedative agents is that the subject can be readily awakened regardless of the drug concentration in plasma, and the depth of anesthesia is easily monitored.

The carbon atom in the middle of the medetomidine molecule is methylated, which results in a stereoisomeric structure. Levomedetomidine is considered functionally inert in doses below 1 mg/kg, while dexmedetomidine is a potent α_2-AR agonist (MacDonald et al., 1991). Dexmedetomidine exhibits dose-dependent α_2-AR selectivity. In animals that receive low to medium doses at slow rates of infusion, high α_2-AR selectivity is observed (Virtanen et al., 1988). According to current knowledge, dexmedetomidine's sedative effect is primarily mediated through α_{2A}-ARs in the LC (Correa-Sales et al., 1992; Mizobe et al., 1996; Bucheler et al., 2002; Ihalainen and Tanila, 2004), i.e. it has been observed that the action potential frequency is reduced in response to α_2-AR activation (Alsene and Bakshi, 2011). Activation of presynaptic α_2-ARs in the CNS induces sympatholysis, which is associated with a biphasic peripheral blood pressure response when dexmedetomidine is administered intravenously. The initial short-lived increase in blood pressure is followed by long-lasting decreases in blood pressure and heart rate. The initial reaction can be explained by activation of peripheral α_{2B}-adrenoceptors in vascular smooth muscle and can be attenuated by administering at a slow infusion rate (Gertler et al., 2001), though even at slower infusion rates, the increase in mean arterial pressure over the first 10 minutes was shown to be in the range of 7 % with a decrease in heart rate between 16 % and 18 % (Hall et al., 2000). It has been reported that the initial response lasts for 5 to 10 minutes and is followed by a decrease in blood pressure of approximately 10 % to 20 % below baseline and stabilization of the heart rate below baseline values (Xu et al., 1998).

Other centrally acting α_2-AR agonists with sympatholytic effects include moxonidine and clonidine, which in Finnish clinical practice are typically used in idiopathic hypertension and hypertensive crisis, respectively. Especially in the US, clonidine is also used for the treatment of ADHD either with concomitant stimulant treatment or as monotherapy (Kornfield et al., 2013).

Close to one hundred patents for different approaches to α_{2C}-AR modulation have been sought during the last ten years (Quaqlia et al., 2011), but until recently, there has been a lack of highly selective α_{2C}-AR ligands, the first candidates are only now entering clinical efficacy and safety trials in humans. As the primary role of α_{2C}-ARs in the CNS is presynaptic autoinhibition of noradrenaline release and reduced noradrenergic signaling has been implicated in the pathogenesis of neurological diseases like PD and AD (see section 2.2.3.), there is a theoretical rationale for using α_{2C}-AR antagonism to treat these conditions. Noradrenaline's immunomodulatory role is manifested via the *in vitro* reductions in inflammatory cytokines and inflammatory gene expression in brain glial cells (Feinstein et al., 2002), which have been speculated to be non-neuronal targets for noradrenergic projections (Stone and Ariano, 1989). This neuroprotective effect appears to counteract Aβ-induced increases in oxidative stress and cell death (Counts and Mufson, 2010), which is at least partially mediated by postsynaptic $\beta_{1/2}$-ARs activating neurotrophic pathways with a subsequent increase in the release of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) (Schwartz and Mischler, 1990; Counts and Mufson, 2010). Evidence has also emerged from work done in transgenic mice to suggest that BDNF is secreted directly from noradrenergic neurons to promote neuronal differentiation and survival in cortical target regions (Fawcett et al., 1998).

α_2-AR antagonism also enhances the antipsychotic efficacy of risperidone, a drug which has low affinity for α_2-ARs on its own (Marcus et al., 2010). Clozapine is known to have the highest affinity for α_2-ARs of all second-generation antipsychotics and it has been claimed to exhibit superior efficacy in treatment-resistant schizophrenia (Kalkman and Loetscher, 2003). While traditional treatment strategies of schizophrenia are relatively successful with regard to the positive symptoms like auditory hallucinations, adjunctive α_2-AR antagonism represents a promising treatment strategy and it is not unreasonable to predict that it may also have efficacy in treating or preventing the development of negative symptoms, such as impaired cognition. Currently there are no effective pharmacological treatments against the negative symptoms of schizophrenia, and furthermore in the elderly, agitation and other behavioural symptoms associated with Alzheimer's type dementia have also been relatively resistant to conventional treatments with second generation antipsychotics like risperidone, which in Finland is the only antipsychotic drug with an official indication for the treatment of agitation/aggression associated with AD.

3. AIMS OF THE STUDY

The main goal of this thesis work was to validate a new tracer for PET imaging in humans. This was achieved by examining the repeatability of [^{11}C]ORM-13070 brain imaging in healthy individuals followed by a study to test the dose-dependent competition of an antagonist with [^{11}C]ORM-13070 binding to α_{2C}-ARs, and finally two studies to evaluate the possibility that [^{11}C]ORM-13070 binding could reflect changes in synaptic concentrations of noradrenaline evoked by different pharmacological and physiological challenges. Prior to this thesis work, there had been a distinct lack of specific and selective PET tracers for CNS adrenoceptors available for human use. The validation process of [^{11}C]ORM-13070 was seen as removing hurdles to the advancement of research on noradrenergic neurotransmission, and it will hopefully benefit the future clinical development of drugs affecting brain noradrenergic neurotransmission.

The specific aims of the individual studies were as follows:

I. To determine the reproducibility [^{11}C]ORM-13070 PET in quantifying cerebral α_{2C}-AR binding *in vivo* in the human brain.

II. To validate [^{11}C]ORM-13070 for α_{2C}-AR occupancy studies in humans by determining the maximal striatal α_{2C}-AR occupancy that could be achieved by administration of the subtype-nonselective α_2-AR antagonist atipamezole, and to explore whether brain uptake of [^{11}C]ORM-13070 would be sensitive to increased release of noradrenaline.

III. To test the sensitivity of [^{11}C]ORM-13070 to increased levels of synaptic endogenous noradrenaline via two different noradrenaline challenges: intravenous ketamine infusion and atomoxetine combined with cold stimulation.

IV. To evaluate the effect of depleted synaptic noradrenaline concentrations on [^{11}C]ORM-13070 uptake by evoking inhibition of noradrenaline release with intravenous infusions of dexmedetomidine.

4. MATERIALS AND METHODS

4.1 ETHICAL ASPECTS

In all four clinical studies, the study protocol, its appendices, and other documents required by the Ethics Committee (EC) were reviewed and approved by the EC of the Hospital District of Southwest Finland before the studies were initiated. Correspondence between the EC and the study site's person responsible for EC correspondence was filed in the Investigator's study file. The Finnish Medicines Agency, as the Competent Authority in Finland, was also notified before the commencement of the studies. The general conduct of all studies followed the regulations and guidance for biomedical research involving human subjects, such as the Declaration of Helsinki, the International Conference on Harmonization's Good Clinical Practice (ICH-GCP) guidelines and relevant national laws and regulations. Adverse events (AEs) were recorded according to GCP guidelines.

As the study subjects were healthy volunteers, no personal medical benefits were expected or provided from participation. The subjects were administered two radioactive tracer injections in study I, four injections in study II, and three injections in studies III and IV. The total effective radiation dose from a single injected 500 MBq dose of [^{11}C]ORM-13070 has been estimated to be 2.0 mSv at the maximum, and thus 2, 3 and 4 injections amount to maximal total doses of 4.0, 6.0 and 8.0 mSv, respectively. None of the subjects were allowed to participate in more than one study, and they were instructed to refrain from further participation as healthy volunteers in any scientific trials involving radiation. According to the Radiation and Nuclear Safety Authority of Finland, the annual radiation dose from normal environmental sources of a person living in Finland is approximately 3.2 mSv per year. Even though the radiation exposure caused by these studies was low, all exposure to ionizing radiation may result in harmful effects. This was adequately explained to the participants and special emphasis was put on the informed consent process and the well-being of the subjects. It was not possible to perform the experiments in any patient group as the validation process of [^{11}C]ORM-13070 needed to be performed in healthy humans before reliable results in different patient populations could be even considered.

4.2. STUDY SUBJECTS

The study subjects were healthy 20-39-year-old males. Good general health was ascertained via interviews, physical examinations, recording of vital signs and ECG and analysis of blood and urine samples. No substances of abuse, excessive alcohol consumption or use of nicotine-containing products more than 5 cigarettes or equivalent/day were allowed. Subjects were allowed to use paracetamol or ibuprofen for occasional pain, if necessary. All other concomitant treatments were prohibited as a general rule. Subjects were recruited with an e-mail campaign targeting medical students in the University of Turku (UTU); these individuals comprised the majority of the study subjects. Notifications on UTU web pages were also used.

4.3. [^{11}C]ORM-13070 AND OTHER STUDY DRUGS

[^{11}C]ORM-13070

The synthetic route of [^{11}C]-labelled ORM-13070 (1-[(*S*)-1-(2,3-dihydrobenzo[1,4]dioxin-2-yl)methyl]-4-(3-^{11}C-methoxymethylpyridin-2-yl)-piperazine) and its precursor ORM-13333 is illustrated in figure 10. The radionuclide required for the production of [^{11}C]ORM-13070 was obtained from a 103 cm isochronous MGC-20 cyclotron (D.V. Efremov Institute, St. Petersburg, Russia) at the Accelerator Laboratory of Turku PET Centre. [^{11}C]ORM-13070 was synthesized by ^{11}C-methylation of *O*-desmethyl ORM-13070 (ORM-13333) with [^{11}C]methyl triflate prepared from cyclotron-produced [^{11}C]methane. [^{11}C]Methyl triflate was bubbled through a solution of the desmethyl precursor in acetonitrile in the presence of tetrabutyl ammonium hydroxide. [^{11}C]ORM-13070 was purified with semi-preparative high performance liquid chromatography (HPLC).

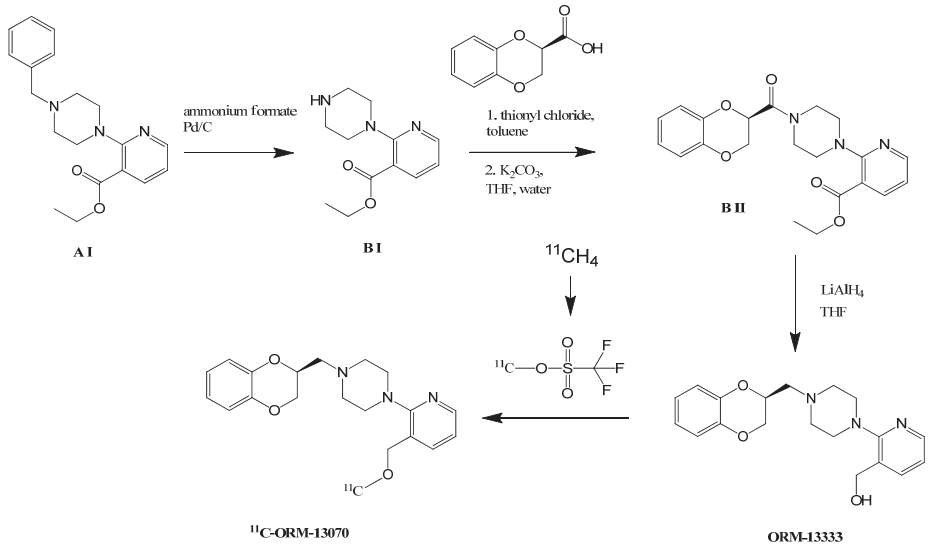

Figure 10. Synthesis of [^{11}C]ORM-13070.

[^{11}C]ORM-13070 is a selective noradrenergic α_{2C}-AR antagonist PET tracer developed by Orion Pharma and Turku PET Centre. ORM-13070 has very high affinity for the α_{2C}-AR according to *in vitro* studies. Receptor binding assays carried out with recombinant human α_2-AR subtypes and ORM-13070 revealed calculated binding affinities of (K_i values and the corresponding 95 % confidence intervals) 3.8 (2.0-7.3) nM, 23 (14-38) nM and 109 (86-138) nM for the α_{2C}-, α_{2B}- and α_{2A}-AR subtypes, respectively (see original communication II for details). Results from functional antagonism studies have also indicated that ORM-13070 is a potent and selective antagonist of α_{2C}-ARs (Orion Pharma, data on file).

A radiation dosimetry study in healthy human subjects revealed that the highest absorbed doses were in the liver and pancreas (Luoto et al., 2014). ORM-13070 is rapidly metabolized in human subjects after intravenous (i.v.) injection. HPLC analyses have revealed that the intact fraction of the administered tracer is around 30-40 % at 30 minutes after an i.v. bolus injection (Luoto et al., 2014). [^{11}C]ORM-13070 has two radioactive metabolites (M1 and M2); their specific binding properties are unclear. It is currently thought that at least M1 can pass through the BBB and enter the brain (Arponen et al., 2014).

Five clinical Phase 1 studies with tracer (<10 µg) doses of [^{11}C]ORM-13070 (clinicaltrials.gov identifier NCT00829907 in addition to original communications I-IV) and one Phase 1 single ascending dose (SAD) study (dose range 3-40 mg) with the non-radiolabelled analog ORM-13070 (also known as ODM-102; see NCT01839019 for details) have so far been conducted. [^{11}C]ORM-13070 has been demonstrated to be safe and well tolerated in all PET studies and it penetrates rapidly into the brain with the most prominent accumulation of radioactivity occurring in the caudate nucleus and putamen, which is in line with the known relative distribution of α_{2C}-ARs in the human brain (Fagerholm et al., 2008). In conclusion, ORM-13070 is a potent and selective α_{2C}-AR antagonist. No pharmacological effects in man have been observed at tracer dose levels.

Ketamine

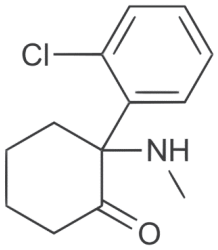

Figure 11. Molecular structure of ketamine

Racemic ketamine (Ketalar®, Pfizer) was administered as a target-controlled infusion (TCI), with a computer containing the pharmacokinetic simulation software StanPump controlling the infusion pump (Harvard apparatus 22 infusion syringe pump 55-2222) inputted with knowledge of each subject's age, height, weight, as well as the predetermined pharmacokinetic (PK) properties of the study drug to maintain a pseudo steady-state plasma concentration of ketamine during the PET scans. The targeted ketamine concentration in plasma was 200 ng/ml in study II and 300 ng/ml in study III. N-methyl-D-aspartate (NMDA) receptor antagonism is regarded as ketamine's primary mechanism of action (Sinner and Graf, 2008), and ketamine administration is known to increase plasma catecholamine concentrations and evoke inotropic cardiac effects as a reflection of increased sympathetic activity (Kienbaum et al., 2000). Ketamine's positive inotropic effect is sometimes utilized in general anesthesia in humans in situations where decreased blood pressure is particularly undesirable, e.g. in hemodynamically compromised patients (Morris et al., 2008). Ketamine-evoked increased neuronal release of noradrenaline (and dopamine) in the brain has been detected in rat brain microdialysis studies (Lorrain et al., 2003; Tose et al., 2009). In studies II and III, ketamine

infusions were started approximately 15 min before the tracer injection and continued until the end of the PET scans.

Atomoxetine

Atomoxetine (Strattera®, Eli Lilly) was used at a dose of approximately 1.2 mg/kg to inhibit striatal noradrenaline re-uptake in studies II and III. Atomoxetine was administered orally as capsules 60 min before the injection of the tracer. Atomoxetine has been associated with many peripheral noradrenergic effects, including increased blood pressure and heart rate (Wernicke et al., 2003). Significant increases in brain extracellular fluid (ECF) noradrenaline concentrations have been detected in rats following acute atomoxetine administration (Montezinho et al., 2010; Koda et al., 2010). Atomoxetine was administered acutely i.e. as a single oral dose, and increases in dose were not expected to have resulted in significantly improved efficacy of noradrenaline re-uptake inhibition. Therefore, in study III, the 1.2 mg/kg dose was combined with cold stimulation of the subject's foot, i.e. the cold pressor test. Previously in study II, cold stimulation had been found to decrease tracer uptake on its own as a sign of increased synaptic noradrenaline levels.

Dexmedetomidine

Dexmedetomidine was administered as a TCI in study IV in an analogous fashion to the ketamine infusions in studies II and III, according to Talke's PK dataset (Talke et al., 2003). The targeted plasma concentrations were 0.2 ng/ml and 0.6 ng/ml. The infusions were started at the beginning of each PET scan, i.e. simultaneously with the tracer administration.

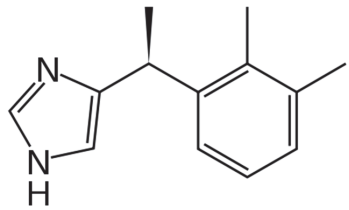

Figure 12. Molecular structure of dexmedetomidine.

4.4. PET METHODS

The data sets in studies I, II, III and IV involved 12, 32, 23 and 18 PET scans, respectively. A total of 85 PET scans were performed in four separate study protocols with the High Resolution Research Tomograph (HRRT) in the Turku PET Centre. Before each scan, the subject's head was fixed with an individually prepared thermoplastic mask and a transmission scan was performed for attenuation correction with a ^{137}Cs rotating point source. Slices of approximately 1.22 mm in thickness covered the whole brain. The camera was used in the 3D mode with scatter correction. The HRRT is capable of registering 4.5x10^{9} lines of response with 120,000 detector crystals and achieves transaxial and axial spatial resolution (full width at half maximum, FWHM) of 2.5 mm. [^{11}C]ORM-13070 was

administered as an intravenous rapid bolus injection with a maximum target activity of 500 MBq. Image reconstruction from list mode data was performed with the OP-OSEM algorithm (Hong et al., 2007) with 8 iterations and 16 subsets.

Images of each dynamic PET scan were aligned for each subject, and the movement-corrected image was then co-registered with the subject's 1.5 T MRI (Philips Intera) scan using the Statistical Parametric Mapping (SPM 2 and SPM8; Department of Cognitive Neurology, University College London, England) software package for MATLAB™ (The MathWorks Inc.). ROIs (see Figure 13 for an example) were drawn manually on the co-registered PET/MRI images using the Carimas (http://www.turkupetcentre.fi/carimas) software to obtain regional time-radioactivity concentration curves. Striatal and other structures were analyzed with reference to the cerebellar cortex. The ratio method assumes that the obtained binding parameter (BP_{ND}) reflects the ratio of specifically bound radioligand over non-displaceable radioligand. The AUC values in the time window of 5-30 minutes from tracer injection were used in studies I-IV, and additional modelling approaches and time windows were tested in studies I and III-IV, respectively. BP_{ND} of [^{11}C]ORM-13070 was calculated according to the equation (where $C_{specifically\ bound} = AUC_{VOI} - AUC_{cerebellum}$):

$$BP_{ND} = \frac{\int C_{specifically\ bound}(t)dt}{\int C_{cerebellum}(t)dt}$$

The dftratio (http://www.turkupetcentre.net/software/show.php?program=dftratio) software was used to obtain the estimates for bound per free tracer (referred to as BP_{ND} in this context) in studies II and III and in the ROI-based analysis portion of studies III and IV.

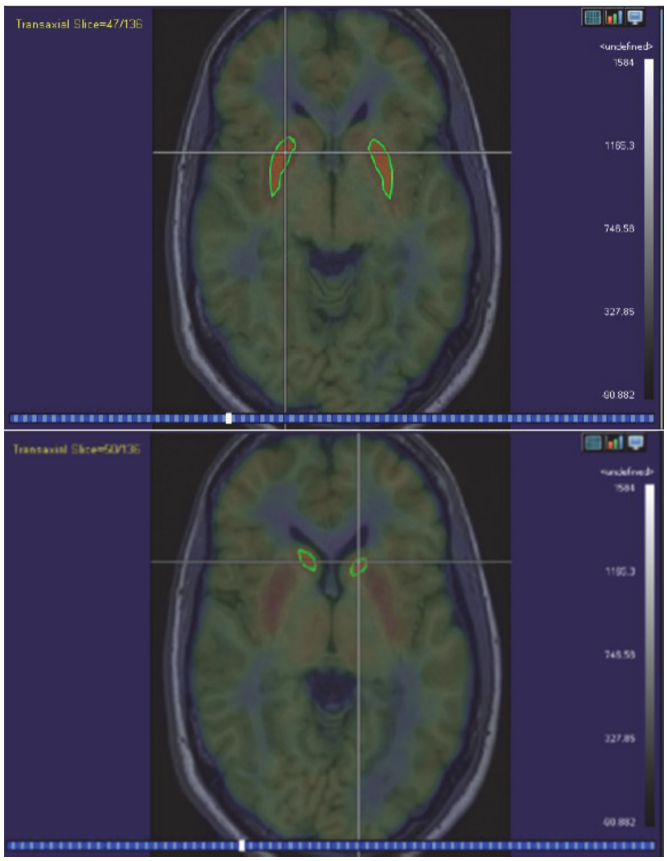

Figure 13. An example of manually defined regions of interest in the putamen (above) and the caudate nucleus (below).

4.5. STATISTICAL METHODS AND STATISTICAL PARAMETRIC MAPPING

In studies II-IV, the statistical testing of the main variables, i.e. the changes in tracer binding potential and receptor occupancy, was conducted with one-way analysis of variance (ANOVA) with no correction for multiple comparisons. The repeatability of [^{11}C]ORM-13070 PET was assessed in study I with intra-class correlation coefficients (ICC) and by calculating the absolute test-retest variability of the BP_{ND} estimates. The statistical analyses were performed with SAS^{TM} software (SAS Institute Inc). Non-linear regression analysis with a sigmoidal E_{max} model was performed with GraphPad Prism^{TM} programs (GraphPad Software, Inc.) to calculate estimates of maximum receptor occupancy (E_{max}) and half maximal inhibitory concentration (IC_{50}), as well as their 95 % confidence intervals in study II.

Statistical parametric mapping was used in studies III-IV to test for differences in specific tracer binding without any pre-defined regions of interest. Parametric B/F images were calculated in Matlab with the following equation: $Ratio(X\text{-}Y)=[AUC_{image}(X\text{-}Y)/AUC_{cerebellum\ TAC}(X\text{-}Y)]\text{-}1$ to obtain whole-brain representations of tracer binding for each treatment and the control condition. B/F

images were spatially normalized into MNI space with SMP8 by utilizing the unified segmentation algorithm (Ashburner and Friston, 2005): T1-weighted MRI images and the MNI template were used create a deformation field which was then applied to project parametric B/F PET images into MNI space. The normalized PET images were smoothed by an 8 x 8 x 8 mm Gaussian kernel. Within-subject ANOVA was used to create a design matrix (Figure 14) in SPM8 and to create parametric maps that visualised significant changes in the B/F tracer.

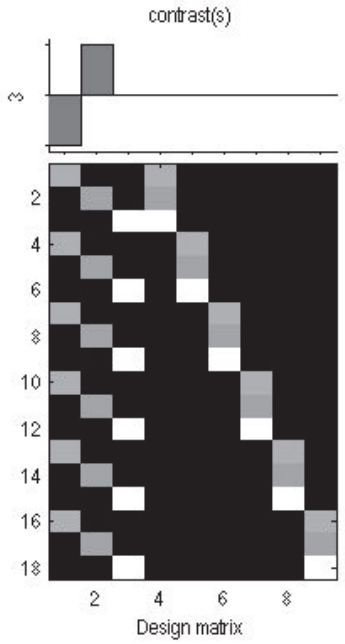

Figure 14. A screenshot from SPM8 displaying a within-subject ANOVA design matrix used in study IV. The control condition (column 1) is subtracted from the high dexmedetomidine condition (column 2).

The anatomical brain area corresponding to the peak coordinates of each significant cluster was determined with SPM Anatomy Toolbox 2.0 (Eickhoff et al., 2005). The effect on tracer binding within the significant clusters was then quantified by applying the clusters as masks in Carimas and then using these masks on the normalized PET images from each subject and treatment.

5. RESULTS

5.1. Repeatability of [^{11}C]ORM-13070 PET in humans (study I)

Six healthy males provided informed consent for the study. The age of the subjects [mean (standard deviation, SD)] was 27 (7) years, their weight was 78 (6) kg, and their body mass index (BMI) was 24 (3). All subjects were non-smokers and were not using any medications. A duration of 90 min of PET imaging with HRRT was performed twice with each subject. The mean (SD) radioactive dose was 478 (63) MBq during PET scan 1 and 486 (28) MBq during PET scan 2.

The tracer entered the brain swiftly, with only perfusion appearing to limit its passage. The plasma-to-brain transport rate (K_1) was estimated to be approximately 0.3 ml plasma/(ml brain tissue x min). Specific tracer binding in the brain reached its maximum at about 15 min after the injection, as determined by visual inspection of the target / reference region ratios of the dorsal striatum. Pseudo-equilibrium of tracer binding in the dorsal striatum occurred approximately 5-10 min post-injection, as estimated by visual inspection of the TACs (cerebellum subtracted from regional TACs).

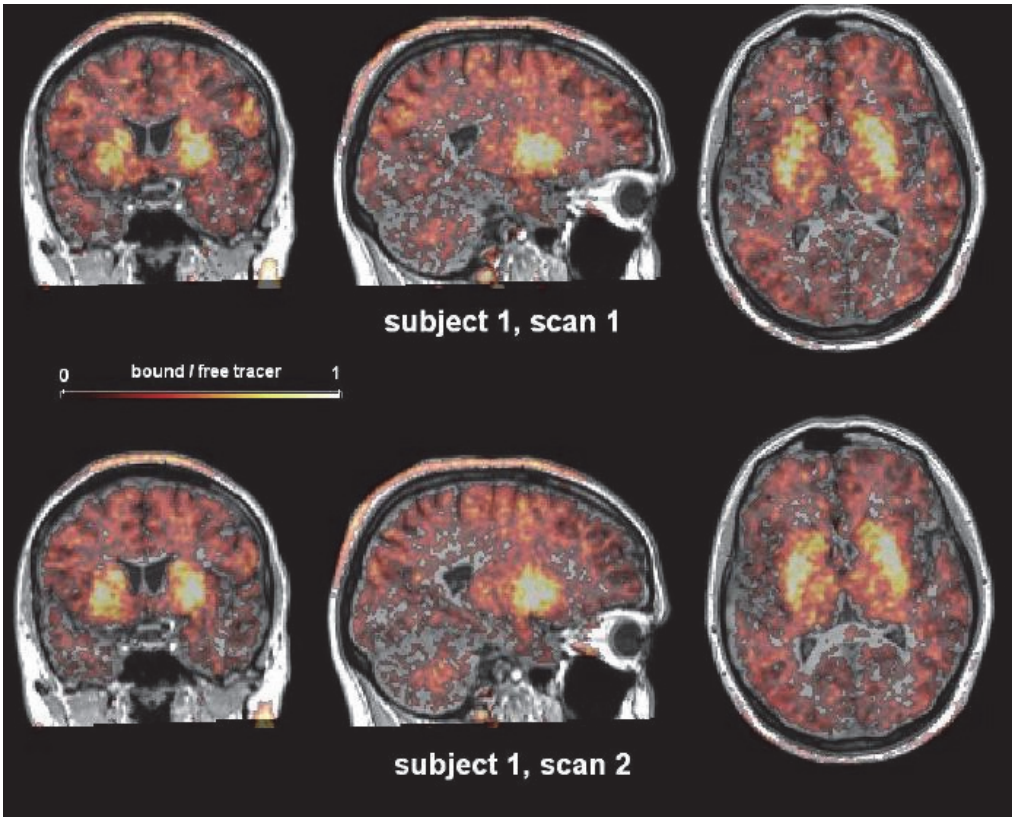

Figure 15. The bound/free ratio of tracer uptake from repeat experiments illustrated at the whole brain level.

The bound/free ratios (BP_{ND}) of tracer uptake were highest in the putamen and caudate nucleus, followed by the thalamus. Lower ratios were observed in all cortical regions and also in the hippocampus. The average BP_{ND} of [^{11}C]-ORM-13070 relative to the cerebellum ranged from 0.77 in the putamen to 0.08 in the hippocampus. The mean values of absolute variability ranged from 4.3 % in the putamen to 29.1 % in the hippocampus; the average variability was < 10 % also in the caudate nucleus and the thalamus (Table 2). The intra-class correlation coefficient ranged from 0.50 in the hippocampus to 0.89 in the thalamus, and was >0.70 also in the caudate nucleus, putamen, lateral frontal cortex and parietal cortex. Test-retest variability was slightly inferior with SRTM: mean absolute variability was 8.5 % and 5.5 % and the ICCs were 0.67 and 0.79 for the caudate nucleus and the putamen, respectively (Table 2).

Table 2. The repeatability of [^{11}C]-ORM-13070 binding in different brain areas

Region	BP_R Scan 1	BP_R Scan 2	Absolute variability % (SD)	ICC
Caudate nucleus	0.58 (0.07)	0.58 (0.07)	6.5 (4.2)	0.76
Putamen	0.76 (0.08)	0.77 (0.10)	4.3 (3.5)	0.88
Thalamus	0.29 (0.06)	0.30 (0.06)	6.6 (6.1)	0.89
Hippocampus	0.11 (0.03)	0.08 (0.04)	29.1 (28.7)	0.50
Lateral frontal cortex	0.19 (0.05)	0.18 (0.05)	13.6 (7.3)	0.84
Occipital cortex	0.19 (0.03)	0.18 (0.04)	13.9 (12.8)	0.63
Parietal cortex	0.18 (0.06)	0.17 (0.06)	13.2 (7.5)	0.89

The ratio of the cerebellum TAC to metabolite-corrected plasma TAC seemed to increase linearly over time, suggestive of additional uptake of radioactivity into the cerebellum, most likely caused by radioactive metabolites (M1 and/or M2). The sum of the parent tracer and both metabolites seemed to best abolish the trend in the ratio curve, but the contribution of M2 was negligible. A compartmental model with a single tissue compartment for the parent tracer and another tissue compartment for both metabolites fitted the data reasonably well. The assumption that the transport rates from plasma to brain tissue should be the same for the parent tracer and the metabolites did not cause any deterioration to the fit. Assuming that either only M1 or both metabolites could pass the BBB resulted in similar fits. The BP_{ND} derived from a compartmental model correlated well with the BP estimated from the tissue ratios.

In conclusion, the results of study I validated the determination of B/F ratios as a reliable way of obtaining a compound estimate of receptor density and affinity with [^{11}C]ORM-13070. High test-retest reliability and good agreement with compartmental model combined with the short scanning times and no requirement for arterial blood sampling all meant that [^{11}C]ORM-13070 represented an appealing option for use in PET studies in humans.

5.2. Estimation of dose-dependent occupancy of α_{2C}-ARs by atipamezole and exploration of sensitivity to endogenous noradrenaline (study II)

The study subjects (n=8) were healthy Caucasian males [average age 28 (SD 5.6), weight 73 kg (SD 8.1), BMI 23 kg/m^2 (SD 2.6)]. Each subject underwent a control PET scan, a scan with atipamezole pre-treatment, and two scans with two different noradrenaline challenges in a balanced, randomized study design. PET imaging with the HRRT camera was used to investigate the capacity of different doses (20–450 µg/kg) of atipamezole to block tracer binding (Fig. 16) and as an exploratory part of the study, the effects of four different noradrenaline challenges on the striatal uptake of [^{11}C]ORM-13070. The noradrenaline challenges included intravenous ketamine injection, peroral administration of atomoxetine capsules, a hypoglycemic insulin clamp protocol and immersion of the subject's left foot in cold water.

The maximal extent of inhibition of striatal [^{11}C]ORM-13070 uptake achievable by the administration of atipamezole, as estimated using non-linear regression analysis of the tracer binding potential and atipamezole plasma concentration data, was 78 % (95 % CI 69–87) in the caudate nucleus and 65 % (95 % CI 53–77) in the putamen. These estimates were similar when the atipamezole dose was plotted against receptor occupancy, with E_{max} estimates of 79 % and 66 %, respectively. EC_{50} was estimated to be 1.6 ng/ml (95 % CI 0.6–2.7) in the caudate nucleus and 2.5 ng/ml (0.3–4.7) in the putamen.

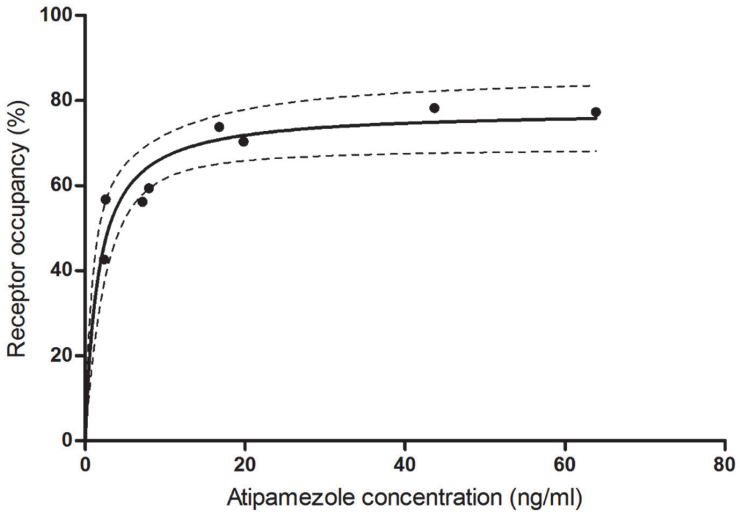

Figure 16. The concentration-effect relationship of atipamezole pre-treatment (n=8) in the caudate nucleus. The solid line represents an unconstrained one-site binding hyperbole. The dotted lines represent the 95 % confidence interval.

The mean (SD) atomoxetine concentrations at the start and end of the PET scans were 346 (205) ng/ml and 345 (171) ng/ml, respectively, and the mean (SD) insulin concentrations were 101 (20) mU/l and 99 (30) mU/l, respectively. Ketamine concentrations were [164 (34) ng/ml] at the end of the PET scan, while the concentrations at the start of the scans were lower [54 (17) ng/ml]. The determined binding affinities of the study drugs for α_{2C}-ARs ranged from negligible (atomoxetine) to

non-existent (ketamine), which confirmed that no direct competition with tracer binding confounded the noradrenaline challenges.

The average reduction in tracer uptake in the dorsal striatum was about 10-12 % after atomoxetine and the cold pressor test. Ketamine was associated with 13-16 % average reductions in tracer uptake. Insulin-induced hypoglycaemia was not associated with any changes in tracer uptake. Even though the atipamezole results pointed to a possible underestimation of receptor occupancy, it was concluded that [^{11}C]ORM-13070 was suitable for clinical imaging of brain α_{2C}-AR occupancy by competing ligands. Preliminary support was also acquired for the capacity of [^{11}C]ORM-13070 PET to reflect endogenous noradrenaline neurotransmission.

5.3. Sensitivity of [^{11}C]ORM-13070 to increased synaptic noradrenaline (study III)

Eight subjects underwent a baseline PET scan with [^{11}C]ORM-13070 and two PET scans after two different noradrenaline challenges, i.e. intravenous administration of ketamine and an oral dose of atomoxetine combined with immersion of the left foot in cold water in a randomized order to test whether tracer binding could be reduced by increased synaptic noradrenaline levels. The subjects were Caucasian males in good general health. The BMI of the subjects was 22–26 kg/m^2 (mean 24, SD 1 kg/m^2), and they weighed 68–94 kg (mean 83, SD 9 kg).

Both noradrenaline challenges caused moderate (up to 20%) reductions in tracer binding (Fig. 17), as estimated by ROI-based analysis in the dorsal striatum. The average (5-30 min) reduction in striatal BP_{ND} of the tracer was 0.13 (p=0.007, 95% CI 0.04–0.21) in the putamen and 0.14 (p=0.010, 95%, CI 0.04–0.24) in the caudate nucleus with the ketamine challenge and 0.15 (p=0.004, 95% CI 0.06–0.24) in the putamen and 0.18 (p=0.004 95 % CI 0.07–0.29) in the caudate nucleus with the atomoxetine+cold challenge. The reductions were slightly more pronounced when measured in the time window of 10-20 min rather than 5-30 min, but nonetheless of a similar overall magnitude.

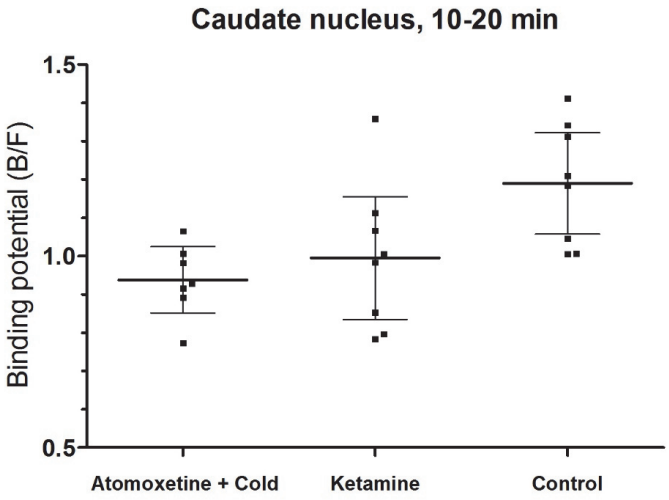

Figure 17. Bound per free tracer (estimated with BPND, n=7 for atomoxetine + cold, n=8 for ketamine and the control condition) in the caudate nucleus in the scanning time interval of 10-20 minutes. Means and 95 % CIs are indicated by the horizontal bars.

Voxel-based analysis (Fig. 18) suggested that the most significant change in B/F tracer with the ketamine challenge took place in the right posterior putamen. Coordinates of the peak voxel [T=9.84, $p=1.08\times10^{-7}$, p(FWE)=0.049] (x, y, z) in MNI space were 30, -3, -7.5, and the cluster size was 523 voxels [p(FEW)=0.00066, p(FDR)=0.010]. A large significant cluster in the dorsal putamen was also seen with the atomoxetine+cold challenge, but the global maximum [T=9.97, $p=9.28\times10^{-8}$, p(FWE)=0.044] was located in coordinates (x, y, z) -16, -72, 0 in the left lingual gyrus. The corresponding cluster consisted of 568 voxels [p(FEW)=0.0004, p(FDR)=0.0014]. Tracer binding in the most significant putamen clusters was reduced by 24 % ($BP_{ND}^{control}$ 1.00, SD 0.15; $BP_{ND}^{ketamine}$ 0.76, SD 0.14) and 23 % ($BP_{ND}^{control}$ 1.02, SD 0.10; $BP_{ND}^{atomoxetine/cold}$ 0.79, SD 0.11) with ketamine and atomoxetine+cold, respectively.

The average atomoxetine concentration in plasma at the time of tracer administration was 501 (SD 215) ng/ml and the average end-of-PET concentration was 414 (SD 159) ng/ml. On average, the targeted plasma concentration of 300 ng/ml was reached during the ketamine scans by end-of-PET, (314 ng/ml, SD 70 ng/ml). The ketamine challenge was associated with statistically significant increases (Baseline - (tracer administration + end-of-PET)/2 = 0.77 nmol/l, p=0.004) in plasma noradrenaline concentrations, but statistical significance was not reached with the atomoxetine+cold challenge. Increases in plasma adrenaline concentrations were also detected during the ketamine challenge (0.30 nmol/l, p=0.002) but not during the atomoxetine+cold challenge (0.01 nmol/l, p=0.88) or the control condition.

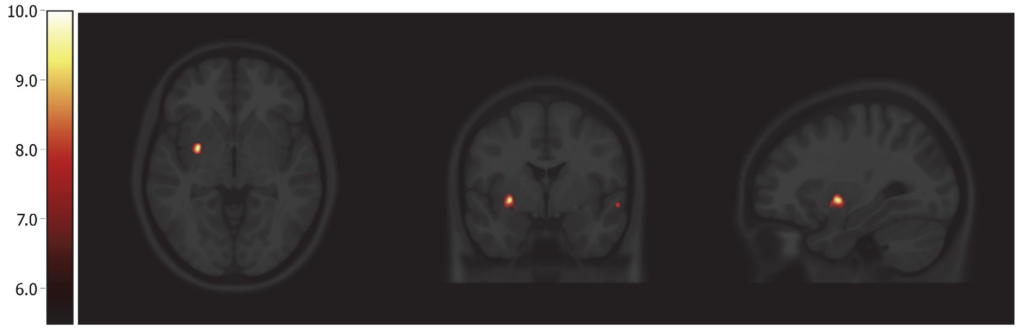

Figure 18. Parametric images of the peak significant cluster in the right dorsal putamen after ketamine administration.

Strong experimental support was obtained confirming the suitability of [^{11}C]ORM-13070 for imaging endogenous synaptic noradrenaline.

5.4. Effect of dexmedetomidine on [^{11}C]ORM-13070 binding in the CNS (study IV)

Six healthy Caucasian male subjects were included in the study. The average (SD) age, weight and body mass index of the subjects were 22 (0.5) years, 75.9 (7.5) kg and 23.2 (1.7) kg/m^2, respectively. Each subject received three injections of [^{11}C]ORM-13070: one without dexmedetomidine [baseline (BL) scan], and two scans with low [0.2 ng/ml (LD)] or high [0.6 ng/ml (HD)] target concentrations of dexmedetomidine in plasma. Dexmedetomidine was employed as a pharmacological challenge to inhibit the neuronal release of noradrenaline. The hypothesis of increased tracer binding in response to a sympatholytic drug was tested with PET. Tiredness was evaluated with visual analog scales (VAS). Cardiovascular effects of the drug infusions were assessed by monitoring of vital signs.

According to VAS, the high dexmedetomidine dose was clearly sedative, while there was no significant sedative effect associated with the low dose. As a sign of centrally mediated peripheral sympatholytic effects, both systolic and diastolic blood pressures were significantly lowered with both dexmedetomidine dose levels compared to the control condition at the end-of-PET time point, while there were no statistically significant differences at 5 min after the start of the infusions. There were no significant differences in heart rate at either time point.

Statistical parametric mapping was performed to identify the brain regions with significant changes in tracer binding. In addition, ROI-based analysis was performed in areas with known high densities of α_{2C}-ARs. Pairwise comparisons LD-BL and HD-BL were made with the uncorrected alpha set to 0.05. Both dose levels were associated with a tendency towards increased values of B/F tracer in the right thalamus (Figure 16, peak MNI x, y, z: 9, -12, 4), 17 % (p=0.138) and 19 % (p=0.054) respectively. A large and statistically significant increase of 42 % (p=0.010) in B/F tracer was associated with the comparison LD-BL in the right superior temporal gyrus (Figure 2, peak MNI x, y, z: 60, -12, 1).

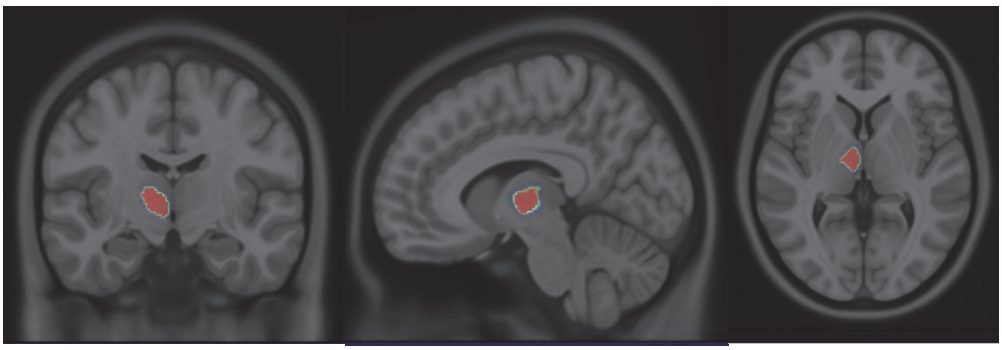

Figure 19. The cluster in the right thalamus (MNI x, y, z: 9, -12, 4) revealed by the comparison HD-BL. The figures are in radiological orientation.

ROI-based analysis in the caudate nucleus and putamen revealed no effect on tracer binding with either dexmedetomidine dose level. The analysis was performed with two slightly different time intervals, 5-30 min and 10-20 min, with similar outcomes. The lack of effect in the areas of dense α_{2C}-AR distribution indicated that direct competition binding by dexmedetomidine did not significantly confound the results in other areas where effects indicative of dexmedetomidine-induced reductions in noradrenaline release were detected. ROI-based analysis in the thalamus revealed a 9 % ($p>0.05$) increase in tracer binding in both hemispheres with the high dexmedetomidine dose, while the global thalamic effect of the low dose was below the absolute test-retest variability established in study I, i.e. negligible.

Analysis of tracer metabolism from venous blood samples at 5, 15 and 30 min after tracer injection revealed that dexmedetomidine reduced the fraction of the intact tracer at the higher dose level, while the low dose had a smaller and statistically insignificant effect. The average fraction of intact [^{11}C]ORM-13070 in blood at 15 min and 30 min after tracer injection was approximately 17 % lower during the high-dose infusions than during the control scan. Average dexmedetomidine concentrations in plasma at the end of the 30-minute infusions were 0.17 ng/ml (SD 0.02) and 0.63 ng/ml (SD 0.13) with the low and high target concentrations, respectively.

6. DISCUSSION

The absolute test-retest variability of [^{11}C]ORM-13070 binding in study I was 4.3 % in the putamen and 6.5 % in the caudate nucleus, with intra-class correlation coefficients of 0.88 and 0.76, respectively. Such high values of test-retest reliability enabled the use of [^{11}C]ORM-13070 for the detection of small endogenous transmitter–induced changes in studies II-IV with relatively small sample sizes. BP_P and BP_{ND} correlated well and were of a similar magnitude, which suggested that non-specific binding in plasma and tissue was not affecting the estimation of specific binding.

The binding estimates obtained with the tissue ratio method and the compartmental model correlated well and the repeatability of tracer binding with the ratio method was superior to SRTM, which is commonly regarded as the standard reference tissue modelling method. Most probably due to the presence of the radioactive metabolites, SRTM did not ideally fit the data. The modelling work indicated that it would be better to use B/F ratios as a compound estimate of receptor density and affinity. The ratio method combined with the rapid kinetics of [^{11}C]ORM-13070 was seen as advantageous with regard to human PET studies since it enabled short scanning times, minimized radiation exposure and the procedure had the added advantage of relative non-invasiveness which meant that there would be no requirement for arterial cannulation in subsequent studies.

[^{11}C]ORM-13070 was dose-dependently displaced by atipamezole from α_{2C}-ARs in the dorsal striatum in study II, but E_{max} remained below 80 %, even though based on its known high *in vitro* affinity, high atipamezole doses would have been anticipated to occupy close to 100 % of all striatal α_{2C}-ARs, and thus the reduction in the binding potential of [^{11}C]ORM-13070 was expected to approach 100 % at high atipamezole concentrations in plasma. The radioactive metabolites of the tracer provide the most likely explanation for this minor discrepancy. Two radioactive metabolites of [^{11}C]ORM-13070 were identified in preclinical experiments and it has been postulated that at least one of them can gain access to the brain. The molecular structures of the metabolites have not been resolved in spite of efforts involving stable isotopes and mass spectroscopy, but they are thought to be volatile, very small molecular weight fragments that are likely to have no affinity for α_{2C}-ARs (Arponen et al., 2014). However, despite the metabolites only exhibiting non-displaceable binding, it is possible that metabolism could cause increased accumulation of radioactivity in the reference region, which may be more pronounced than the non-displaceable uptake of radioactivity in the target region. This would explain why atipamezole was apparently unable to fully displace the tracer from α_{2C}-ARs in the human brain. With the proviso of the aforementioned scenario, the ratio method is robust in terms of possible bias caused by tracer metabolism, as long as there are no treatment-related alterations compared to the control condition, which was confirmed with venous blood sampling during the PET scans.

It is possible that [^{11}C]ORM-13070 could be binding to other specific sites in addition to α_2-ARs that would be more abundant in the striatum than in the cerebellum. Another alternative explanation involves the cellular localization of α_{2C}-ARs: a significant proportion of α_{2C}-ARs may have an intracellular location (Olli-Lähdesmäki et al., 1999), and the presence of the antagonist atipamezole could cause increased cell surface expression of the receptors (Olli-Lähdesmäki et al., 2003). In the event of increased cell surface receptor expression, then one would predict that the value of B_{max} would increase, which would result in larger apparent BP values.

From a pure PET modelling standpoint, a bolus + infusion approach where true equilibrium in tracer kinetics is achieved would theoretically be better suited for binding estimation with tissue ratios. Unfortunately, certain practical aspects currently prevent the use of [^{11}C]ORM-13070 infusions in studies on humans, as the currently available radiosynthetic methods are not able to consistently produce more than 1 GBq of [^{11}C]ORM-13070 at one time without sacrificing high specific activity. Due to the 20 min half-life of carbon-11, true equilibrium would be reached at a relatively late stage when the overall radioactivity would be low, which would in turn reduce the ratio of true signal-to-noise. The radioactive metabolites could also accumulate sufficiently to further decrease this ratio, as was indicated during earlier bolus + infusion studies with [^{11}C]ORM-13070 in cynomolgus monkeys (Finnema et al., unpublished data), and furthermore the required imaging time would be at least doubled.

Bolus injections of radiotracers result in a transient equilibrium (also referred to as pseudo equilibrium; Farde et al., 1989), and analysis with the ratio method is typically performed by including parts of both the upward and downward slopes of the radioactivity curves, as well as the theoretical point of equilibrium where the derivative for specific binding is zero. Thus, the bolus method also includes a measure of radiotracer wash-out (k_2), which could be sensitive to changes in regional cerebral blood flow (CBF). If the employed pharmacological (or other) challenges were to alter CBF significantly, this could confound the results or, in extreme cases, even fully account for the observed effects. The observed reductions in [^{11}C]ORM-13070 binding in studies II, III are not, however, regarded as a result of increased CBF for reasons which will be discussed below.

The exploratory findings found in study II of reduced tracer binding in response to noradrenergic challenges were seen across different mechanisms of action and the findings were repeated in study III with larger numbers of subjects and slightly modified challenge designs, where tracer binding in the striatum was reduced by up to 24 % and 23 % with ketamine and atomoxetine+cold stimulation, respectively. The use of anesthesia has been demonstrated to affect binding of other PET tracers such as [^{11}C]raclopride in test animals (Ginovart et al., 2002, McCormick et al., 2011), but the ketamine concentrations employed in studies II and III were clearly sub-anesthetic, and similar reductions in tracer binding were also seen with cold exposure and atomoxetine. Although no *in vivo* data are available, it is very unlikely that NET inhibition would significantly affect CBF. Increased regional CBF has been demonstrated by PET in humans in response to i.v. ketamine in frontal, orbital-frontal, and anterior cingulate cortical areas, but not in the dorsal striatum (Rowland et al., 2010). Furthermore, even though there was no effect on [^{11}C]ORM-13070 binding, insulin-induced hypoglycemia has been reported to significantly increase regional CBF in the human striatum, as demonstrated with both pulsed arterial spin labelling-MRI (pASL-MRI) and PET (Arbeláez et al., 2013). This would suggest, although not conclusively, that the estimation of [^{11}C]ORM-13070 binding with tissue ratios is not particularly sensitive to changes in regional blood flow.

Since ketamine is believed to release also other brain monoamines in addition to noradrenaline, most notably dopamine, a striatal dopamine D2 receptor binding study was used as a reference for the targeted ketamine concentration in studies II and III. Ketamine administration did not decrease striatal [^{11}C]raclopride uptake at an average plasma ketamine concentration of approximately 300 ng/ml (Aalto et al., 2002). Even though earlier studies with plausible methodological weaknesses have reported decreased [^{11}C]raclopride binding in response to ketamine, the finding of unaffected

tracer binding to D_2/D_3 receptors has been subsequently repeated across species i.e. in humans (Kegeles et al., 2002) and test animals (Hassoun et al., 2003), and ketamine's negligible effect on striatal dopamine has also been confirmed in microdialysis experiments performed in non-human primates (Adams et al., 2002).

Atomoxetine reduced striatal [^{11}C]ORM-13070 uptake to a similar small extent as ketamine in study II and the same was true for the cold pressor test. Until recently, atomoxetine was regarded as a highly selective inhibitor of neuronal noradrenaline re-uptake, and the doses used in studies II and III were assumed to produce near-complete NET occupancy (Takano et al., 2009). There is no evidence or indication of a direct excitatory effect on dopamine neurotransmission. A recent PET study with non-human primates revealed that atomoxetine achieves almost equal occupancy of SERT compared to NET after acute administration of clinically relevant doses (Ding et al., 2014) but this would theoretically reduce, rather than increase, striatal dopaminergic activity by activating 5-HT_{2C} receptors (Alex et al., 2005; Egerton et al., 2008). It is therefore very unlikely that the reductions in tracer binding following atomoxetine administration were caused by increased extracellular (synaptic) dopamine.

The effects of both atomoxetine and ketamine on extracellular noradrenaline levels have been well documented. Previously, NET inhibition by atomoxetine has been shown to result in up to eight-fold increases in brain ECF noradrenaline concentrations following acute exposure (Montezinho et al., 2010; Umehara et al., 2013). Up to five-fold increases in rat brain ECF noradrenaline levels have been detected with microdialysis following ketamine administration (Kubota et al., 1999; Lorrain et al., 2003; Tose et al., 2009). Now for the first time, results consistent with those obtained in test animals have been achieved by *in vivo* imaging results from human subjects.

Atomoxetine was combined with the cold pressor test in study III. It is acknowledged that this challenge protocol is somewhat lacking in specificity with respect to brain neurotransmitters and the contributing effects of monoamine transmitters other than noradrenaline cannot be excluded. The combination challenge was associated with approximately equal reductions in striatal [^{11}C]ORM-13070 uptake as observed with ketamine, and the decrease in tracer uptake was somewhat larger than that seen with the two challenges employed separately in study II. The consistent and complementary findings obtained with four different challenge protocols across studies II and III supported the conclusion that striatal [^{11}C]ORM-13070 uptake can reflect increased extracellular (synaptic) noradrenaline concentrations in the brain, but a possible contribution of synaptic dopamine levels to the observed changes in striatal [^{11}C]ORM-13070 binding cannot be completely excluded.

Catecholamine concentrations in peripheral venous plasma were not significantly increased in conjunction with any of the noradrenaline challenges in study II and the atomoxetine + cold pressor combination challenge, but ketamine administration was associated with significantly increased plasma concentrations of both noradrenaline and adrenaline in study III. The discrepancy in ketamine-evoked responses between studies II and III is in part explained by the increase in dose and possibly also by improved control of sampling times in study III. Blood sampling for the baseline noradrenaline sample was always performed at least 20 min after i.v. cannulation in a well-controlled resting state in study III, whereas there was more variation in study II due to some

difficulties in blood sampling, which quite possibly resulted in elevated baseline noradrenaline levels during certain visits. It is not apparent why the atomoxetine + cold pressor challenge in study III did not evoke similar plasma catecholamine responses as ketamine, as the CNS effects of the two challenges were similar in magnitude. It is unclear how well peripheral i.e. venous, noradrenaline levels actually reflect noradrenaline release in the brain. The fact that both noradrenaline and adrenaline were elevated peripherally while there seems to be no connection to the perceived CNS effects would point to adrenomedullary activation being associated with the ketamine challenge, but this peripheral contribution should not influence the CNS findings and vice versa, unfortunately further exploration of this phenomenon was beyond the scope of this thesis work.

The PET imaging results also provided evidence of reduced noradrenaline release in the brain associated with i.v. dexmedetomidine infusion in study IV, even although there were no statistically significant effects in the brain regions with highly repeatable tracer binding. Binding in the right superior temporal gyrus was significantly increased by the low dose dexmedetomidine infusion, and furthermore a clear indication of a treatment effect was also detected in the right thalamus with both dose levels. The increase in tracer binding was primarily interpreted as evidence of a reduction in extracellular (synaptic) noradrenaline levels, i.e. in accordance with the original hypothesis based on the occupancy model.

The results of study IV are also in agreement with other studies, which have implicated the thalamus as an important anatomic location in both disruption (He et al., 2014) and recovery (Gummadavelli et al., 2014) of consciousness. Dexmedetomidine infusions have been linked to decreases in CBF and brain glucose metabolism with pASL-MRI and FDG-PET, respectively (Akeju et al., 2014), and the initiation of dexmedetomidine-induced loss of consciousness has been shown to affect the thalamus and cortical areas concurrently (Baker et al., 2014). It has also been postulated that the afferent neurons from the LC are involved in a thalamic gating mechanism of nociception (Vogt et al., 2008).

Decreased regional CBF may have affected the tracer binding estimates in study IV by decreasing k_2, i.e. an opposite effect compared to that encountered in studies II and III. The results of Akeju et al. (2014) indicated that there can be decreases in thalamic CBF caused by dexmedetomidine, but this was seen at anesthetic drug concentrations. Binding in the right superior temporal gyrus in study IV was only increased by the low-dose dexmedetomidine infusion ($p<0.05$), while the higher dose had no effect. The higher dose should have had a more pronounced effect (if any) on CBF, while the low dose, considered to be non-anesthetic by the subjects in their VAS assessments, was not expected to affect CBF similarly to anesthesia. The lack of cortical effects with the higher dose may have been at least partially explained by the reduced fraction of the parent drug during the higher dose, which reduced specific binding in reference to the cerebellum. It is possible that a more pronounced inhibitory effect on noradrenaline release was only partially able to overcome for the negative bias caused by the reduced parent fraction in the thalamus, and thus only moderate increases ($p>0.05$) were seen with both dose levels in the voxel-based analysis, although the effect of the high dose was slightly more pronounced ($p>0.05$). ROI-based analysis in the thalamus revealed an approximately 9 % ($p>0.05$) increase in tracer binding in both hemispheres with the high dexmedetomidine dose, while the global thalamic effect of the low dose was within the absolute test-retest variability established in study I, i.e. it was regarded as negligible.

The small sample size (n=6) restricted the interpretation of the results of study IV. Despite the previously demonstrated high repeatability of thalamic [^{11}C]ORM-13070 binding (absolute variability 6.6 %; see results section for details), a larger sample size would have been required to reach statistical significance. The relevance of the (statistically significant) isolated cortical finding is unclear with regard to previously published data on dexmedetomidine and the CNS effects of anesthetic agents in general, and the repeatability of cortical [^{11}C]ORM-13070 binding is questionable, with an absolute test-retest variability of 13-14 % (see results section for details).

Even though the effects of dexmedetomidine are known to be primarily mediated via the α_{2A}-AR subtype (Bucheler et al., 2002, Lähdesmäki et al., 2003; Ihalainen and Tanila, 2004), it was considered possible that the higher dose, while being more likely to cause significant inhibition of noradrenaline release, might also be able to significantly reduce tracer binding by direct competition with the tracer for binding to α_{2C}-ARs. The lower dose, which caused similar peripheral sympatholytic effects, was not expected to result in any detectable α_{2C}-AR occupancy. Significant competition binding with the tracer did not occur in study IV as this would have become manifested in the dorsal striatum where the majority of α_{2C}-ARs are located, and where [^{11}C]ORM-13070 binding was unaffected regardless of the dexmedetomidine dosage. Thus, the binding estimates in other brain areas were not likely to be significantly affected by direct competition binding by dexmedetomidine, which would have counteracted the indirect pharmacodynamic effect, i.e. reduced synaptic noradrenaline levels. The thalamus is a well-documented noradrenergic projection area of the LC (Vogt et al., 2008; Devilbiss et al., 2012), whereas relatively little is known about noradrenergic innervation of the dorsal striatum. Even if dexmedetomidine might efficiently reduce synaptic concentrations of noradrenaline in the brain, the effect size on tracer binding might remain relatively small; in contrast, potent monoamine releasing drugs like ketamine, which was used in studies II and III, may evoke abrupt and dramatic increases compared to the basal transmitter levels, more easily detectable by PET (Kubota et al., 1999; Lorrain et al., 2003; Tose et al., 2009). While the LC sends projections across the neuraxis and cortical areas (Keren et al., 2009), detecting a treatment effect also requires sufficient binding sites for the tracer, and the cortical distribution of α_{2C}-ARs outside striatal and thalamic areas is scarce.

Based on physiological responses to knock-outs of genes encoding the α_{2A} -and α_{2C} –subtypes in mice (Hein et al., 1999; Altman et al., 1999), the primary function of the α_2-ARs is regarded to be pre-synaptic auto-inhibition. When measured from rat CSF after sub-cutaneous administration of dexmedetomidine, the concentration of the main metabolite MHPG was dose-dependently reduced (MacDonald et al., 1991). Major reductions in plasma concentrations of both noradrenaline (up to 66-85 %) and adrenaline have been detected in humans with dexmedetomidine plasma concentrations of 0.5-8.0 ng/ml (Ebert et al., 2000). Significant attenuations of noradrenaline release caused by injection stress *in vivo* (Ihalainen and Tanila, 2004) and electrical stimulation *in vitro* in cortical slices (Bucheler et al., 2002) have been demonstrated in response to two α_2-AR agonists, dexmedetomidine and brimonidine in wild-type mice but not in α_{2A}-AR knock-out mice. However, no significant differences in baseline levels of noradrenaline were detected in the ventral striatum (nucleus accumbens) of mice in response to α_{2A}-AR knock-out, as determined by ECF microdialysis (Ihalainen and Tanila 2004). Dexmedetomidine's effect on synaptic noradrenaline may become more apparent under stressful conditions, and inhibition of stimulated noradrenaline release could be tested by combining dexmedetomidine with a physiological challenge, e.g. cold exposure, which decreased tracer binding in study II.

Generally, a lack of effect in one brain area is not conclusive evidence of an overall lack of effect, and conversely synaptic changes in transmitter levels in response to systemic drug infusions may vary between brain areas, and microdialysis (or CSF sampling) may not be able to reliably detect these effects. An endogenous dopamine displacement protocol performed with amphetamine and methylphenidate to increase synaptic dopamine concentrations, which utilized [^{11}C]raclopride PET combined with ECF microdialysis in rhesus monkeys, could detect no linear correlation between the the ECF and PET findings: An approximately 25 % decrease in striatal [^{11}C]raclopride binding was seen with both drugs, while the (ECF) concentration of dopamine was increased fourfold more with amphetamine compared to methylphenidate (Schiffer et al., 2006). The pharmacodynamic properties of the investigational medicinal products seem to determine whether there is a correlation between microdialysis and binding measured by PET e.g. a linear correlation has been observed with amphetamine (Narendran et al., 2014).

A low basal level of receptor occupancy by endogenous noradrenaline may prevent the detection of subtle changes in transmitter release. Inhibition of stimulated neurotransmitter release has been tested with moderate success in humans by administering amphetamine and reserpine with the dopamine D_2/D_3 receptor tracer IBZM (Innis et al., 1992; Laruelle et al., 1996). The noradrenergic hypothesis of the pathogenesis of memory impairing neurological diseases states that there is disturbed LC function and cell loss (Marien et al., 2004). Thus, inhibition of stimulated release by α_{2A}-AR-agonists like dexmedetomidine should be disturbed in neurological conditions where normal LC function is impaired. As a result of the reduced LC cell count, the basal noradrenaline level in the projection areas should be reduced concurrently with an impaired capacity for drug-induced autoinhibition due to a reduction in the number of binding sites, i.e. α_{2A}-ARs.

Prior to studies in AD/PD patients, the feasibility of this type of modified challenge protocol needs to be tested initially in healthy volunteers. Subsequently, one could conduct follow-up PET imaging in MCI/AD/PD patients to assess whether disturbances LC function actually predict the development of cognitive impairment or even precede other biomarkers of AD such as the brain amyloid load or CSF biomarkers. This type of costly and time-consuming follow-up is not feasible based on the present understanding of the topic, which is mostly based on theoretical considerations. However, should possible pilot experiments with AD patients confirm a noradrenergic disturbance, the situation would change. A straightforward approach would be to measure [^{11}C]ORM-13070 binding in patient groups compared with age-matched controls. The result would represent a combination of changes in receptor density and possible alterations in competing transmitter levels, in fact these two factors could not be reliably differentiated, but would reveal a disturbance at the level of noradrenergic transmission in specific parts of the brain. Furthermore, this approach would also provide valuable information on whether [^{11}C]ORM-13070 PET would be useful for studying CNS pathology in general. Interestingly, in addition to cortical areas, the presence of significant Aβ deposits that precede the onset of dementia in patients with autosomal dominant (i.e. hereditary) AD has been detected in the thalamus and particularly in the caudate nucleus (Bateman et al., 2012), which are also areas with significant and highly reproducible levels of [^{11}C]ORM-13070 binding.

7. CONCLUSIONS

[^{11}C]ORM-13070 was validated as a new radiotracer for use in PET imaging studies in humans. The repeatability of [^{11}C]ORM-13070 binding in healthy individuals was found to be very high. The binding properties of the tracer enabled reliable estimation of α_{2C}-AR occupancy by using the reference tissue ratio method; with this approach there was low test-retest variability. Even though there may have been some degree of apparent underestimation involved in the receptor occupancy estimates when assessed with competing ligands, [^{11}C]ORM-13070 is still considered to be suitable for use in the clinical development process of novel α_{2C}-AR ligands e.g. to determine the best doses and dosing intervals for clinical trials in patients.

By utilizing different pharmacological and physiological challenges, convincing experimental evidence was gained to support the suitability of [^{11}C]ORM-13070 PET for detecting increases in endogenous synaptic noradrenaline levels in the human brain. The PET imaging results were less conclusive with regard to detecting reductions in synaptic noradrenaline levels. While significant cortical effects indicative of drug-induced inhibition of noradrenaline release were seen, detection of inhibitory effects on stimulated release in the dorsal striatum and/or the thalamus would provide even further support for the suitability of [^{11}C]ORM-13070 PET for monitoring reductions in noradrenergic neurotransmission.

The thesis work revealed new information as well as supported existing hypotheses on the CNS effects of the investigated agents. For the first time in humans, ketamine and atomoxetine were shown to increase synaptic noradrenaline levels in striatal as well as cortical brain areas; furthermore a simultaneous increase in peripheral noradrenaline concentrations was also detected when ketamine was administered.

The successful validation of [^{11}C]ORM-13070 PET imaging will benefit the future clinical development of drugs affecting brain noradrenergic neurotransmission. Potential therapeutic areas include neurology and psychiatry, where [^{11}C]ORM-13070 PET can be employed in the development of new treatments e.g. for Alzheimer's disease and schizophrenia. [^{11}C]ORM-13070 PET also has potential to be a useful new clinical tool for research investigating the pathophysiology of various brain disorders involving disturbances in noradrenergic neurotransmission.

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REFERENCES

Aalto S, Hirvonen J, Kajander J, Scheinin H, Nagren K, Vilkman H *et al* (2002). Ketamine does not decrease striatal dopamine D2 receptor binding in man. *Psychopharmacology (Berl)* **164:** 401-406.

Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M *et al* (1998). Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry* **155:** 761-767.

Adams B and Moghaddam B (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* **18:** 5545-5554.

Adams BW, Bradberry CW, Moghaddam B (2002). NMDA antagonist effects on striatal dopamine release: microdialysis studies in awake monkeys. *Synapse* **43:** 12-18.

Ago Y, Umehara M, Higashino K, Hasebe S, Fujita K, Takuma K *et al* (2014). Atomoxetine-induced increases in monoamine release in the prefrontal cortex are similar in spontaneously hypertensive rats and Wistar-Kyoto rats. *Neurochem Res* **39:** 825-832.

Airaksinen AJ, Finnema SJ, Balle T, Varnas K, Bang-Andersen B, Gulyas B *et al* (2013). Radiosynthesis and evaluation of new alpha1-adrenoceptor antagonists as PET radioligands for brain imaging. *Nucl Med Biol* **40:** 747-754.

Akeju O, Loggia ML, Catana C, Pavone KJ, Vazquez R, Rhee J *et al* (2014). Disruption of thalamic functional connectivity is a neural correlate of dexmedetomidine-induced unconsciousness. *Elife* **3:** 10.7554/eLife.04499.

Alex KD, Yavanian GJ, McFarlane HG, Pluto CP, Pehek EA (2005). Modulation of dopamine release by striatal 5-HT2C receptors. *Synapse* **55:** 242-251.

Alsene KM and Bakshi VP (2011). Pharmacological stimulation of locus coeruleus reveals a new antipsychotic-responsive pathway for deficient sensorimotor gating. *Neuropsychopharmacology* **36:** 1656-1667.

Alsene KM, Rajbhandari AK, Ramaker MJ, Bakshi VP (2011). Discrete forebrain neuronal networks supporting noradrenergic regulation of sensorimotor gating. *Neuropsychopharmacology* **36:** 1003-1014.

Altman JD, Trendelenburg AU, MacMillan L, Bernstein D, Limbird L, Starke K *et al* (1999). Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice. *Mol Pharmacol* **56:** 154-161.

Arbelaez AM, Su Y, Thomas JB, Hauch AC, Hershey T, Ances BM (2013). Comparison of regional cerebral blood flow responses to hypoglycemia using pulsed arterial spin labeling and positron emission tomography. *PLoS One* **8:** e60085.

Arnsten AF (1997). Catecholamine regulation of the prefrontal cortex. *J Psychopharmacol* **11:** 151-162.

Arnsten AF (2006). Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. *J Clin Psychiatry* **67 Suppl 8:** 7-12.

Arponen E, Helin S, Marjamaki P, Gronroos T, Holm P, Loyttyniemi E *et al* (2014). A PET Tracer for Brain alpha2C Adrenoceptors, 11C-ORM-13070: Radiosynthesis and Preclinical Evaluation in Rats and Knockout Mice. *J Nucl Med* **55:** 1171-1177.

Ashburner J and Friston KJ (2005). Unified segmentation. *Neuroimage* **26:** 839-851.

Aston-Jones G, Rajkowski J, Cohen J (2000). Locus coeruleus and regulation of behavioral flexibility and attention. *Prog Brain Res* **126:** 165-182.

Aston-Jones G, Rajkowski J, Cohen J (1999). Role of locus coeruleus in attention and behavioral flexibility. *Biol Psychiatry* **46:** 1309-1320.

Baker R, Gent TC, Yang Q, Parker S, Vyssotski AL, Wisden W *et al* (2014). Altered activity in the central medial thalamus precedes changes in the neocortex during transitions into both sleep and propofol anesthesia. *J Neurosci* **34:** 13326-13335.

Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC *et al* (2012). Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* **367:** 795-804.

Bondareff W, Mountjoy CQ, Roth M, Rossor MN, Iversen LL, Reynolds GP *et al* (1987). Neuronal degeneration in locus ceruleus and cortical correlates of Alzheimer disease. *Alzheimer Dis Assoc Disord* **1:** 256-262.

Boyajian CL, Loughlin SE, Leslie FM (1987). Anatomical evidence for alpha-2 adrenoceptor heterogeneity: differential autoradiographic distributions of [3H]rauwolscine and [3H]idazoxan in rat brain. *J Pharmacol Exp Ther* **241:** 1079-1091.

Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A *et al* (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc Natl Acad Sci U S A* **94:** 2569-2574.

Bruck A, Virta JR, Koivunen J, Koikkalainen J, Scheinin NM, Helenius H *et al* (2013). 11C]PIB, [18F]FDG and MR imaging in patients with mild cognitive impairment. *Eur J Nucl Med Mol Imaging* **40:** 1567-1572.

Bucheler MM, Hadamek K, Hein L (2002). Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. *Neuroscience* **109:** 819-826.

Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O (2012). Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry* **69:** 98-106.

Budai D, Harasawa I, Fields HL (1998). Midbrain periaqueductal gray (PAG) inhibits nociceptive inputs to sacral dorsal horn nociceptive neurons through alpha2-adrenergic receptors. *J Neurophysiol* **80:** 2244-2254.

Burnstock G (2006). Historical review: ATP as a neurotransmitter. *Trends Pharmacol Sci* **27:** 166-176.

Chacko TK, Zhuang H, Nakhoda KZ, Moussavian B, Alavi A (2003). Applications of fluorodeoxyglucose positron emission tomography in the diagnosis of infection. *Nucl Med Commun* **24:** 615-624.

Charnay Y, Leger L, Vallet PG, Hof PR, Jouvet M, Bouras C (1995). 3H]Nisoxetine binding sites in the cat brain: an autoradiographic study. *Neuroscience* **69:** 259-270.

Cipriani A, Furukawa TA, Salanti G, Geddes JR, Higgins JP, Churchill R *et al* (2009). Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. *Lancet* **373:** 746-758.

Cipriani A, Koesters M, Furukawa TA, Nose M, Purgato M, Omori IM *et al* (2012). Duloxetine versus other anti-depressive agents for depression. *Cochrane Database Syst Rev* **10:** CD006533.

Cipriani A, Purgato M, Furukawa TA, Trespidi C, Imperadore G, Signoretti A *et al* (2012). Citalopram versus other anti-depressive agents for depression. *Cochrane Database Syst Rev* **7:** CD006534.

Colasanti A, Searle GE, Long CJ, Hill SP, Reiley RR, Quelch D *et al* (2012). Endogenous opioid release in the human brain reward system induced by acute amphetamine administration. *Biol Psychiatry* **72:** 371-377.

Corman SL, Fedutes BA, Culley CM (2004). Atomoxetine: the first nonstimulant for the management of attention-deficit/hyperactivity disorder. *Am J Health Syst Pharm* **61:** 2391-2399.

Correa-Sales C, Rabin BC, Maze M (1992). A hypnotic response to dexmedetomidine, an alpha 2 agonist, is mediated in the locus coeruleus in rats. *Anesthesiology* **76:** 948-952.

Counts SE and Mufson EJ (2010). Noradrenaline activation of neurotrophic pathways protects against neuronal amyloid toxicity. *J Neurochem* **113:** 649-660.

Coyle JT, Price DL, DeLong MR (1983). Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* **219:** 1184-1190.

Czernin J (2002). Clinical applications of FDG-PET in oncology. *Acta Med Austriaca* **29:** 162-170.

Daunt DA, Hurt C, Hein L, Kallio J, Feng F, Kobilka BK (1997). Subtype-specific intracellular trafficking of alpha2-adrenergic receptors. *Mol Pharmacol* **51:** 711-720.

Devilbiss DM, Waterhouse BD, Berridge CW, Valentino R (2012). Corticotropin-releasing factor acting at the locus coeruleus disrupts thalamic and cortical sensory-evoked responses. *Neuropsychopharmacology* **37:** 2020-2030.

Devoto P and Flore G (2006). On the origin of cortical dopamine: is it a co-transmitter in noradrenergic neurons? *Curr Neuropharmacol* **4:** 115-125.

Devoto P, Flore G, Pira L, Longu G, Gessa GL (2004). Mirtazapine-induced corelease of dopamine and noradrenaline from noradrenergic neurons in the medial prefrontal and occipital cortex. *Eur J Pharmacol* **487:** 105-111.

Devoto P, Flore G, Vacca G, Pira L, Arca A, Casu MA *et al* (2003). Co-release of noradrenaline and dopamine from noradrenergic neurons in the cerebral cortex induced by clozapine, the prototype atypical antipsychotic. *Psychopharmacology (Berl)* **167:** 79-84.

Di Carli MF (2002). Assessment of myocardial viability after myocardial infarction. *J Nucl Cardiol* **9:** 229-235.

Ding YS, Naganawa M, Gallezot JD, Nabulsi N, Lin SF, Ropchan J *et al* (2014). Clinical doses of atomoxetine significantly occupy both norepinephrine and serotonin transports: Implications on treatment of depression and ADHD. *Neuroimage* **86:** 164-171.

DosSantos MF, Love TM, Martikainen IK, Nascimento TD, Fregni F, Cummiford C *et al* (2012). Immediate effects of tDCS on the mu-opioid system of a chronic pain patient. *Front Psychiatry* **3:** 93.

Dringenberg HC (2000). Alzheimer's disease: more than a 'cholinergic disorder' - evidence that cholinergic-monoaminergic interactions contribute to EEG slowing and dementia. *Behav Brain Res* **115:** 235-249.

Easwaramoorthy B, Pichika R, Collins D, Potkin SG, Leslie FM, Mukherjee J (2007). Effect of acetylcholinesterase inhibitors on the binding of nicotinic alpha4beta2 receptor PET radiotracer, (18)F-nifene: A measure of acetylcholine competition. *Synapse* **61:** 29-36.

Ebert TJ, Hall JE, Barney JA, Uhrich TD, Colinco MD (2000). The effects of increasing plasma concentrations of dexmedetomidine in humans. *Anesthesiology* **93:** 382-394.

Egerton A, Ahmad R, Hirani E, Grasby PM (2008). Modulation of striatal dopamine release by 5-HT2A and 5-HT2C receptor antagonists: [11C]raclopride PET studies in the rat. *Psychopharmacology (Berl)* **200:** 487-496.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K *et al* (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* **25:** 1325-1335.

Faber JE, Yang N, Xin X (2001). Expression of alpha-adrenoceptor subtypes by smooth muscle cells and adventitial fibroblasts in rat aorta and in cell culture. *J Pharmacol Exp Ther* **298:** 441-452.

Farde L, Eriksson L, Blomquist G, Halldin C (1989). Kinetic analysis of central [11C]raclopride binding to D2-dopamine receptors studied by PET--a comparison to the equilibrium analysis. *J Cereb Blood Flow Metab* **9:** 696-708.

Fawcett JP, Bamji SX, Causing CG, Aloyz R, Ase AR, Reader TA *et al* (1998). Functional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *J Neurosci* **18:** 2808-2821.

Feinstein DL, Heneka MT, Gavrilyuk V, Dello Russo C, Weinberg G, Galea E (2002). Noradrenergic regulation of inflammatory gene expression in brain. *Neurochem Int* **41:** 357-365.

Ferrer-Lorente R, Cabot C, Fernandez-Lopez JA, Alemany M (2005). Combined effects of oleoyl-estrone and a beta3-adrenergic agonist (CL316,243) on lipid stores of diet-induced overweight male Wistar rats. *Life Sci* **77:** 2051-2058.

Finnema SJ, Scheinin M, Shahid M, Lehto J, Borroni E, Bang-Andersen B *et al* (2015). Application of cross-species PET imaging to assess neurotransmitter release in brain. *Psychopharmacology (Berl)*.

Finnema SJ, Varrone A, Hwang TJ, Gulyas B, Pierson ME, Halldin C *et al* (2010). Fenfluramine-induced serotonin release decreases [11C]AZ10419369 binding to 5-HT1B-receptors in the primate brain. *Synapse* **64:** 573-577.

Flower DR (1999). Modelling G-protein-coupled receptors for drug design. *Biochim Biophys Acta* **1422:** 207-234.

Friedman AM, DeJesus OT, Revenaugh J, Dinerstein RJ (1984). Measurements in vivo of parameters of the dopamine system. *Ann Neurol* **15 Suppl:** S66-76.

Fujimura Y, Ito H, Takahashi H, Yasuno F, Ikoma Y, Zhang MR *et al* (2010). Measurement of dopamine D2 receptors in living human brain using [11C]raclopride with ultra-high specific radioactivity. *Nucl Med Biol* **37:** 831-835.

Fulton B and Benfield P (1996). Moclobemide. An update of its pharmacological properties and therapeutic use. *Drugs* **52:** 450-474.

Gertler R, Brown HC, Mitchell DH, Silvius EN (2001). Dexmedetomidine: a novel sedative-analgesic agent. *Proc (Bayl Univ Med Cent)* **14:** 13-21.

Ginovart N, Hassoun W, Le Cavorsin M, Veyre L, Le Bars D, Leviel V (2002). Effects of amphetamine and evoked dopamine release on [11C]raclopride binding in anesthetized cats. *Neuropsychopharmacology* **27:** 72-84.

Goldstein DJ, Lu Y, Detke MJ, Lee TC, Iyengar S (2005). Duloxetine vs. placebo in patients with painful diabetic neuropathy. *Pain* **116:** 109-118.

Gummadavelli A, Motelow JE, Smith N, Zhan Q, Schiff ND, Blumenfeld H (2015). Thalamic stimulation to improve level of consciousness after seizures: Evaluation of electrophysiology and behavior. *Epilepsia* **56:** 114-124.

Gwak YS and Hulsebosch CE (2011). GABA and central neuropathic pain following spinal cord injury. *Neuropharmacology* **60:** 799-808.

Hajos M, Fleishaker JC, Filipiak-Reisner JK, Brown MT, Wong EH (2004). The selective norepinephrine reuptake inhibitor antidepressant reboxetine: pharmacological and clinical profile. *CNS Drug Rev* **10:** 23-44.

Hall JE, Uhrich TD, Barney JA, Arain SR, Ebert TJ (2000). Sedative, amnestic, and analgesic properties of small-dose dexmedetomidine infusions. *Anesth Analg* **90:** 699-705.

Hannestad J, Gallezot JD, Planeta-Wilson B, Lin SF, Williams WA, van Dyck CH *et al* (2010). Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo. *Biol Psychiatry* **68:** 854-860.

Hassoun W, Le Cavorsin M, Ginovart N, Zimmer L, Gualda V, Bonnefoi F *et al* (2003). PET study of the [11C]raclopride binding in the striatum of the awake cat: effects of anaesthetics and role of cerebral blood flow. *Eur J Nucl Med Mol Imaging* **30:** 141-148.

He JH, Cui Y, Song M, Yang Y, Dang YY, Jiang TZ *et al* (2014). Decreased functional connectivity between the mediodorsal thalamus and default mode network in patients with disorders of consciousness. *Acta Neurol Scand* .

Hein L, Altman JD, Kobilka BK (1999). Two functionally distinct alpha2-adrenergic receptors regulate sympathetic neurotransmission. *Nature* **402:** 181-184.

Hertel P, Mathe JM, Nomikos GG, Iurlo M, Mathe AA, Svensson TH (1995). Effects of D-amphetamine and phencyclidine on behavior and extracellular concentrations of neurotensin and dopamine in the ventral striatum and the medial prefrontal cortex of the rat. *Behav Brain Res* **72:** 103-114.

Hillmer AT, Wooten DW, Farhoud M, Higgins AT, Lao PJ, Barnhart TE *et al* (2013). PET imaging of acetylcholinesterase inhibitor-induced effects on alpha4beta2 nicotinic acetylcholine receptor binding. *Synapse* **67:** 882-886.

Holmberg M, Fagerholm V, Scheinin M (2003). Regional distribution of alpha(2C)-adrenoceptors in brain and spinal cord of control mice and transgenic mice overexpressing the alpha(2C)-subtype: an autoradiographic study with [(3)H]RX821002 and [(3)H]rauwolscine. *Neuroscience* **117:** 875-898.

Hong IK, Chung ST, Kim HK, Kim YB, Son YD, Cho ZH (2007). Ultra fast symmetry and SIMD-based projection-backprojection (SSP) algorithm for 3-D PET image reconstruction. *IEEE Trans Med Imaging* **26:** 789-803.

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A *et al* (2012). The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry* **69:** 776-786.

Hume S, Hirani E, Opacka-Juffry J, Myers R, Townsend C, Pike V *et al* (2001). Effect of 5-HT on binding of [(11)C] WAY 100635 to 5-HT(IA) receptors in rat brain, assessed using in vivo microdialysis nd PET after fenfluramine. *Synapse* **41:** 150-159.

Hume SP, Hirani E, Opacka-Juffry J, Osman S, Myers R, Gunn RN *et al* (2000). Evaluation of [O-methyl-11C]RS-15385-197 as a positron emission tomography radioligand for central alpha2-adrenoceptors. *Eur J Nucl Med* **27:** 475-484.

Hurt CM, Feng FY, Kobilka B (2000). Cell-type specific targeting of the alpha 2c-adrenoceptor. Evidence for the organization of receptor microdomains during neuronal differentiation of PC12 cells. *J Biol Chem* **275:** 35424-35431.

Ihalainen JA and Tanila H (2004). In vivo regulation of dopamine and noradrenaline release by alpha2A-adrenoceptors in the mouse nucleus accumbens. *J Neurochem* **91:** 49-56.

Ihalainen JA and Tanila H (2002). In vivo regulation of dopamine and noradrenaline release by alpha2A-adrenoceptors in the mouse prefrontal cortex. *Eur J Neurosci* **15:** 1789-1794.

Ingman K, Hagelberg N, Aalto S, Nagren K, Juhakoski A, Karhuvaara S *et al* (2005). Prolonged central mu-opioid receptor occupancy after single and repeated nalmefene dosing. *Neuropsychopharmacology* **30:** 2245-2253.

Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN *et al* (2007). Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* **27:** 1533-1539.

Innis RB, Malison RT, al-Tikriti M, Hoffer PB, Sybirska EH, Seibyl JP *et al* (1992). Amphetamine-stimulated dopamine release competes in vivo for [123I]IBZM binding to the D2 receptor in nonhuman primates. *Synapse* **10:** 177-184.

Ito H, Hietala J, Blomqvist G, Halldin C, Farde L (1998). Comparison of the transient equilibrium and continuous infusion method for quantitative PET analysis of [11C]raclopride binding. *J Cereb Blood Flow Metab* **18:** 941-950.

Jain D and He ZX (2008). Direct imaging of myocardial ischemia: a potential new paradigm in nuclear cardiovascular imaging. *J Nucl Cardiol* **15:** 617-630.

Jakobsen S, Pedersen K, Smith DF, Jensen SB, Munk OL, Cumming P (2006). Detection of alpha2-adrenergic receptors in brain of living pig with 11C-yohimbine. *J Nucl Med* **47:** 2008-2015.

Jellinger K, Danielczyk W, Fischer P, Gabriel E (1990). Clinicopathological analysis of dementia disorders in the elderly. *J Neurol Sci* **95:** 239-258.

Kalkman HO and Loetscher E (2003). alpha2C-Adrenoceptor blockade by clozapine and other antipsychotic drugs. *Eur J Pharmacol* **462:** 33-40.

Kaminska K, Golembiowska K, Rogoz Z (2014). The effect of risperidone on the mirtazapine-induced changes in extracellular monoamines in the rat frontal cortex. *Pharmacol Rep* **66:** 984-990.

Karhuvaara S, Kallio A, Scheinin M, Anttila M, Salonen JS, Scheinin H (1990). Pharmacological effects and pharmacokinetics of atipamezole, a novel alpha 2-adrenoceptor antagonist--a randomized, double-blind cross-over study in healthy male volunteers. *Br J Clin Pharmacol* **30:** 97-106.

Kast RE (2001). Mirtazapine may be useful in treating nausea and insomnia of cancer chemotherapy. *Support Care Cancer* **9:** 469-470.

Kawamura K, Akiyama M, Yui J, Yamasaki T, Hatori A, Kumata K *et al* (2010). In vivo evaluation of limiting brain penetration of probes for alpha(2C)-adrenoceptor using small-animal positron emission tomography. *ACS Chem Neurosci* **1:** 520-528.

Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, Slifstein M *et al* (2010). Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch Gen Psychiatry* **67:** 231-239.

Kegeles LS, Martinez D, Kochan LD, Hwang DR, Huang Y, Mawlawi O *et al* (2002). NMDA antagonist effects on striatal dopamine release: positron emission tomography studies in humans. *Synapse* **43:** 19-29.

Keren NI, Lozar CT, Harris KC, Morgan PS, Eckert MA (2009). In vivo mapping of the human locus coeruleus. *Neuroimage* **47:** 1261-1267.

Kienbaum P, Heuter T, Michel MC, Peters J (2000). Racemic ketamine decreases muscle sympathetic activity but maintains the neural response to hypotensive challenges in humans. *Anesthesiology* **92:** 94-101.

Kitson S, Cuccurullo V, Ciarmiello A, Salvo D, Mansi L (2009). Clinical Applications of Positron Emission Tomography (PET) Imaging in Medicine: Oncology, Brain Diseases and Cardiology. *Current Radiopharmaceuticals* **2:** 224-253.

Knaapen P, Germans T, Knuuti J, Paulus WJ, Dijkmans PA, Allaart CP *et al* (2007). Myocardial energetics and efficiency: current status of the noninvasive approach. *Circulation* **115:** 918-927.

Koda K, Ago Y, Cong Y, Kita Y, Takuma K, Matsuda T (2010). Effects of acute and chronic administration of atomoxetine and methylphenidate on extracellular levels of noradrenaline, dopamine and serotonin in the prefrontal cortex and striatum of mice. *J Neurochem* **114:** 259-270.

Kornfield R, Watson S, Higashi AS, Conti RM, Dusetzina SB, Garfield CF *et al* (2013). Effects of FDA advisories on the pharmacologic treatment of ADHD, 2004-2008. *Psychiatr Serv* **64:** 339-346.

Laakso A and Hietala J (2000). PET studies of brain monoamine transporters. *Curr Pharm Des* **6:** 1611-1623.

Lahdesmaki J, Sallinen J, MacDonald E, Sirvio J, Scheinin M (2003). Alpha2-adrenergic drug effects on brain monoamines, locomotion, and body temperature are largely abolished in mice lacking the alpha2A-adrenoceptor subtype. *Neuropharmacology* **44:** 882-892.

Lammertsma AA and Hume SP (1996). Simplified reference tissue model for PET receptor studies. *Neuroimage* **4:** 153-158.

Landau AM, Doudet DJ, Jakobsen S (2012). Amphetamine challenge decreases yohimbine binding to alpha2 adrenoceptors in Landrace pig brain. *Psychopharmacology (Berl)* **222:** 155-163.

Laruelle M (2000). Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab* **20:** 423-451.

Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J *et al* (1996). Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci U S A* **93:** 9235-9240.

Laruelle M, Slifstein M, Huang Y (2002). Positron emission tomography: imaging and quantification of neurotransporter availability. *Methods* **27:** 287-299.

Lee A, Rosin DL, Van Bockstaele EJ (1998). Ultrastructural evidence for prominent postsynaptic localization of alpha2C-adrenergic receptors in catecholaminergic dendrites in the rat nucleus locus coeruleus. *J Comp Neurol* **394:** 218-229.

Leverenz J and Sumi SM (1986). Parkinson's disease in patients with Alzheimer's disease. *Arch Neurol* **43:** 662-664.

Levin CS and Hoffman EJ (1999). Calculation of positron range and its effect on the fundamental limit of positron emission tomography system spatial resolution. *Phys Med Biol* **44:** 781-799.

Levitt P, Rakic P, Goldman-Rakic P (1984). Region-specific distribution of catecholamine afferents in primate cerebral cortex: a fluorescence histochemical analysis. *J Comp Neurol* **227:** 23-36.

Li XM, Perry KW, Wong DT, Bymaster FP (1998). Olanzapine increases in vivo dopamine and norepinephrine release in rat prefrontal cortex, nucleus accumbens and striatum. *Psychopharmacology (Berl)* **136:** 153-161.

Lindefors N, Barati S, O'Connor WT (1997). Differential effects of single and repeated ketamine administration on dopamine, serotonin and GABA transmission in rat medial prefrontal cortex. *Brain Res* **759:** 205-212.

Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL (1996). Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab* **16:** 834-840.

Lorrain DS, Schaffhauser H, Campbell UC, Baccei CS, Correa LD, Rowe B *et al* (2003). Group II mGlu receptor activation suppresses norepinephrine release in the ventral hippocampus and locomotor responses to acute ketamine challenge. *Neuropsychopharmacology* **28:** 1622-1632.

Lundquist P, Roman M, Syvanen S, Hartvig P, Blomquist G, Hammarlund-Udenaes M *et al* (2007). Effect on [11C]DASB binding after tranylcypromine-induced increase in serotonin concentration: positron emission tomography studies in monkeys and rats. *Synapse* **61:** 440-449.

Luoto P, Suilamo S, Oikonen V, Arponen E, Helin S, Herttuainen J *et al* (2014). (11)C-ORM-13070, a novel PET ligand for brain alpha2C-adrenoceptors: radiometabolism, plasma pharmacokinetics, whole-body distribution and radiation dosimetry in healthy men. *Eur J Nucl Med Mol Imaging* **41:** 1947-1956.

MacDonald E, Kobilka BK, Scheinin M (1997). Gene targeting--homing in on alpha 2-adrenoceptor-subtype function. *Trends Pharmacol Sci* **18:** 211-219.

MacDonald E, Scheinin M, Scheinin H, Virtanen R (1991). Comparison of the behavioral and neurochemical effects of the two optical enantiomers of medetomidine, a selective alpha-2-adrenoceptor agonist. *J Pharmacol Exp Ther* **259:** 848-854.

Maeda J, Suhara T, Ogawa M, Okauchi T, Kawabe K, Zhang MR *et al* (2001). In vivo binding properties of [carbonyl-11C]WAY-100635: effect of endogenous serotonin. *Synapse* **40:** 122-129.

Marcus MM, Wiker C, Franberg O, Konradsson-Geuken A, Langlois X, Jardemark K *et al* (2010). Adjunctive alpha2-adrenoceptor blockade enhances the antipsychotic-like effect of risperidone and facilitates cortical dopaminergic and glutamatergic, NMDA receptor-mediated transmission. *Int J Neuropsychopharmacol* **13:** 891-903.

Marien MR, Colpaert FC, Rosenquist AC (2004). Noradrenergic mechanisms in neurodegenerative diseases: a theory. *Brain Res Brain Res Rev* **45:** 38-78.

Masana M, Castane A, Santana N, Bortolozzi A, Artigas F (2012). Noradrenergic antidepressants increase cortical dopamine: potential use in augmentation strategies. *Neuropharmacology* **63:** 675-684.

Maura G, Bonanno G, Raiteri M (1992). Presynaptic alpha 2-adrenoceptors mediating inhibition of noradrenaline and 5-hydroxytryptamine release in rat cerebral cortex: further characterization as different alpha 2-adrenoceptor subtypes. *Naunyn Schmiedebergs Arch Pharmacol* **345:** 410-416.

McCormick PN, Ginovart N, Wilson AA (2011). Isoflurane anaesthesia differentially affects the amphetamine sensitivity of agonist and antagonist D2/D3 positron emission tomography radiotracers: implications for in vivo imaging of dopamine release. *Mol Imaging Biol* **13:** 737-746.

Mika J, Zychowska M, Makuch W, Rojewska E, Przewlocka B (2013). Neuronal and immunological basis of action of antidepressants in chronic pain - clinical and experimental studies. *Pharmacol Rep* **65:** 1611-1621.

Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V *et al* (2003). The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine2C receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther* **306:** 954-964.

Millan MJ, Lejeune F, Gobert A (2000). Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. *J Psychopharmacol* **14:** 114-138.

Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ (1984). A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol* **15:** 217-227.

Mirfeizi L, Rybczynska AA, van Waarde A, Campbell-Verduyn L, Feringa BL, Dierckx RA *et al* (2014). (18)F]-(fluoromethoxy)ethoxy)methyl)-1H-1,2,3-triazol-1-yl)propan-2-ol ([(18)F FPTC) a novel PET-ligand for cerebral beta-adrenoceptors. *Nucl Med Biol* **41:** 203-209.

Mitchell JM, O'Neil JP, Janabi M, Marks SM, Jagust WJ, Fields HL (2012). Alcohol consumption induces endogenous opioid release in the human orbitofrontal cortex and nucleus accumbens. *Sci Transl Med* **4:** 116ra6.

Mizobe T, Maghsoudi K, Sitwala K, Tianzhi G, Ou J, Maze M (1996). Antisense technology reveals the alpha2A adrenoceptor to be the subtype mediating the hypnotic response to the highly selective agonist, dexmedetomidine, in the locus coeruleus of the rat. *J Clin Invest* **98:** 1076-1080.

Mongeau R, Blier P, de Montigny C (1997). The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. *Brain Res Brain Res Rev* **23:** 145-195.

Mongeau R, Weiss M, de Montigny C, Blier P (1998). Effect of acute, short- and long-term milnacipran administration on rat locus coeruleus noradrenergic and dorsal raphe serotonergic neurons. *Neuropharmacology* **37:** 905-918.

Montezinho LP, Miller S, Plath N, Jensen NH, Karlsson JJ, Witten L *et al* (2010). The effects of acute treatment with escitalopram on the different stages of contextual fear conditioning are reversed by atomoxetine. *Psychopharmacology (Berl)* **212:** 131-143.

Morris C, Perris A, Klein J, Mahoney P (2009). Anaesthesia in haemodynamically compromised emergency patients: does ketamine represent the best choice of induction agent? *Anaesthesia* **64:** 532-539.

Morrison JH and Foote SL (1986). Noradrenergic and serotoninergic innervation of cortical, thalamic, and tectal visual structures in Old and New World monkeys. *J Comp Neurol* **243:** 117-138.

Munk OL and Smith DF (2011). PET kinetics of radiolabeled antidepressant, [N-methyl-11C]mirtazapine, in the human brain. *EJNMMI Res* **1:** 36-219X-1-36.

Murray RM, Paparelli A, Morrison PD, Marconi A, Di Forti M (2013). What can we learn about schizophrenia from studying the human model, drug-induced psychosis? *Am J Med Genet B Neuropsychiatr Genet* **162:** 661-670.

Muzyk AJ, Kerns S, Brudney S, Gagliardi JP (2013). Dexmedetomidine for the treatment of alcohol withdrawal syndrome: rationale and current status of research. *CNS Drugs* **27:** 913-920.

Nai Q, Dong HW, Hayar A, Linster C, Ennis M (2009). Noradrenergic regulation of GABAergic inhibition of main olfactory bulb mitral cells varies as a function of concentration and receptor subtype. *J Neurophysiol* **101:** 2472-2484.

Narendran R, Frankle WG, Keefe R, Gil R, Martinez D, Slifstein M *et al* (2005). Altered prefrontal dopaminergic function in chronic recreational ketamine users. *Am J Psychiatry* **162:** 2352-2359.

Narendran R, Jedema HP, Lopresti BJ, Mason NS, Gurnsey K, Ruszkiewicz J *et al* (2014). Imaging dopamine transmission in the frontal cortex: a simultaneous microdialysis and [11C]FLB 457 PET study. *Mol Psychiatry* **19:** 302-310.

Nicholas AP, Hokfelt T, Pieribone VA (1996). The distribution and significance of CNS adrenoceptors examined with in situ hybridization. *Trends Pharmacol Sci* **17:** 245-255.

Nutt DJ (2014). The role of the opioid system in alcohol dependence. *J Psychopharmacol* **28:** 8-22.

Oksuz MO, Dittmann H, Wicke C, Mussig K, Bares R, Pfannenberg C *et al* (2011). Accuracy of parathyroid imaging: a comparison of planar scintigraphy, SPECT, SPECT-CT, and C-11 methionine PET for the detection of parathyroid adenomas and glandular hyperplasia. *Diagn Interv Radiol* **17:** 297-307.

Olli-Lahdesmaki T, Kallio J, Scheinin M (1999). Receptor subtype-induced targeting and subtype-specific internalization of human alpha(2)-adrenoceptors in PC12 cells. *J Neurosci* **19:** 9281-9288.

Olli-Lahdesmaki T, Scheinin M, Pohjanoksa K, Kallio J (2003). Agonist-dependent trafficking of alpha2-adrenoceptor subtypes: dependence on receptor subtype and employed agonist. *Eur J Cell Biol* **82:** 231-239.

Ordway GA, Schenk J, Stockmeier CA, May W, Klimek V (2003). Elevated agonist binding to alpha2-adrenoceptors in the locus coeruleus in major depression. *Biol Psychiatry* **53:** 315-323.

Oreland L, Wiberg A, Asberg M, Traskman L, Sjostrand L, Thoren P *et al* (1981). Platelet MAO activity and monoamine metabolites in cerebrospinal fluid in depressed and suicidal patients and in healthy controls. *Psychiatry Res* **4:** 21-29.

Passchier J, Gee A, Willemsen A, Vaalburg W, van Waarde A (2002). Measuring drug-related receptor occupancy with positron emission tomography. *Methods* **27:** 278-286.

Paterson LM, Tyacke RJ, Nutt DJ, Knudsen GM (2010). Measuring endogenous 5-HT release by emission tomography: promises and pitfalls. *J Cereb Blood Flow Metab* **30:** 1682-1706.

Philbin KE, Bateman RJ, Mendelowitz D (2010). Clonidine, an alpha2-receptor agonist, diminishes GABAergic neurotransmission to cardiac vagal neurons in the nucleus ambiguus. *Brain Res* **1347:** 65-70.

Piascik MT and Perez DM (2001). Alpha1-adrenergic receptors: new insights and directions. *J Pharmacol Exp Ther* **298:** 403-410.

Pike VW (2009). PET radiotracers: crossing the blood-brain barrier and surviving metabolism. *Trends Pharmacol Sci* **30:** 431-440.

Purgato M, Papola D, Gastaldon C, Trespidi C, Magni LR, Rizzo C *et al* (2014). Paroxetine versus other anti-depressive agents for depression. *Cochrane Database Syst Rev* **4:** CD006531.

Quaglia W, Del Bello F, Giannella M, Piergentili A, Pigini M (2011). alpha2C-adrenoceptor modulators: a patent review. *Expert Opin Ther Pat* **21:** 455-481.

Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD *et al* (2002). A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normal male volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. *Neuroimage* **15:** 620-632.

Ramsberg J, Asseburg C, Henriksson M (2012). Effectiveness and cost-effectiveness of antidepressants in primary care: a multiple treatment comparison meta-analysis and cost-effectiveness model. *PLoS One* **7:** e42003.

Rapoport SI, Horwitz B, Grady CL, Haxby JV, DeCarli C, Schapiro MB (1991). Abnormal brain glucose metabolism in Alzheimer's disease, as measured by position emission tomography. *Adv Exp Med Biol* **291:** 231-248.

Reivich M, Amsterdam JD, Brunswick DJ, Shiue CY (2004). PET brain imaging with [11C](+)McN5652 shows increased serotonin transporter availability in major depression. *J Affect Disord* **82:** 321-327.

Ressler KJ and Nemeroff CB (2000). Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. *Depress Anxiety* **12 Suppl 1:** 2-19.

Reyes BA, Chavkin C, van Bockstaele EJ (2009). Subcellular targeting of kappa-opioid receptors in the rat nucleus locus coeruleus. *J Comp Neurol* **512:** 419-431.

Rinne JO, Brooks DJ, Rossor MN, Fox NC, Bullock R, Klunk WE *et al* (2010). 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol* **9:** 363-372.

Robertson IH (2013). A noradrenergic theory of cognitive reserve: implications for Alzheimer's disease. *Neurobiol Aging* **34:** 298-308.

Roe CM, Mintun MA, D'Angelo G, Xiong C, Grant EA, Morris JC (2008). Alzheimer disease and cognitive reserve: variation of education effect with carbon 11-labeled Pittsburgh Compound B uptake. *Arch Neurol* **65:** 1467-1471.

Rowland LM, Beason-Held L, Tamminga CA, Holcomb HH (2010). The interactive effects of ketamine and nicotine on human cerebral blood flow. *Psychopharmacology (Berl)* **208:** 575-584.

Roy A, Pickar D, De Jong J, Karoum F, Linnoila M (1988). Norepinephrine and its metabolites in cerebrospinal fluid, plasma, and urine. Relationship to hypothalamic-pituitary-adrenal axis function in depression. *Arch Gen Psychiatry* **45:** 849-857.

Russell VA, Lamm MC, Allin R, de Villiers AS, Searson A, Taljaard JJ (1989). Effect of selective noradrenergic denervation on noradrenaline content and [3H]dopamine release in rat nucleus accumbens slices. *Neurochem Res* **14:** 169-172.

Sallinen J, Holappa J, Koivisto A, Kuokkanen K, Chapman H, Lehtimaki J *et al* (2013). Pharmacological characterisation of a structurally novel alpha2C-adrenoceptor antagonist ORM-10921 and its effects in neuropsychiatric models. *Basic Clin Pharmacol Toxicol* **113:** 239-249.

Sallinen J, Link RE, Haapalinna A, Viitamaa T, Kulatunga M, Sjoholm B *et al* (1997). Genetic alteration of alpha 2C-adrenoceptor expression in mice: influence on locomotor, hypothermic, and neurochemical effects of dexmedetomidine, a subtype-nonselective alpha 2-adrenoceptor agonist. *Mol Pharmacol* **51:** 36-46.

Sasaki T, Ito H, Kimura Y, Arakawa R, Takano H, Seki C *et al* (2012). Quantification of dopamine transporter in human brain using PET with 18F-FE-PE2I. *J Nucl Med* **53:** 1065-1073.

Scheinin H, MacDonald E, Scheinin M (1988). Behavioural and neurochemical effects of antipamezole, a novel alpha 2-adrenoceptor antagonist. *Eur J Pharmacol* **151:** 35-42.

Scheinin M, Karhuvaara S, Ojala-Karlsson P, Kallio A, Koulu M (1991). Plasma 3,4-dihydroxyphenylglycol (DHPG) and 3-methoxy-4-hydroxyphenylglycol (MHPG) are insensitive indicators of alpha 2-adrenoceptor mediated regulation of norepinephrine release in healthy human volunteers. *Life Sci* **49:** 75-84.

Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Caron MG, Lefkowitz RJ *et al* (1994). Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Brain Res Mol Brain Res* **21:** 133-149.

Scheinin M, Sallinen J, Haapalinna A (2001). Evaluation of the alpha2C-adrenoceptor as a neuropsychiatric drug target studies in transgenic mouse models. *Life Sci* **68:** 2277-2285.

Schiffer WK, Volkow ND, Fowler JS, Alexoff DL, Logan J, Dewey SL (2006). Therapeutic doses of amphetamine or methylphenidate differentially increase synaptic and extracellular dopamine. *Synapse* **59:** 243-251.

Schwartz JP and Mishler K (1990). Beta-adrenergic receptor regulation, through cyclic AMP, of nerve growth factor expression in rat cortical and cerebellar astrocytes. *Cell Mol Neurobiol* **10:** 447-457.

Sekine M, Arakawa R, Ito H, Okumura M, Sasaki T, Takahashi H *et al* (2010). Norepinephrine transporter occupancy by antidepressant in human brain using positron emission tomography with (S,S)-[18F]FMeNER-D2. *Psychopharmacology (Berl)* **210:** 331-336.

Seneca N, Finnema SJ, Farde L, Gulyas B, Wikstrom HV, Halldin C *et al* (2006). Effect of amphetamine on dopamine D2 receptor binding in nonhuman primate brain: a comparison of the agonist radioligand [11C]MNPA and antagonist [11C]raclopride. *Synapse* **59:** 260-269.

Servan-Schreiber D, Printz H, Cohen JD (1990). A network model of catecholamine effects: gain, signal-to-noise ratio, and behavior. *Science* **249:** 892-895.

Sevy S, Papadimitriou GN, Surmont DW, Goldman S, Mendlewicz J (1989). Noradrenergic function in generalized anxiety disorder, major depressive disorder, and healthy subjects. *Biol Psychiatry* **25:** 141-152.

Shiue C, Pleus RC, Shiue GG, Rysavy JA, Sunderland JJ, Cornish KG *et al* (1998). Synthesis and biological evaluation of [11C]MK-912 as an alpha2-adrenergic receptor radioligand for PET studies. *Nucl Med Biol* **25:** 127-133.

Slavikova K, Montravers F, Treglia G, Kunikowska J, Kaliska L, Vereb M *et al* (2013). What is currently the best radiopharmaceutical for the hybrid PET/CT detection of recurrent medullary thyroid carcinoma? *Curr Radiopharm* **6:** 96-105.

Smiley JF, Subramanian M, Mesulam MM (1999). Monoaminergic-cholinergic interactions in the primate basal forebrain. *Neuroscience* **93:** 817-829.

Smith DF, Dyve S, Minuzzi L, Jakobsen S, Munk OL, Marthi K *et al* (2006). Inhibition of [11C]mirtazapine binding by alpha2-adrenoceptor antagonists studied by positron emission tomography in living porcine brain. *Synapse* **59:** 463-471.

Smith DF, Stork BS, Wegener G, Ashkanian M, Jakobsen S, Bender D *et al* (2009). 11C]Mirtazapine binding in depressed antidepressant nonresponders studied by PET neuroimaging. *Psychopharmacology (Berl)* **206:** 133-140.

Stenkrona P, Halldin C, Lundberg J (2013). 5-HTT and 5-HT(1A) receptor occupancy of the novel substance vortioxetine (Lu AA21004). A PET study in control subjects. *Eur Neuropsychopharmacol* **23:** 1190-1198.

Stone EA and Ariano MA (1989). Are glial cells targets of the central noradrenergic system? A review of the evidence. *Brain Res Brain Res Rev* **14:** 297-309.

Streel E, Dan B, Campanella S, Meyvaert A, Hanak C, Pelc I *et al* (2006). A pharmacological modulation of opiate withdrawal using an up-/down-regulation of the noradrenergic system in opiate-dependent rats. *Int J Neuropsychopharmacol* **9:** 621-626.

Sumiyoshi T, Kunugi H, Nakagome K (2014). Serotonin and dopamine receptors in motivational and cognitive disturbances of schizophrenia. *Front Neurosci* **8:** 395.

Takamatsu Y, Hagino Y, Sato A, Takahashi T, Nagasawa SY, Kubo Y *et al* (2015). Improvement of learning and increase in dopamine level in the frontal cortex by methylphenidate in mice lacking dopamine transporter. *Curr Mol Med* **15:** 245-252.

Takano A, Gulyas B, Varrone A, Maguire RP, Halldin C (2009). Saturated norepinephrine transporter occupancy by atomoxetine relevant to clinical doses: a rhesus monkey study with (S,S)-[(18)F]FMeNER-D (2). *Eur J Nucl Med Mol Imaging* **36:** 1308-1314.

Takano A, Halldin C, Farde L (2013). SERT and NET occupancy by venlafaxine and milnacipran in nonhuman primates: a PET study. *Psychopharmacology (Berl)* **226:** 147-153.

Takano A, Suzuki K, Kosaka J, Ota M, Nozaki S, Ikoma Y *et al* (2006). A dose-finding study of duloxetine based on serotonin transporter occupancy. *Psychopharmacology (Berl)* **185:** 395-399.

Talbot PS, Frankle WG, Hwang DR, Huang Y, Suckow RF, Slifstein M *et al* (2005). Effects of reduced endogenous 5-HT on the in vivo binding of the serotonin transporter radioligand 11C-DASB in healthy humans. *Synapse* **55:** 164-175.

Talke P, Lobo E, Brown R (2003). Systemically administered alpha2-agonist-induced peripheral vasoconstriction in humans. *Anesthesiology* **99:** 65-70.

Tellez S, Colpaert F, Marien M (1997). Acetylcholine release in the rat prefrontal cortex in vivo: modulation by alpha 2-adrenoceptor agonists and antagonists. *J Neurochem* **68:** 778-785.

Tose R, Kushikata T, Yoshida H, Kudo M, Furukawa K, Ueno S *et al* (2009). Orexin A decreases ketamine-induced anesthesia time in the rat: the relevance to brain noradrenergic neuronal activity. *Anesth Analg* **108:** 491-495.

Tuominen L, Nummenmaa L, Keltikangas-Jarvinen L, Raitakari O, Hietala J (2014). Mapping neurotransmitter networks with PET: an example on serotonin and opioid systems. *Hum Brain Mapp* **35:** 1875-1884.

Umehara M, Ago Y, Fujita K, Hiramatsu N, Takuma K, Matsuda T (2013). Effects of serotonin-norepinephrine reuptake inhibitors on locomotion and prefrontal monoamine release in spontaneously hypertensive rats. *Eur J Pharmacol* **702:** 250-257.

Van der Mey M, Windhorst AD, Klok RP, Herscheid JD, Kennis LE, Bischoff F *et al* (2006). Synthesis and biodistribution of [11C]R107474, a new radiolabeled alpha2-adrenoceptor antagonist. *Bioorg Med Chem* **14:** 4526-4534.

Vazquez GH, Baldessarini RJ, Tondo L (2014). Co-occurrence of anxiety and bipolar disorders: clinical and therapeutic overview. *Depress Anxiety* **31:** 196-206.

Vernaleken I, Klomp M, Moeller O, Raptis M, Nagels A, Rosch F *et al* (2013). Vulnerability to psychotogenic effects of ketamine is associated with elevated D2/3-receptor availability. *Int J Neuropsychopharmacol* **16:** 745-754.

Virtanen R, Savola JM, Saano V, Nyman L (1988). Characterization of the selectivity, specificity and potency of medetomidine as an alpha 2-adrenoceptor agonist. *Eur J Pharmacol* **150:** 9-14.

Visser FC (2001). Imaging of cardiac metabolism using radiolabelled glucose, fatty acids and acetate. *Coron Artery Dis* **12 Suppl 1:** S12-8.

Vogt BA, Hof PR, Friedman DP, Sikes RW, Vogt LJ (2008). Norepinephrinergic afferents and cytology of the macaque monkey midline, mediodorsal, and intralaminar thalamic nuclei. *Brain Struct Funct* **212:** 465-479.

Wernicke JF, Faries D, Girod D, Brown J, Gao H, Kelsey D *et al* (2003). Cardiovascular effects of atomoxetine in children, adolescents, and adults. *Drug Saf* **26:** 729-740.

Xia ZQ, Chen SQ, Yao X, Xie CB, Wen SH, Liu KX (2013). Clinical benefits of dexmedetomidine versus propofol in adult intensive care unit patients: a meta-analysis of randomized clinical trials. *J Surg Res* **185:** 833-843.

Xu H, Aibiki M, Seki K, Ogura S, Ogli K (1998). Effects of dexmedetomidine, an alpha2-adrenoceptor agonist, on renal sympathetic nerve activity, blood pressure, heart rate and central venous pressure in urethane-anesthetized rabbits. *J Auton Nerv Syst* **71:** 48-54.

Yamamoto S, Onoe H, Tsukada H, Watanabe Y (2007). Effects of increased endogenous serotonin on the in vivo binding of [11C]DASB to serotonin transporters in conscious monkey brain. *Synapse* **61:** 724-731.

Yavich L, Lappalainen R, Sirvio J, Haapalinna A, MacDonald E (1997). Alpha2-adrenergic control of dopamine overflow and metabolism in mouse striatum. *Eur J Pharmacol* **339:** 113-119.

Yu S (2006). Review of F-FDG Synthesis and Quality Control. *Biomed Imaging Interv J* **2:** e57.

Zarow C, Lyness SA, Mortimer JA, Chui HC (2003). Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch Neurol* **60:** 337-341.

Zauner C, Schneeweiss B, Kranz A, Madl C, Ratheiser K, Kramer L *et al* (2000). Resting energy expenditure in short-term starvation is increased as a result of an increase in serum norepinephrine. *Am J Clin Nutr* **71:** 1511-1515.