TURUN YLIOPISTO

VISUALIZATION AND QUANTIFICATION OF NEUROKININ-1-RECEPTORS IN HUMAN BRAIN WITH POSITRON EMISSION TOMOGRAPHY

Mikko Nyman

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University of Turku

Faculty of Medicine
Department of Psychiatry and Department of Radiology
Turku Doctoral Programme in Clinical Research
Turku PET Centre

Supervised by

Professor Jarmo Hietala
Department of Psychiatry
University of Turku
Turku, Finland

Reviewed by

Professor Eero Castrén, Neuroscience Center, University of Helsinki, Helsinki Finland

Professor Koen van Laere, Department Nuclear Medicine, University Hospital Leuven, Leuven, Belgium

Opponent

Professor Lars Farde, Centre for Psychiatry Research, Karolinska Institutet, Stockholm, Sweden

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To my mother, father and sister
To my wife and my sons

ABSTRACT

University of Turku
Faculty of Medicine
Department of Psychiatry and Department of Radiology
Turku PET Centre
Mikko Nyman: **Visualization and Quantification of Neurokinin-1-receptors in Human Brain With Positron Emission Tomography**
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Substance P (SP) is a neurotransmitter serving numerous different functions in human central and peripheral nervous system. SP acts mainly through neurokinin-1 receptors (NK1R). SP and NK1R are widely distributed in the human central nervous system. This neurotransmitter system modulates other neurotransmitter systems and adjusts network balances. The SP system regulates a wide variety of functions such as mood, stress, pain, inflammation, anxiety, and satiety. Therefore, this system is implicated in many pathological conditions such as major depression, anxiety, nausea and emesis, addiction and obesity. Our knowledge of the SP and NK1R systems derive mostly from animal studies, whereas human data is lacking., Direct in vivo research of SP and NK1R functions in man has become possible by using molecular imaging techniques such as positron emission tomography and specific neurokinin receptor tracers.

We had three main aims in this study: First, we validated a method to visualize and quantify NK1 receptors in human brain *in vivo* using PET and a fluorine-18 labeled substance P antagonist receptor ligand [18F]SPA-RQ. Second, we studied normal healthy subjects and tested if age or gender affect the availability of these receptors in human brain. Third, as this neurotransmitter system, and more specifically, NK1R antagonists, have been implicated in the treatment of major depressive disorder (MDD), we studied patients with MDD to see if these patients have changes in their brain NK1R availability.

We validated a method to image and quantify NK1R in human brain in vivo using [18F]SPA-RQ. Reasonable scan time was determined as 240 minutes, cerebellum was available as a reference region as it was devoid of NK1Rs, and simplified reference tissue model (SRTM) was validated as a reliable method to model the data. Healthy volunteers had age-related decrease of 7% per decade in NK1R availability in many brain regions. Females had lower brain NK1R1 availability than males with no statistical evidence for regional specificity. Patients with MDD did not have differences in their NK1R availability compared to healthy controls, but their rated symptoms on overall depression and anxiety correlated with NK1R binding in some brain regions.

These studies validated a method to image and quantify NK1R availability in human brain *in vivo* using PET. The application potential of this method is obvious in drug development (mechanism of action and dose-finding) and *in vivo* studies of human physiology and disease processes related to the substance P system. In applied studies of this thesis, we found a decrease in NK1R availability during aging that follows the same rate that has been previously reported for many other receptor systems, such as the dopamine system. The detailed mechanisms in NK1 receptor sex differences are not know, but hormonal differences are likely to contribute. We found no difference on NK1R availability between patients with MDD and matched healthy controls, consistent with lack of efficacy of substance P antagonists in clinical trials in MDD and anxiety disorders. However, NK1 receptors did modulate the affective symptom domain in patients with MDD, however, NK1 receptor antagonism alone may be insufficient for clinical treatment of MDD.

Keywords: substance P, neurokinin-1 receptors, positron emission tomography, major depressive disorder

TIIVISTELMÄ

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Substanssi P (SP) on hermoston välittäjäaine, joka on osallisena useissa eri keskus- ja perifeerisen hermoston toiminnoissa. Sen vaikutuksen välittyvät pääasiassa neurokiniini-1 reseptoreiden kautta (NK1R). Tämän välittäjäaine toimii toisten välittäjäaineisysteemeiden modulaattorina ja säätää niiden tasapainoa vastaamaan elimistön kulloistakin tilaa riippuen esimerkiksi mielialasta, stressistä, tulehdustiloista ja kylläisyydestä. Tällä välittäjäaineella on mahdollisesti merkitystä monissa patologisissa tiloissa kuten masennuksessa, ahdistuneisuudessa, pahoinvoinnissa, riippuvuudessa ja lihavuudessa. SP:tä ja NK1R:ta esiintyy laajasti koko keskushermoston alueella. Suurin osa tiedostamme SP:stä ja NK1 toiminnoista perustuu eläintutkimuksiin ja ihmisillä tehtyjä tutkimuksia on vähän. Molekylaarikuvantaminen, esimerkiksi positroniemissiotomografialla ja spesifisillä neurokiniinireseptorien merkkiaineilla, on mahdollistanut SP:n ja NK1R:n toimintojen suoran *in vivo* tutkimisen.

Tutkimuksessamme oli kolme tavoitetta. Ensimmäinen tavoite oli kehittää menetelmä NK1-reseptoreiden kuvantamiseen ja mittaamiseen ihmisen aivoissa *in vivo* käyttäen PET-kuvausta ja substanssi P antagonisti-merkkiainetta ([18]SPA-RQ). Toinen tavoite oli tutkia terveillä vapaaehtoisilla koehenkilöillä iän ja sukupuolen vaikutuksia näiden reseptoreiden määrään ihmisen aivoissa. Kolmanneksi, koska tämä välittäjäaine on aikaisemmissa tutkimuksissa liitetty masennuksen patofysiologiaan, tutkimme miten masennus vaikuttaa NK1R määrään masennuspotilaiden aivoissa.

Tutkimuksessa validoimme menetelmän NK1R:en kuvantamiseen ja mittaamiseen *in vivo* käyttäen PET:aa ja [18F]SPA-RQ-merkkiainetta. Riittäväksi kuvaus ajaksi määritettiin 240 minuuttia, pikkuaivot osoittautuivat sopivaksi vertailualueeksi, ja yksinkertaistettu vertailukudosmalli validoitiin luotettavaksi tavaksi mallintaa saatua dataa. Terveillä vapaaehtoisilla havaittiin tapahtuvan NK1R sitoutumisen vähenemistä iän mukana noin 7% vuosikymmenessä monilla aivoalueilla. Naisilla NK1R sitoutumisen oli vähäisempää kuin miehillä monilla aivoalueilla. Masennuspotilailla NK1R:n sitoutuminen ei eronnut terveistä kontrolleista millään aivoalueella, mutta muutamilla alueilla reseptorien määrä oli yhteydessä potilaiden raportoimiin ahdistuneisuuteen ja masennuksen oireiden kokonaismäärään.

Tässä tutkimuksessa kehitettiin ja validoitiin menetelmä substanssi P-järjestelmän keskeisen vaikutuskohdan, NK1 reseptorin kuvantamiseen ja mittaamiseen PET:lla ihmisen aivoissa *in vivo*. Selkein mahdollinen käyttökohde tälle menetelmälle on lääkekehitys, kuten toimintamekanismit ja annoksen etsintä, ja ihmisen fysiologian ja patologian *in vivo* tutkimukset. Käytimme tätä menetelmää onnistuneesti jatkotutkimuksissamme. Metodin suurimpana ongelmana on pitkä kuvausaika. Löytämämme ikään liittyvä NK1R sitoutumisen väheneminen on vastaavaa kuin esimerkiksi dopamiinireseptoreista on aikaisemmin raportoitu. Myöskään sukupuoleen liittyvien NK1 reseptorisitoutumiserojen syytä ei tiedetä, mutta esimerkiksi erot hormonitoiminnoissa ovat todennäköisesti näiden taustalla. Terveiden verrokkien ja masennuspotilaiden NK1R sitoutumisessa ei tutkimuksessamme havaittu eroja. Tämä tulos on linjassa sen kanssa, että substanssi P antagonisteilla ei ole havaittu olevan tehoa masennustilojen tai ahdistuneisuushäiriöiden hoidossa. NK1R sitoutumisella vaikuttaisi olevan yhteys masennuksen ja ahdistukseen oireisiin, mutta tämä yhteys on liian heikko, jotta masennus- tai ahdistuneisuushäiriöiden lääkehoidossa voitaisiin käyttää NK1R antagonisteja yksinään.

Avainsanat: substanssi P, neurokiniini-1 reseptori, positroniemissiotomografia, masennus

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ABBREVIATIONS AND DEFINITIONS

[11C]GR205171	carbon-11 labeled neurokinin-1 receptor radiotracer
[11C]R116301	carbon-11 labeled neurokinin-1 receptor radiotracer
[18F]SPA-RQ	fluorine-18 labeled substance P antagonist receptor quantifier
5-HT1A	serotonin 1A receptor
BBB	blood brain barrier
BDNF	brain-derived neurotrophic factor
BMI	body mass index
BP	binding potential
CJ 012,255	substance P antagonist
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computer tomography
DA	dopamine
DRN	dorsal raphe nucleus
EC	entorhinal cortex
EKA	endokinin A
EKB	endokinin B
EKC	endokinin C
EKD	endokinin D
FWE	family-wise error
GABA	gamma-aminobutyric acid
GDP	guanosine diphosphate
GPCR	G protein-coupled receptor
GR205171	substance P antagonist
GSK1144814	substance P antagonist
GTP	guanosine triphosphate
HAM-D	Hamilton Rating Scale for Depression
HK-1	hemokinin-1
IL-8	interleukin 8
L-597274	substance P antagonist
LC	locus coeruleus
LY686017	substance P antagonist
MBq	megabecquerel
MCL	mesocorticolimbic system

MDD	major depressive disorder
[18F]MK-0999	same as [18F]SPA-RQ
MK869	substance antagonist, aprepitant
MRI	magnetic resonance imaging
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK1R	neurokinin-1 receptor
NKA	neurokinin A
NKB	neurokinin B
NPK	neuropeptide K
NKγ	neuropeptide γ
PET	positron emission tomography
PPT	preprotachykinin
PPT-1	preprotachykinin-1
PTSD	post-traumatic stress-disorder
R116301	carbon-11 labeled neurokinin-1 receptor radiotracer
rCBV	regional cerebral blood flow
RTCM	reference tissue compartment model (full)
SAD	social anxiety disorder
SP	Substance P
SPA	Substance P antagonists
SRTM	simplified reference tissue model
TAC1	a gene that encode tachykinins SP, NKA, NPK and NPγ
TAC3	a gene that encode tachykinin NKB
TAC4	a gene that encode tachykinins HK-1, EKA, EKB, EKC,EKD
TLC	thin layer chromatography
TrkB	tropomyosin receptor kinase B
VTA	ventral tegmental area

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on following original publications. They are referred to in text by Roman numerals I-III.

I. Hietala, J., M.J. Nyman, O. Eskola, A. Laakso, T. Grönroos, V. Oikonen, J. Bergman, et al. 2005. “Visualization and Quantification of Neurokinin-1 (NK1) Receptors in the Human Brain.” Molecular Imaging and Biology 7 (4): 262–72.

II. Nyman, M. J., O. Eskola, J. Kajander, T. Vahlberg, S. Sanabria, D. Burns, R. Hargreaves, O. Solin, and J. Hietala. 2007. “Gender and Age Affect NK1 Receptors in the Human Brain - a Positron Emission Tomography Study with [F-18]SPA-RQ.” The International Journal of Neuropsychopharmacology 10 (2): 219–29.

III. Nyman, M, O. Eskola, J. Kajander, R. Jokinen, J. Penttinen, T. Karjalainen, L. Nummenmaa, et al. 2019. “Brain Neurokinin-1 Receptor Availability in Never-Medicated Patients with Major Depression - A Pilot Study.” Journal of Affective Disorders, 242, 188–194.

In addition to the publications, unpublished data are also presented.

1 INTRODUCTION

Substance P (SP) was found accidentally almost 90 years ago. The researchers Von Euler and Gaddum purified this substance from horse intestines and the name was derived from the powder-like presence it had. The substance Powder was found to cause decrease in blood pressure and make intestinal smooth muscle to contract. Substance P was purified, sequenced and synthesized in the 1970's and found to be a peptide. Several other similar peptides were also found 1980's and the peptide group was called tachykinins, a name inspired from their fast onset of action. These tachykinins were all acting as neurotransmitters and were then later called neuropeptides.

Before the surge to study substance P and neurokinin-1 receptors (NK1R) in humans for drug development in last 20 years, our knowledge in SP and NK1R system was mainly based on large literature on animal studies. In these animals studies, most importantly for psychiatric research, acute and chronic stress was found to increases SP production and release in brain areas known to be related to stress responses (Brodin et al., 1994; Ebner et al., 2004; Herpfer et al., 2012; Rosen et al., 1992; Siegel et al., 1984). Also, prevention of NK1R gene expression (NK1R knockout) produced animal behavior that resembled anxiolytic or antidepressant medication (De Felipe et al., 1998; Herpfer et al., 2005; Rupniak et al., 2000; Santarelli et al., 2001).

In subsequent human studies it was shown that intravenous infusion of SP in healthy males decreased mood, induces anxiety symptoms, causes sleep disturbances and worsening in short-term memory functions (Herpfer et al., 2007; Lieb et al., 2002). Substance P antagonists (SPA) was shown to increase neural activity caused by processing positive emotional information in healthy volunteers (Chandra et al., 2010; McCabe et al., 2009).

Major clinical breakthrough in neuropeptide research in psychiatry happened when Kramer and colleagues demonstrated that blocking substance P action by substance P antagonist, or a neurokinin-1 receptor antagonist, a receptor that substance P is an major agonist for, has an distinct antidepressive effect in patients (Kramer et al., 1998). They proposed that this antidepressive effect was not related to serotonin or dopamine neurotransmission and that it might be more potent and better tolerated drug than antidepressants now are in use. This led to major increase in research activities in the field on neuropeptides, and especially in the substance P research, for the next ten years.

Several substance P antagonists were developed, and many clinical trials were done in hope to find new cure for depression, anxiety or addiction.

At this time, several drug companies as well as academic centers started to develop positron emission tomography (PET) methods to study the substance P system and its major receptor site, the NK1 receptor, in the brain in vivo (Solin et al., 2004). The goal was to verify the mechanism of action of NK1R antagonists *in vivo* in man as well as to help in dose-finding using NK1R occupancy studies in drug development programs (Wallius et al., 2007). Although later controlled clinical studies on NK1R antagonists in major depression and anxiety disorders were disappointing (Keller et al., 2006; Michelson et al., 2013; Rupniak and Kramer, 2017), this wave of studies created novel methods for imaging NK1 receptors *in vivo*.

The current series of studies was aimed at developing NK1R imaging methods in Turku PET Centre. This projects started in Turku already in late 1990s with a selective and specific Nk1 receptor tracer, 18F-labeled PET tracer SPA-RQ. Over the years we also carried out a vast amount of applied studies using the developed method, predominantly related to NK1 receptor drug development programs. Some of these applied studies are presented in this thesis. In particular, a study on NK1 receptor availability in patients with drug-naïve major depression is described.

2 REVIEW OF LITERATURE

2.1 Tachykinins and neurokinin receptors

2.1.1 History of substance P and mammalian tachykinin family

Substance P was discovered in 1931 from horse intestine extracts by two scientists working in National Institute for Medical Research, London. Back then, they didn't know what the substance was that they managed to extract (V Euler and Gaddum, 1931). They were studying recently discovered acetylcholine, when they discovered an extract that was different from it. The extract was like powder and they called it preparation *P* and powder *P*. It caused a decrease on blood pressure, stimulated the contraction of intestinal tissue but did not cause a contraction of a denervated skeletal muscle. They suspected that these effects could be caused by histamine still present in the powder. That early name later evolved to substance P.

After the early work, substance P was not purified or sequenced for decades. In the 1970's, pure substance P was recognized (Chang and Leeman, 1970), sequenced (Chang et al., 1971) and synthetized (Tregear et al., 1971). Substance P was recognized as a peptide and a neurotransmitter, a neuropeptide. Later it was shown that two other mammalian neuropeptides exits: neurokinin A (NKA, previously called substance K, neurokinin α and neuromedin L) and neurokinin B (NKB, previously called neurokinin β and neuromedin K). These neuropeptides were discovered by several groups in about the same time (Kangawa et al., 1983; Kimura et al., 1983; Minamino et al., 1984; Nawa et al., 1983) and later named accordingly. These neuropeptides shared fast onset of action in gut tissues and were labeled "tachykinins" (and the slower acting ones were named bradykinins). All tachykinins share a carboxyl terminal sequence: -Phe-X-Gly-Leu-Met-NH2 where in mammalian tachykinins X is Phe in Substance P and Val neurokinin A and B.

NKA was later found to have two N-terminally extended forms that were called neuropeptide K (NKP) and neuropeptide γ (NKγ) (Carter and Krause, 1990). These are also biologically active peptides (Kage et al., 1988; Tatemoto et al., 1985). Even more tachykinins were discovered in 2000's when third preprotachykinin gene was found. It encodes peptides hemokinin-1 (HK-1) and endokinins A-D (EKA, EKB, EKC, EKD) (Page et al., 2003; Zhang et al., 2000).

Three genes that encode tachykinins have been discovered. TAC1 encodes SP, NKA, NPK and NPγ. TAC3 gene encodes NKB and TAC4 encodes HK-1, EKA, EKB, EKC and EKD (https://www.genenames.org/). There are also numerous non-mammalian tachykinins (Maggio, 1988). Some of these tachykinins, like eledoisin and physalaemin, are used in mammalian research.

2.1.2 History of neurokinin receptors

As there was evidence that several mammalian tachykinins exits, evidence of several different tachykinin receptors were also surfacing. In first studies reported, different tachykinins were acting with different potencies to different tissues (Watson et al., 1983). These binding sites were further characterized by using SP and Neurokinin B (NKB) ligands. SP was found to have higher affinity to SP binding sites and NKB was shown to have higher affinity to eledoisin / NKB binding sites. The rank order for SP binding sites was SP > eledoisin > NKA > kassinin = NKB and rank order for NKB binding sites was NKB > eledoisin > kassinin > NKA > SP (Beaujouan et al., 2004). SP binding sites was named as Neurokinin-1 receptors (NK1R) and NKB binding sites as Neurokinin-3 receptors (NK3R). The neurokinin-2 receptor (NK2R) was also characterized in rat duodenum smooth muscle using Neurokinin A ligand (NKA) which showed a receptor subtype having different rank order of affinity to mammalian tachykinins (NKA > NKB > SP) (Bergström et al., 1987). The distribution of NK2R in rat brain was also later reported (Saffroy et al., 2003).

2.1.3 Substance P

Substance P is a peptide that is coded by TAC1 gene. It was the first neuropeptide discovered and considered a ‘pioneering neuropeptide’. It is extensively distributed in the mammalian central nervous system and peripheral tissues. In human CNS, substance P is most abundant in basal ganglia, cortical regions, hypothalamus and substantia innominata. SP is also present in regions and nuclei that receive direct or indirect somatosensory inputs like ventral thalamic nuclei and nucleus of the solitary tract (Mai et al., 1986). In mammals, SP can be found in the neuron cell bodies, axon fibers and terminals. SP coexist regularly with other neuropeptides like neurokinins, but also with other neurotransmitters, like dopamine, serotonin, GABA and noradrenaline, acetylcholine, and glutamate (Merighi, 2002).

Substance P in the plasma can cross the blood brain barrier (BBB) (Freed et al., 2002) and plasma and cerebrospinal fluid (CSF) concentrations seem to be well correlated (Clark et al., 1994). Substance P is also abundant in the gut and it is produced there by several cell types (Severini et al., 2002). Peptides released from the gut have an important role in body homeostasis (Badman and Flier, 2005). SP is found in stomach, small bowel, hypothalamus and striatum which are important areas in digestion and nutrient uptake. NK1R are also found in adipose tissue, hypothalamus and striatum (Karagiannides et al., 2006; Mantyh et al., 1984). Substance P antagonist CJ 012,255 prevents weight gain by western type diet (high in fat, energy) in lean mice and causes weight loss in obese mice (Karagiannides et al., 2008). It also improves insulin sensitivity in lean and obese mice, suggesting that SP might be promoting weight gain and appetite. SPA treated mice have also lower serum insulin and leptin levels suggesting a role for SP in signaling pathways that regulate responses to insulin in fat cells. Because SP deficiency is related to decreased insulin levels also without affecting to the weight gain, SP may regulate insulin signaling on fat tissue at the peripheral level (Karagiannides et al., 2011).

TAC1 null mice have lower body mass in adulthood and are resistant to weight gain in high fat diet (Maguire et al., 2017a). Those mice also have a better circadian control of feeding and more active brown fat. Tachykinins have also been shown to have a role in ghrelin regulated fat deposit control (Trivedi et al., 2015). Human studies have found that obese subjects have higher blood SP levels than controls (Baroncelli et al., 1989; Fu et al., 2011). These studies suggest that overexpression of SP might also be important factor in development of obesity and type 2 diabetes. BMI and major depression are also positively correlated (de Wit et al., 2009), which may relate to obesity-related alteration in SP which subsequently translates to mood alteration. Chronic stress promotes development of these syndromes and coexistence of these syndromes with affective disorders might be related to NK1R-SP system.

Substance P could be considered as acute responder to stressors that pose a threat to biological system. Substance P effect in CNS and periphery could be seen to aim to immediate survival of the host system when confronted by acute harm. These effects are usually beneficial acutely but might lead to pathological effects when became chronic for some reason.

2.1.4 Neurokinin-1 receptors

Neurokinin-1 receptor is G protein-coupled receptor (GPCR) and it is coded by the TACR1 gene. Almost all mammalian tachykinins, SP, NKA, NKB, NPK, NPγ, HK-1, EKA and EKB have a high affinity on the NK1R and only EKC and EKD seem not to have affinity for this receptor. SP is the main endogenous ligand for NK1R in CNS and it has been suggested that EKA and EKB might be endogenous ligands for NK1R in peripheral non-innervated tissues (Page et al., 2003).

NK1R is part of the rhodopsin-like like family of GPCRs. The receptor has three extracellular and intracellular loops. It has an extracellular N-terminus and seven transmembrane domains and possible fourth intracellular loop. It also has intracellular C-terminus. The receptor binds to intracellular G proteins which transmit the signal from the receptor to enzymes and ion channels.

Shared carboxy-terminal of all tachykinins interacts with all tachykinin receptors and alternating amino-terminal makes the peptide specific for the receptor. Second and third loop of the receptor makes the binding agonist or antagonist. The third cytoplasmic loop binds to the G protein. C-terminus of the receptor gets phosphorylated when the receptor has been activated by the agonist repeatedly and this causes desensitization of the receptor. Activation of the NK1R by tachykinin causes the G protein Gα linked to the Gβγ complex to exchange its guanosine diphosphate (GDP) to guanosine triphosphate (GTP) permitting Gα and Gβγ dissociation. These Gα and Gβ subunits can then begin their own signaling cascade regulating enzymes or ion channels of the cell. Different Gα subunits have different consequences in the cell when activated. Gαs subunit activation leads to increased levels of adenosine monophosphate and it activates protein kinase A. Protein kinase A can inhibit NF-κB and this leads to anti-inflammatory effects. On the other hand, subunit Gαq can activate NF-κB via increased levels of intracellular calcium causing proinflammatory effects. Gαq can also stimulate cell proliferation, have antiapoptotic effects and increase the levels of cytokines. Gαi subunit stimulates DNA synthesis, antiapoptotic effects and cell proliferation. Activated $G\alpha_{12/13}$ leads to cytoskeletal reorganization and cell migration. Effects of NK1R activation are very different depending of type of the Gα and Gβγ subunits and the cell stimulated (Garcia-Recio and Gascón, 2015).

NK1R has two isoforms, long and short. In human central nervous system long splice variant is more abundant in most brain regions than short (Caberlotto et al., 2003; Mantyh et al., 1996). Short isoform seems to have lower affinity to SP than long (Fong et al., 1992). Secondary signaling and cell activation is also different between long and short isoforms (Lai et al., 2008). Receptor internalization is probably slower for the short isoform than the long which might lead to prolonged response for the ligand (Garcia-Recio and Gascón, 2015).

NK1R amino terminal glycosylation also affects the receptor cell membrane dynamics. Nonglycosylated receptors are internalized faster than glycosylated receptors. Glycosylated NK1R is more stable on the cell membrane and second messenger pathway might be altered on nonglycosylated receptors (Tansky et al., 2007). There is also evidence that different conformations of NK1R exits. These receptors have been named “septide-sensitive”, “new NK1-sensitive” and “classic”. SP has high affinity to all these receptors, but NKA and NKB have higher affinity to “new NK1-sensitive” and “septide-sensitive” subtypes. Septide (a short substance P C-terminal analogue) has affinity only to “septide-sensitive” subtype (Beaujouan et al., 2004; Maggi and Schwartz, 1997; Pennefather et al., 2004). Functional role is these different conformations are not known.

2.1.5 Distribution of Neurokinin-1 receptors in mammalian CNS

NK1R distribution in mammalian CNS has been studied using immunocytochemistry (Nakaya et al., 1994), studying mRNA encoding expression for NK1R protein (Maeno et al., 1993; Whitty et al., 1995) and by autoradiography (Dam and Quirion, 1986; Danks et al., 1986; Saffroy et al., 1988; Solin et al., 2004; Wolf et al., 1985). In these studies, high levels of NK1Rs have been found in striatum, nucleus accumbens, hippocampus, interpeduncular nucleus, tractus solitarius nucleus and raphe nuclei. High levels have been found also in medulla oblongata. Guinea pigs have similar regional distribution of NK1Rs as rats in the basal ganglia, but rats have more NK1Rs for example in cortical regions, dorsal raphe nuclei, vermis and locus coeruleus than guinea pigs (Dam and Quirion, 1986; Saffroy et al., 1994). Guinea pigs have more NK1Rs for example in nucleus accumbens, hypothalamus and some nuclei in mesencephalon. Regional distribution differences were present most clearly in thalamus, hippocampus and amygdala (Saffroy et al., 1994). Species difference in the affinity on NK1Rs to non-peptide antagonists have also been reported (Petitet et al., 1993). Different species, including humans, seem to have similar distribution of NK1Rs in basal ganglia (Caberlotto et al., 2003). Comparing to

rodents, human NK1R distribution is closer to rat than guinea pig with dissimilarities in cortex, hippocampus and dorsal raphe nuclei (Caberlotto et al., 2003) compared to guinea pigs. Humans also have distinct distribution of NK1Rs in amygdala compared to rodents which seem to have more similar amygdala NK1R organization than humans (Caberlotto et al., 2003). Functional significance of this these distribution differences is not known, but SPAs that have anxiolytic activity in rodents, have been negative in large human trials (detailed in chapter 2.4.1). It might be that interpretation of SP-NK1R functions in humans should be done very cautiously when based on rodent data.

In humans, NK1Rs are present in the striatum, globus pallidus, in the cortical regions, especially in the occipital cortex, amygdala, hippocampus, parahippocampal gyrus, hypothalamus, locus coeruleus and medulla oblongata (Caberlotto et al., 2003). Distribution of NK1Rs in the human striatum has been studied in more detail. NK1Rs are located mainly near the cell bodies and not in the terminal projections (Caberlotto et al., 2003). In the striatum, NK1Rs have found from aspiny interneurons and local collaterals of spiny projection neurons (Mounir and Parent, 2002; Parent et al., 1995). These striatal aspiny interneurons coexpress choline acetyltransferase and somatostatin linking them indirectly to both GABAergic inhibitory and cholinergic excitatory system in striatal level (Mounir and Parent, 2002). This striatal NK1R mediated balance is possible modulated by NK1R second messenger system which is discussed in previous chapter. In rat hippocampus, NK1Rs are also located only in interneurons containing GABA and possible glutamate (Stacey et al., 2002a, 2002b). NK1R-SP interactions with other neurotransmitters are detailed in chapters 2.2.2-5.

SP is not always present near the NK1Rs and NK1Rs not always presents near SP containing axon terminals and there is a mismatch between these locations. NK1Rs are found not only in synaptic cleft but also in non-synaptic areas. SP can then bind to the near synaptic NK1Rs, but also to NK1Rs in extrasynaptic sites in larger distances via volume transmission (Li et al., 2000). This volume transmission and extrasynaptic receptors are the basis of neuromodulator role of the SP-NK1R system.

2.2 Central nervous functions of the SP-NK1R system

SP and NK1R are associated with numerous functions in mammalian CNS such as stress response, emotion, depression, neuronal survival, neurogenesis, pain, emesis, vigilance, addiction and cancer progression (Czeh et al., 2005; De Felipe et al., 1998; Gadd et al., 2003; George et al., 2008; Kramer et al., 1998; Lorente et al., 2016; Morcuende et al.,

2003; Muñoz and Coveñas, 2013; Murtra et al., 2000; Navari et al., 1999). Substance P levels are, in general, increased in many pathological and stressful conditions in CNS (Bondy et al., 2003; Geracioti et al., 2006; Lorente et al., 2016). This has led to development of drugs that block NK1R-SP system (substance P antagonist, SPA) in hope to alleviate the pathological conditions caused by the increased levels of SP after chronic or acute stress conditions. The only SPAs that have made to market so far (aprepitant, netupitant and rolapitant) are for treatment of chemotherapy-induced nausea and vomiting (Rapoport, 2017). SPAs studied for the treatment of MDD have not been effective and the NK1R-SP system involvement in MDD and anxiety are detailed in chapter 2.4.1.

2.2.1 NK1R-SP in healthy humans

SP in plasma can cross the BBB to the brain as previously mentioned (see chapter 2.1.3). Effect of stress on SP levels in plasma in healthy humans has produced mixed results and SP might be more elevated only in subset of subjects (Ebner et al., 2009). Intravenous infusion of SP in healthy males decreased mood, induces anxiety symptoms, causes sleep disturbances and worsening in short-term memory functions (Herpfer et al., 2007; Lieb et al., 2002). SP infusion also increases cortisol plasma levels (Coiro et al., 1992; Lieb et al., 2002). These effects closely resemble those seen on MDD patients. SPAs increase neural activity caused by processing positive emotional information in healthy volunteers without effect in mood. This suggest that SPAs modulate processing of emotional information directly (Chandra et al., 2010; McCabe et al., 2009). These results are consistent with effects caused by conventional MDD medication like SSRIs, although after SSRIs, attention towards positive stimulus is stronger.

2.2.2 Interaction with dopamine system

Interactions between SP-NK1R and dopamine (DA) system are well characterized. SP is present in the human striatal medium-sized spiny neurons of the striatonigral pathway (Beaujouan et al., 2004) and NK1R are found in midbrain dopaminergic neurons in humans (Whitty et al., 1997). In striatum, SP is found in striosomes and its boundary areas and it is able to modulate DA release in center-surround manner (Brimblecombe and Cragg, 2015). SPAs can modulate cocaine evoked DA overflow and methamphetamine induced loss of DA transporters in the rat striatum (Loonam et al., 2003). Increased levels of SP increase dopaminergic cell death, motor symptoms and inflammation in a rat model of early Parkinson's disease and SPA is neuroprotective in this setting (Thornton and Vink, 2012). SPA is able to attenuate gain anticipation induce

increase of blood flow in nucleus accumbens in healthy volunteers (Saji et al., 2013). These findings indicate close functional connections between the NK1R-SP and DA-systems and NK1R-SP system seems to play a role in reward, early dopaminergic degeneration and addiction.

2.2.3 Interaction with serotonin system

Interactions between NK1R-SP and serotonergic system are also well documented. NK1R are located in dorsal raphe nucleus (DRN) and locus coeruleus (LC) in mammal brain (Léger et al., 2002) and SP has been localized also in these same neurons (Nakaya et al., 1994; Sergeyev et al., 1999). SP acts via local circuits in DRN to alter serotonergic neurotransmission (Commons and Valentino, 2002; Lacoste et al., 2006; Liu et al., 2002; Valentino et al., 2003). SPAs and genetic disruption of NK1R results increase neuronal activity in DRN and this leads to enhanced serotonergic neurotransmission (Conley et al., 2002; Haddjeri and Blier, 2008, 2001; Santarelli et al., 2001). SPAs and genetic disruption of NK1R also leads to downregulation of 5-HT1A receptors (Froger et al., 2001; Guiard et al., 2005).

2.2.4 Interaction with noradrenaline system

Ascending noradrenergic neurons in LC are innervated by SP and NK1R containing neurons (Caberlotto et al., 2003; Chen et al., 2000; Ribeiro-da-Silva and Hökfelt, 2000). SPAs enhance the activity of adrenergic neurons and genetic disruption of NK1R results in increased basal activity of these neurons in non-stressed conditions (Fisher et al., 2007; Millan et al., 2001). In contrast, in stressed situation, SPAs attenuated firing rate of these neurons in LC and lowered the release of noradrenaline in prefrontal cortex (Renoldi and Invernizzi, 2006).

2.2.5 Interaction with GABA and glutamate

In rat entorhinal cortex (EC) NK1Rs are located in GABAergic interneurons and SP promotes the release of GABA from these interneurons and inhibit pyramidal cell activation (Maubach et al., 1998; Stacey et al., 2002a; Wolansky et al., 2007). NK1R agonists can also promote glutamate release onto EC principal neurons (Stacey et al., 2002b). NK1Rs are located in EC only in interneurons which contain GABA and possible also glutamate (Stacey et al., 2002b). By stimulating GABA release from these NK1R

containing interneurons these cells inhibit principal neurons and by stimulating glutamate from these possible excitatory interneurons principal neurons are stimulated (Stacey et al., 2002b). These data suggest that NK1R-SP system is controlling the overall balance of cortical stimulation and output. NK1R-SP system also modulate GABA and glutamate mediated activity related to anxiogenic action of SP in the amygdala (Sosulina et al., 2015; Sreepathi and Ferraguti, 2012).

2.3 Imaging neurokinin-1 receptors in vitro and *in vivo* in humans

2.3.1 Positron emission tomography (PET)

PET is an imaging modality that utilizes specific tracer molecules labeled with a positron emitting isotope. The positron emitting isotopes are made using a cyclotron, for example fluorine-18 can be made by high energy proton beam to oxygen-18 enriched water. The isotope made in cyclotron is attached to a molecule with desired function, for example to neurokinin-1 receptor antagonist. This combination of positron emitting nucleus with biologically active molecule is called a radiotracer.

After intravenous injection, the radiotracer is delivered from the bloodstream to the target tissue, where it can bind to NK1Rs (specific binding). The unstable isotope decays via positron emission. The emitted positron travels in the tissue for a while, usually a millimeter, depending on positron energy received from the decay process. When the travelling positron has lost all of its kinetic energy it will annihilate with its antiparticle, an electron. This annihilation produces two annihilation photons with 511 keV of energy and these particles move in almost opposite directions (180 ± 0.5°) forming a gamma ray.

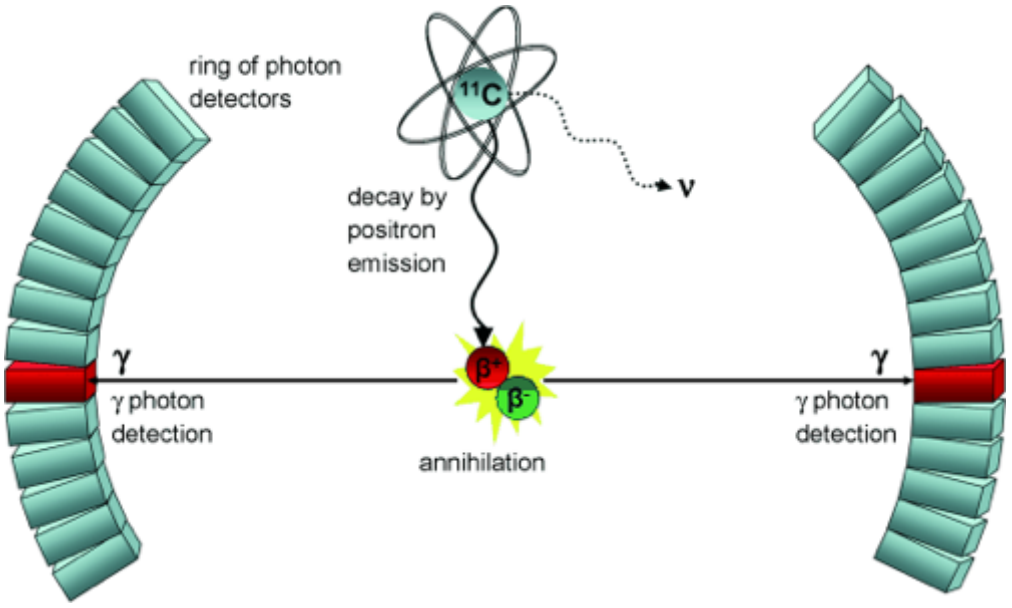

Figure 1. The basic principle behind PET with positron emission, annihilation and detection (Miller et al., 2008).

These photons / gamma rays are detected by PET scanners detectors. As these two photons are detected at almost the same time, they can be assumed to be from the same annihilation event, a coincidence event. These coincidence events are collected to projection images called sinograms. From one scan there are multiple sinograms, each representing a projection of the coincidence events from different angle and tilt. These projections are then reconstructed to an image volume representing the locations of the annihilations in the tissue that was in the PET scanner. Usually the PET data are collected dynamically at multiple time points (frames) to allow kinetic modelling. All this makes PET imaging the most sensitive and specific molecular imaging method available today. Numerous different tracers can be used to image almost anything interesting in human body and new tracer inventions like the one discussed in this thesis even widen the possibilities of this novel imaging method.

2.3.2 Limitations of PET

As the image reconstructed from the sinograms represents location of the detected coincidence events, which are annihilations of the positron with electron, there is an inherited mismatch with the original location of the radiotracer and the detected

annihilation. This mismatch depends on the radioisotope used as different radioisotopes give different amount kinetic energy to the emitted positron. Fluorine-18 has low positron kinetic energy and this leads to short travel distance (around 1mm) before annihilation and a good spatial resolution of the image finally reconstructed.

Gamma rays emitted from the annihilation are able to ionize other atoms (ionizing radiation). In biological tissue, this is mostly harmful. Exposure to ionizing radiation on low doses (like in PET) leads to stochastic effects in the tissue which are hard to predict. Most commonly feared stochastic effect is the induction of cancer. Lifetime risk increase after a fluoride-18 injection is difficult to predict and depends on the injected dose and ligand attached. For a 20-year-old, having received an effective dose of 6,9mSv from 18F injection, is estimated to result in additional lifetime cancer risk of 0.08% (American Society of Radiologic Technologist risk calculator, http://www.xrayrisk.com).

PET is labor intensive, time consuming, and fairly expensive. For example, PET scanners, cyclotrons and hot-cells of radiopharmaceutical laboratory and quality control are expensive with high maintenance involved. All these also require a number of highly educated staff to operate and maintain. The isotopes used in radiotracers have a short half-life (fluorine-18 half-life is 110 min) and must be prepared for each scan individually. Any technical or other problem that delays the scan will result in loss of radiotracer produced.

2.3.3 Human *in vivo* neurokinin-1 radiotracers

Several different NK1R radiotracers for human use have been developed. In this thesis we will present our results in using fluorine-18 labeled substance P antagonist receptor quantifier [18F]SPA-RQ (also called [18F]MK-0999). [18F]SPA-RQ has high affinity to NK1R (IC_{50} = 67 pM, K_d has not been published) (Solin et al., 2004). Binding is also highly selective to NK1Rs as will be discussed in the chapter 5.1. In addition to the studies discussed here, this radiotracer has been used in other human studies also. Test-retest study of reference tissue models using [18F]SPA-RQ has been reported (Yasuno et al., 2007). NK1R occupancy studies of SPA L-759274 and aprepitant in humans have also been performed (Bergström et al., 2004; Michelson et al., 2013; Van Laere et al., 2012; Wallius et al., 2007). Patients with panic disorder (Fujimura et al., 2009) and chronic visceral pain have been studied (Jarcho et al., 2013), and distribution of NK1Rs in human body has also been studied (Sprague et al., 2007). A fluoroethyl analog of SPA-RQ has been developed (FE-SPA-RQ) and it is reported to have slower rate of defluorination than SPA-RQ which should result in lower accumulation of 18F to bone (Okumura et al., 2008).

Carbon-11 labeled GR205171, a highly specific NK1R antagonist (Bergström et al., 2000) is another commonly used NK1R radiotracer. The GR205171 is based on the same pharmacophore as SPA-RQ and has a high affinity and specificity to NK1Rs (K_d = 16 pM) (Fridén et al., 2014). The effect of age and gender in NK1Rs have been studied also using this radiotracer (Engman et al., 2012) and these results are discussed later in chapter 6.2. This radiotracer has also been used to study symptom provocation effects in NK1R availability specific phobias (Michelgård et al., 2007), NK1R availability in temporal lobe epilepsy (Danfors et al., 2011), in chronic pain (Linnman et al., 2010), in chronic tennis elbow (Linnman et al., 2016; Peterson et al., 2013), in SAD (Frick et al., 2015) and in PTSD (Frick et al., 2016b). A NK1R occupancy of NK1R antagonist casopitant, netupitant, GSK1144814, LY686017 and rolapitant have been studied using this radiotracer (Ridler et al., 2014; Spinelli et al., 2014; Tauscher et al., 2010; Wang et al., 2017; Zamuner et al., 2012).

Another carbon-11 labeled NK1R radiotracer is R116301, with lower affinity NK1Rs than two previous tracers mentioned (Van der Mey et al., 2005; Wolfensberger et al., 2009). First evaluation of the [11C]R116301 has been published and also a more detailed description about the quantification of the radiotracer (Wolfensberger et al., 2011, 2009).

2.3.4 Methods of analyzing the PET data

After the PET scan is completed, the data must be modelled to transform the raw radioactivity counts into biologically meaningful information. The resulting outcome images must be analysed to get the time activity information for different brain areas. Different brain areas must be first defined and this can done manually for each subject or automatically after the PET-images have been transformed to standard anatomical space. Subsequently, the measured region specific time activity represents total activity detected and must be further modeled to get an estimate of specific binding to the receptor of interest.

2.3.4.1 Manually drawn regions of interests

In this method brain regions are defined for each subject individually. This is usually done using magnetic resonance images (MRI) as anatomical reference. MR-images are first registered to PET-image so that the anatomical regions are in the same space in both

images. Registration is usually done using algorithms that measure similarity of the intensity patterns of the two images. When registering two images of different modalities, like PET- with MR-images, normalized mutual information registration is commonly used (Maes et al., 1997). Then regions of interest are drawn based on MR-images anatomical landmarks and transferred to PET-images. This method is depended on good coregistration between imaging modalities, good anatomical knowledge of the brain and precise drawing of these regions (Bremner et al., 1998).

2.3.4.2 Atlas based definition of regions of interests

In atlas based method, brain regions are derived from labeled atlas which is in standard anatomical space. Individual PET and MRI-images are first normalized to standard anatomical space. The conversion to standard anatomical space is done in two steps: (1) linear registration is used to get source image in the same position and zoom as the standard image and (2) nonlinear registration where deformations to the source image is also done to correct for the differences in head (and brain) shape between an individual and the standard space brain (Ashburner and Friston, 1999; Friston et al., 1995). This normalization performs well in healthy normal brain, even in the small deep regions (Salmond et al., 2002). Normalization of deep brain regions affected by some form of pathology might not be that good (Krishnan et al., 2006). Then selected ROIs from this atlas defining brain areas are used to calculate time activity curves from normalized dynamic PET-images. The method has an advantage of having anatomically consistent ROIs in all individual subjects. The location of the ROIs is also commonly known and the comparison of the results between studies is easier.

2.3.4.3 Statistical parametric mapping (SPM)

In contrast to previous method where all images were analyzed by predefined regions of interest, in statistical parametric mapping (SPM) all images are analyzed in standard anatomical space voxel-by-voxel. Usually normalized parametric images calculated from PET-images (see next chapter) are used. Then the parametric images can be analyzed as a group using generalized linear model.

2.3.5 Modeling the PET data in the brain receptor studies

When the radiotracer (ligand) has been injected to the venous circulation it will be distributed evenly to the whole body. Radioligand arrives to the brain via the arterial circulation distributed in the plasma. From there, part of the ligand will cross the BBB and distribute to the tissue water. When in the tissue, the ligand starts to bind to the receptors if available. This dynamic process of ligand distribution leading to radiotracer binding to the specific brain receptor can be modeled by different ways. Most commonly used model is a compartment model.

In the compartment model, the radiotracer is assumed to be uniformly distributed in the artificial 'compartments' and movement of the tracer between these compartments is characterized by a rate constants. If the concentration of the radiotracer in the plasma is known, then it can be used as an input function and assumed as one compartment. In the brain receptor studies three different tissue compartments are usually assumed. First compartment is the free radiotracer in the tissue, C_F. When the radioligand is transferred from the plasma to the tissue it is assumed to be in this compartment. From this compartment, the radioligand can specifically bound to the receptor and move to the C_B compartment or it can be not specifically bound to the receptor and be in the C_{NS} compartment. Rate constants between these compartments can been seen in figure 2. This four compartment model usually leads to too complicated model and C_F and C_{NS} are usually assumed to form one compartment with fast transfer between.

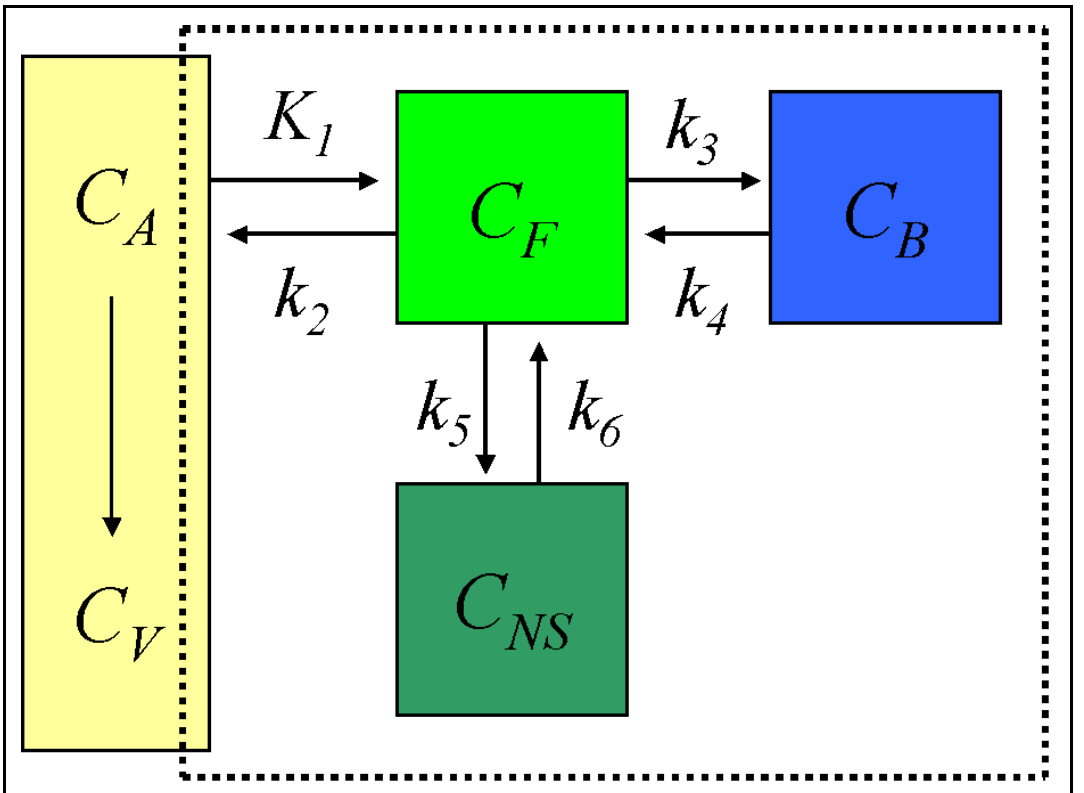

Figure 2. The four compartment model.
Modified from Vesa Oikonen (http://www.turkupetcentre.net/petanalysis/).

When the concentration of the radiotracer in the plasma is not available, or it is not feasible to use it, a reference region input compartment models can be used. In these models, a region with no specific uptake (binding) must be available. The model is usually used to estimate binding potential BP_{ND}. In these models, BP is depended on the fraction of the radioligand that is freely available in the tissue:

$$BP_{ND} = f_{ND} * B_{avail} / K_d = k_3/k_4$$

, where B_{avail} refers to receptors available for binding, f_{ND} to free fraction of the radioligand in the tissue and K_d to affinity of the radiotracer to the receptor.

The golden standard of the reference tissue compartment models is the full model (RTCM, (Cunningham et al., 1991). In reference tissue models, rate between plasma and tissue (K_1/k_2) is considered constant in all regions. R_1 is the ratio of the K1 value of the regions of interest and reference region ($R_1 = K_1 / K_1'$) and it can be seen as an index of

the perfusion and transport of the radiotracer to the tissue. R1, k2, k3 and k4 are estimated from the model using nonlinear fitting. See figure 3.

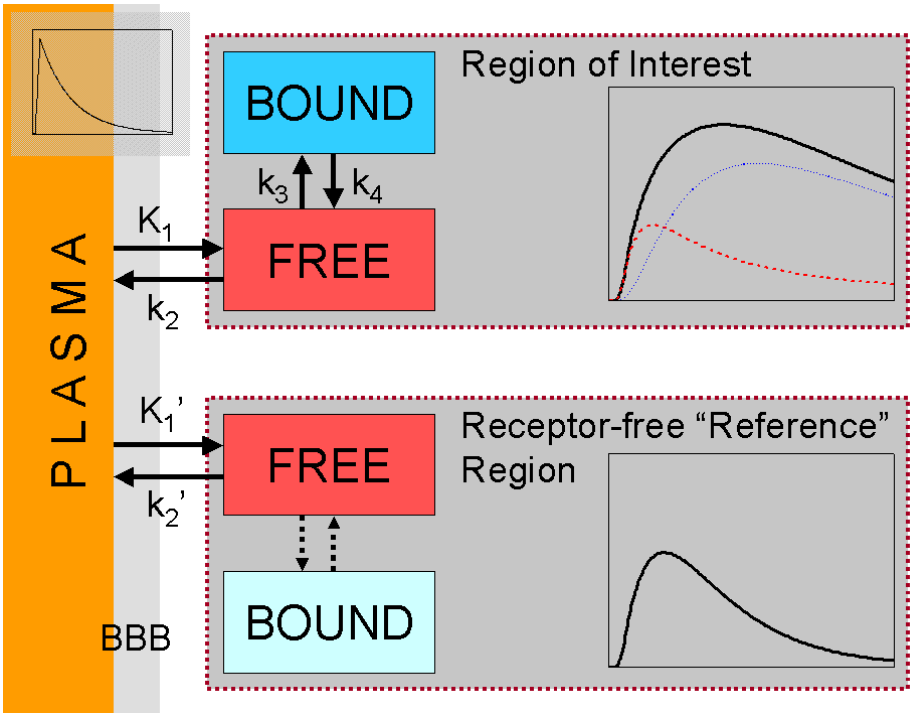

Figure 3. Full reference tissue compartment model (RTCM).
Modified from Vesa Oikonen (http://www.turkupetcentre.net/petanalysis/).

If the full model is too complex for the noisy PET data, a simplified reference tissue model (SRTM) is also available (Lammertsma and Hume, 1996). This model assumes that the radiotracer kinetics can be described by two compartments: plasma Cp and tissue compartment containing free radiotracer C_{FT}, non specifically bound radiotracer C_{NS} and specifically bound radiotracer C_S. In the reference region (for example in the cerebellum) C_S can be assumed to be 0. See figure 4.

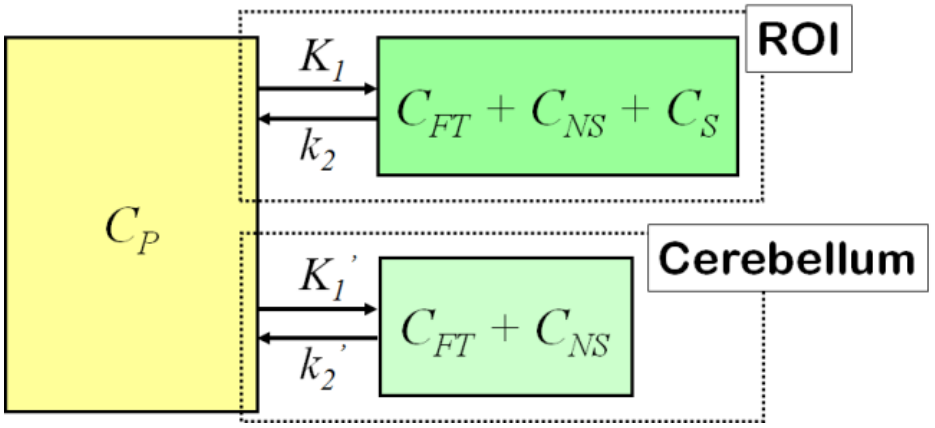

Figure 4. The simplified reference tissue model (SRTM).
Modified from Vesa Oikonen (http://www.turkupetcentre.net/petanalysis/).

As the full model, this simplified model assumes that the reference tissue has no specific binding and that the K_1/k_2 is same in all regions. Simplified model also assumes that radiotracer kinetics in all brain regions is fast and simple. This model has then three parameters to solve: R_1, k_2 and BP_{ND} and these parameters can be solved using nonlinear fitting and using basis function implementation of the SRTM (Gunn et al., 1997). Using the basis function implementation parametric image can also be calculated.

2.3.6 Human brain receptor digital autoradiography

In human post-mortem brain receptor autoradiography the tissue is sliced to thin samples and then the radioligand alone or with a high concentration of cold displacing agent is applied on the tissue. After the incubation, the sample is washed and dried to remove any nonspecific binding. Then the thin samples are placed on a digital imaging plate and the distribution of gamma rays is detected by a phosphor imaging plate. The gamma rays stimulate electrons in the phosphor crystals and these electrons are trapped by crystals F centers forming a metastable state in them. When laser light is focused on these F centers they emit blue-violet light. This light is emitted in proportion to the gamma rays emitted from to the sample. The light is collected by a photomultiplier tube and is then converted to a quantitative digital image of e.g. total and non-specific radioligand binding resulting in specific regional radioligand binding.

2.3.7 Effect of age and gender to NK1R distribution

As discussed in the chapter 2.2.2, NK1R-SP and DA systems are closely related. Functional imaging studies have documented decreased striatal and cortical dopamine activity during aging (Inoue et al., 2001; Rinne et al., 1993, 1990; Suhara et al., 1991; Wang et al., 1998; Wong et al., 1997) as well as gender differences in dopamine activity (Kaasinen et al., 2001; Laakso et al., 2002; Pohjalainen et al., 1998). Availability of dopamine receptors decrease by 5-10% per decade in striatal and extrastriatal regions respectively (Kaasinen et al., 2000; Wang et al., 1998). Male rats have shown to have 10-40% higher SP levels than females in posterior caudate, globus pallidus and nucleus accumbens (Pittenger et al., 2016) . NK1R availability may be affected by age and gender in a manner similar to that seen in the DA-system i.e. NK1R availability decreases with age and females have higher NK1R availability than males. The effect of aging and gender to NK1R availability in the human brain had not been studied before our study.

2.4 Major depressive disorder

Major depressive disorder (MDD) is a mental disorder that affects more than 300 million people globally and 30 million in the EU area (Wittchen et al., 2011). The global point prevalence of the disease is 5,9% for females and 3,8% for males, although it varies from 0,05% for over 65 year old Japanese males to 73% for over 15 years old females living in Afghanistan (Ferrari et al., 2013).

The core symptoms of the MDD are depressed mood, feelings of guilt, inability to experience pleasure from activities usually found enjoyable (anhedonia) and psychic anxiety. Loss of energy, psychomotor retardation, somatic anxiety and suicidal ideation are also considered as core symptoms.

MDD is diagnosed by a doctor who records patient's current symptoms and history. Blood test are usually performed to rule metabolic and other causes for depressed mood. Patients substance abuse is assessed. Brain computer tomography scan (CT) or magnetic resonance imaging (MRI) can be done if patient presents with unusually rapid or severe symptoms. When recording patient's symptoms, a rating scale such as Hamilton Rating

Scale for Depression (Hamilton, 1960) or Beck Depression Inventory (Beck et al., 1961) can be used. These scales provide information about the severity of the symptoms.

Treatments for MDD include psychotherapies (like cognitive behavioral therapy, CBT), pharmacological treatments and lifestyle adjustments in exercise, substance abuse, smoking, diet and sleep.

Neurobiological processes that lead to MDD is still unclear. Functional changes in cortical-striatal-limbic circuit are widely reported. Alterations in prefrontal areas and hippocampus are related to altered cognition, changes in amygdala to emotional processing and changes in nucleus accumbens to feelings of reward. Most commonly cited neurotransmitter theory is the monoamine theory which states that disturbances in monoamine neurotransmission are related to MDD (Schildkraut, 1965). Although most commonly used antidepressant are, at least partly, in line with this theory, the theory is not able to explain the slow onset of actions of monoamine based antidepressant. Neuronal atrophy and cell loss in limbic areas and alterations of other neurotransmitter systems are also not explained by this theory.

Newer theory, called 'neurotrophin hypothesis of depression' or 'network hypothesis' have related MDD to changes in neuroplasticity and neurogenesis in prefrontal cortex, amygdala and hippocampus (Castrén, 2013; Duman and Monteggia, 2006). Alterations in neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin receptor kinase B (TrkB), are related to these changes (Berton et al., 2006; Castrén and Rantamäki, 2010; Nibuya et al., 1995; Saarelainen et al., 2003; Sairanen et al., 2005). Chronic stress decreases BDNF levels in animals (Duman and Monteggia, 2006). Patients with MDD have lower serum BDNF levels than healthy controls and antidepressive medication increases serum BDNF levels (Sen et al., 2008). Patients with MDD have reduction in hippocampal volume (Campbell et al., 2004; MacQueen et al., 2003; Sheline et al., 2003, 1999). Antidepressive medication increases BDNF production and restores hippocampal volume (Nibuya et al., 1995; Russo-Neustadt et al., 1999). Hippocampal neurogenesis is also requirement for behavioral effects of antidepressants (Santarelli et al., 2003). Antidepressants are not effective in mice with reduced BDNF and TrkB function (Monteggia et al., 2004; Saarelainen et al., 2003). Subjects that have committed suicide have reduced BDNF and TrkB expression in postmortem brain (Dwivedi et al., 2003). The studies suggest that antidepressants induce neuronal plasticity in brain areas related to mood regulation. This plasticity seems to be depended on positive stimulus from the environment and increased neural plasticity with positive stimulus allows neural networks to return to its 'normal mood' -state.

Other factors that are recognized important for pathophysiology of depression include gut microbiota (Slyepchenko et al., 2014), disturbed HPA-axis regulation (de Kloet et al., 2005), inflammatory reactions (Yamagata et al., 2017) and genetics (Sun et al., 2013).

Conventional pharmacological treatments for MDD include selective serotonin reuptake inhibitors (SSRI) and serotonin-noradrenalin reuptake inhibitors (SNRIs). These drugs increase the levels of extracellular serotonin and noradrenalin by inhibiting the reuptake of these molecules to the presynaptic cell. These lead to prolonged stimulation of postsynaptic cell and, in chronic use, modulation of 5-HT and NA receptors in pre- and postsynaptic cells and increase the expression of BDNF in the brain.

2.4.1 NK1R-SP in major depression disorder (MDD) and anxiety disorders

Substance P and NK1Rs are widely distributed in deep brain and cortical areas. Some of these areas are essential for stress responses and emotional and affective functions as previously mentioned (Ebner et al., 2009). These areas are for example cingulate cortex, striatum, nucleus accumbens, hippocampus, amygdala, hypothalamus, periaqueductal gray and dorsal raphe nuclei. In these areas, SP acts as a neurotransmitter and neuromodulator and some neurons that express SP, coexpress serotonin and noradrenalin, monoamines that are known to be involved in the regulation of mood and anxiety (see chapters 2.2.3-4).

2.4.2 NK1R-SP in animal models of depression, anxiety and antidepressant and anxiolytic drug action

Acute and chronic stress in animals increases SP production and release in brain areas known to be related to stress responses (Brodin et al., 1994; Ebner et al., 2004; Herpfer et al., 2012; Rosen et al., 1992; Siegel et al., 1984). Intracerebral injection of SP is anxiogenic in animals (Ebner et al., 2009; Kramer et al., 1998; Teixeira et al., 1996). NK1R antagonists have anxiolytic and antidepressive like effects in animal behavioral models (Dableh et al., 2005; Heldt et al., 2009; Kramer et al., 1998; Teixeira et al., 1996; Wallace-Boone et al., 2008). Prevention of NK1R gene expression (NK1R knockout) produces animal behavior that resembles anxiolytic or antidepressant medication (De Felipe et al., 1998; Herpfer et al., 2005; Rupniak et al., 2000; Santarelli et al., 2001).

As discussed in chapter 2.4, increased neural plasticity seems to be a requirement for antidepressant action. Increased neural plasticity is strongly related to increased BDNF levels and TrkB signaling. Reduced BDNF levels are associated with reduced TAC1 gene expression (Tripp et al., 2012). NK1R knockout mice have increased neurogenesis and increased levels of BDNF in hippocampus (Morcuende et al., 2003) but the genetic background of these NK1R knockout mice also influences the behavioral and molecular consequences of stress (McCutcheon et al., 2008). Early life stress has been shown to induce increased levels of SP and delayed impairment of long-term synaptic potentiation in adult mice suggesting a role of SP in the modulation of brain plasticity (Herpfer et al., 2012). Continuous infusion of SP in the rat lateral ventricles was reported to increase proliferation of neural progenitor cells in subventricular zone and in hippocampus dentate gyrus and infusion of SPA L-703,606 prevented it (Park et al., 2007). SP induced microglial proliferation in the subventricular zone and dentate gyrus following traumatic brain injury. SPAs inhibited this and this inhibition was associated with functional improvement (Carthew et al., 2012). SPAs might not promote neural proliferation directly but possible through increased levels of BDNF (Morcuende et al., 2003; Park et al., 2007).

2.4.3 NK1R-SP in patients with MDD and anxiety related disorders

Patients with MDD have elevated SP concentrations in plasma (Bondy et al., 2003) and in CSF (Geracioti et al., 2006). Decreased levels of SP were reported in treatment resistant depression with chronic medication (Carpenter et al., 2008). Patients that develop MDD after stroke have elevated SP concentrations in their CSF (Li et al., 2009; Lorente et al., 2016).

In human post-mortem autoradiographic studies, decreased NK1R binding in patients with MDD has been found in orbitofrontal cortex and in superficial layer of anterior cingulate cortex (Burnet and Harrison, 2000; Stockmeier et al., 2002). In another post-mortem autoradiographic study, down regulation of TAC1 mRNA was found in basolateral amygdala in patients with unipolar and bipolar depression (Carletti et al., 2005).

SPA MK869 (aprepitant) was effective in treatment of MDD in phase II trial (Kramer et al., 1998) but failed in phase III trial (Keller et al., 2006). SPA L-597274 produced antidepressive and anxiolytic effects in patients with severe MDD (Kramer et al., 2004) but was later discontinued. SPA casopitant was effective in treatment of MDD in phase II trial (Ratti et al., 2011).

From 36 patients suffering from social anxiety disorder, 42% responded to SPA GR205171 (Furmark et al., 2005). Compared to placebo, patients had lower regional cerebral blood flow (rCVF) after SPA treatment in left inferior temporal cortex including amygdala during stressful public speaking. SPA LY686017 was not effective in treatment of SAD in a proof-of-concept trial (Tauscher et al., 2010) and SPA L-759274 was not effective in treatment of generalized anxiety disorder (Michelson et al., 2013). In these studies, PET was first used to assess receptor occupancy and to choose correct dosing. Patients with panic disorder had decreased NK1R availability in several brain areas but doxapram induced panic attacks had no effect on NK1R availability (Fujimura et al., 2009). Female subjects with snake or spider phobia had decreased NK1R availability following phobogenic stimulation in right amygdala, but not in the left (Michelgård et al., 2007). This decrease in NK1R availability probably reflected increased endogenous SP release due the fear provocation. Patients with post-traumatic stress-disorder (PTSD) had increased CSF SP concentrations during and after symptom provocation (Geracioti et al., 2006). Patients with chronic pain have decreased NK1R availability in several brain areas related to affective processing (Jarcho et al., 2013). Taken together these studies demonstrate that stressful states or stimuli seem to induce substance P release in humans and it could to lead to decrease in NK1R availability probably due receptor internalization. Blocking this effect by substance P antagonist has not been enough to produce anxiolytic effect in humans.

Excessive alcohol consumption increases sensitivity to stress and SPA LY686017 suppressed spontaneous as well as challenge-induced cravings in anxious alcoholics (George et al., 2008). SPA aprepitant had no effect PTSD symptoms or stress and alcohol cues in patients with in comorbid PTSD and alcoholism (Kwako et al., 2015). The role of SP in human addiction is unclear.

3. AIMS OF THE PRESENT STUDY

The overall goal of this study was to develop and validate a quantitative method for imaging NK1Rs *in vivo* in humans using PET. Using this new imaging method, distribution of NK1Rs in normal healthy brain and the effect of age and gender to the distribution was studied. Finally, as there is a vast evidence on the involvement of NK1Rs in the mood disorders, a study on patients with MDD was performed.

Specific aims and hypotheses of the study were:

1. To validate a quantitative method for imaging NK1Rs in man in vivo with PET using a novel tracer [18F]SPA-RQ.

2. To study the effects of aging and gender on NK1-receptors in human brain. Our hypothesis was that aging decreases NK1 receptor availability and gender associates with NK1R availability in man.

3. To map brain NK1R availability in patients with MDD and matched healthy volunteers. Our original hypothesis was that patients with major depression disorder have an overactive NK1R system, in particular in in brain areas known to be involved in mood disorders.

4. SUBJECTS AND METHODS

4.1 Study design

This study consists of three sub-studies (I-III). In the first study (I) the aim was to validate a method for visualization and quantification of NK1R in human brain *in vivo* with PET. In the second study (II) the effect of age and gender to the availability of NK1Rs n healthy subjects was studied. The third study (III) focused to patients with MDD and effect of mood disorders to the NK1R availability.

The study was performed in accordance with the Declaration of Helsinki. The PET and MRI scans were performed in Turku PET Centre and Turku University Hospital between 1998 and 2005, including data for study III which was published later in 2019. The study protocols were reviewed and accepted by the local ethics committee. All participants gave written informed consent prior inclusion to the study.

4.2 Study subjects

Healthy volunteers in this study had no history of psychiatric disorders, substance abuse, or somatic illnesses. They had no clinically significant abnormalities in their physical examination or in blood and urine tests. All had negative urine drug screens. T1-weighted MR images with 1 mm^3 isotropic voxels were acquired with 1.5 T Siemens Magnetom (Iselin) device. The MR images were screened for anatomical abnormalities in the brain and were also used for anatomical reference.

For study I ten male volunteers were recruited. All were non-smokers with ages ranging from 19-33 years (25 ± 4 years, given as mean ± SD) and weights from 61-81 kg (71 ± 7 kg). In this study six patient formed a group one. They were scanned for six hours without arterial blood sampling. Group two was scanned for four hours and arterial blood samples were collected.

For study II another 25 male and 10 female healthy volunteers were recruited and volunteers from study I was also included this study. Mean age for male subjects was 27±8 years (range: 19-55 years) and mean age for female subjects was 37±11 years (range: 21-52 years). Age difference between genders was statistically significant

(p=0.002). Subject weights were between 55-87 kg and 55-80 kg for male and female, respectively.

For study III we recruited nine patients with MDD. Healthy age and sex matched controls were selected from study II. These patients with MDD were studied with same protocol, including PET-scanner as were the healthy controls. Inclusion criteria for patients with major depression were: diagnosed major depression based on DSM-IV criteria, Hamilton Rating Scale for Depression (HAM-D17) score > 18, Clinical Global Impressions of Severity of Illness Scale (CGI-S) score > 5, and no previous antidepressant medication. SCID-I interview was carried out by an experienced clinician to exclude other axis I disorders. Patients were also screened by a physician, and they were tested with laboratory tests of blood and urine. Substance abuse was excluded by blood and urine tests as with healthy controls. A MRI scan was performed also to patients.

4.3 Radiosynthesis of [18F]SPA-RQ

The NK1 receptor antagonist, SPA-RQ (MW = 450 g/mol) was labeled with the positron emitter fluorine-18 (18F; T½=109.8 min). Precursor was provided by Merck, USA. [18F]SPA-RQ, (S,S)-[2-[18F]Fluoromethoxy-5-(5-Trifluoromethyltetrazol-1-yl)-benzyl]-(2-phenylpiperidin-3-yl)-amine was prepared by [18F]fluoroalkylation of a deprotonated phenolic hydroxyl group with [18F]FCH2Br in dimethylformamide followed by removal of the BOC protecting group. [18F]FCH2Br was prepared from [18F]F- and dibromomethane and purified via preparative gas chromatography (Bergman et al., 2001). After fluoroalkylation, dimethylformamide was removed in ~ 7 min by applying a stream of helium over the surface of the solution in the reaction vessel which was simultaneously heated at 110 °C. The t-BOC protecting group was removed by treatment with trifluoroacetic acid at room temperature for 1-2 min, and the trifluoroacetic acid was evaporated by passing a stream of helium through the reaction vessel. The resulting [18F]SPA-RQ was purified via gradient preparative HPLC. The final product was formulated in ethanolic D-glucose solution buffered to pH 7. The specific radioactivity was high (between 72-4150 GBq/µmol) at the time of injection. A detailed description and radiosynthesis and quality control can be found in Solin et al 2004.

4.4 PET data acquisition

The PET experiments were performed using a PET scanner (GE advance, Milwaukee, WI, USA) in 3D mode. This scanner acquires 35 slices of 4.25 mm thickness covering the

whole brain. The basic performance tests indicate that the scanner has a spatial resolution (FWHM) of 4.3 mm. Head was fixated using a commercial head holder (GE) supporting the head from the top, back and sides. Two beams of laser light were used in the head positioning according to canthomeatal and sagittal lines. Several points were marked on the skin of the subjects aligned with the laser beams to control for any movement during the image acquisition and for repositioning the subjects in the scanner FOV in each scanning segment. For the purpose of attenuation correction of the emission scans, a transmission scan was obtained with twin 400 MBq germanium-68 pin sources prior to tracer injection.

The right antecubital vein was cannulated for injection of [18F]SPA-RQ. Group two in study I had left radial artery also cannulated for arterial blood sampling. [18F]SPA-RQ was given as a rapid bolus injection and flushed with 10 cc of saline. Mean injected activity was 131 MBq (range 122-143 MBq). The specific radioactivity of [18F]SPA-RQ was on average 1035 GBq/μmol (range 72 – 4150) at the time of the injection corresponding a very low mass of injected tracer, on average 185 ng (range 14-772 ng). Usually, PET tracer-concept means that the ligand occupies only 1% or less of the receptors inducing no pharmacological effects. This is most likely the case here.

For the group one in study I, brain radioactivity was measured for 87 min (3 x 1 min, 6 x 3 min frames and thereafter using 6 min frames) followed by additional dynamic scans consisting of six 10 min frames from 120-180 min, from 210 – 270 min and 300 – 360 min. Three dynamic scans were conducted for group two in study I and for all subjects in study II and III. The first scan, 87 min in duration, was acquired immediately after injection followed by a 40 minute scan from 120 min after injection and a third, 60 min scan, from 180 min after injection.

4.5 [18F]SPA-RQ and radiolabeled metabolite analysis in plasma

For group two in study I an automated arterial blood sampling system was used for the first 3.5 min after which arterial blood samples were taken manually up to 90 min. Unchanged [18F]SPA-RQ in arterial plasma was determined with planar chromatography and digital autoradiography. Deproteinized plasma samples were applied to a high performance thin layer chromatography (TLC) plate with an automatic TLC sampler. After the migration, the TLC plates were exposed to a phosphorimaging plate (Fuji BAS-TR2025, Fuji Photo Film Co., Ltd., Japan) for 4±0.5 hours and analyzed for photostimulated luminescence (Tina 2.1, Raytest Isotopenmessgeräte GmbH, Straubenhardt, Germany).

The arterial plasma input function for [18F]SPA-RQ was generated by curve fitting to measurements of the unchanged tracer fraction using a Hill function of the general form:

$$Y(L)/Y(0) = 1- L^h / (K^h + L^h)$$

where L is the free unbound ligand concentration and K^h is apparent dissociation constant. These routines are described by (Gunn et al., 1998).

4.6 Region-of-interest definition and time activity curve calculation

An integrated PET image was calculated for every subject using part of the first dynamic image (3-87 min). Dynamic images were frame-to-frame motion corrected to the integral image using automatic image registration (AIR) available MedX software package (study II) or using normalized mutual information coregistration (study III). No frame-to-frame motion correction was done in study I.

MR-images were coregistered to the PET integrated image using normalized mutual information coregistration. Regions of interests (ROIs) were drawn individually to the coregistered MR-images on three to four consecutive slices. ROI total activity curves (TACs) were calculated from PET-images using in-house software. To control for unequal sampling over time, TACs were weighted according to frame duration and true counts in each frame (Gunn et al., 1998) using in-house software.

4.7 Binding potential estimates for ROIs

For group two in study I, TACs were analyzed with kinetic modeling using a reference region and metabolite corrected arterial plasma as inputs. Cerebellum was selected as a reference region as there was no specific update (see results). Rate constants (k1, k2, k3 and k4) were estimated with a nonlinear least squares global optimization procedure using tissue and metabolite-corrected plasma time-radioactivity curves. Total blood activity was used to account for the vascular radioactivity and tissue time-radioactivity curves were corrected assuming a fixed 4% vascular volume. After 90 min the radioactivity in blood was relatively low and analysis was constrained to the first 90 min. Regional binding potential (BP_{ND}) was defined as k3/k4.

First regional binding potentials were estimated using an indirect approach: calculation of regional distribution volumes (V_T) from three compartment model using the constants K1, k2, k3 and k4.

$$V_T = K_1/k_2(1 + k_3/k_4)$$

And then binding potential BP_{ND} can be calculated as:

$$BP_{ND} = (V_T \text{ ROI}/V_T \text{ cerebellum})-1$$

In this analysis k1, k2 or K1/k2 were not fixed to cerebellum.

Secondly, for group two in study I, regional BP_{ND} (k3/k4) were estimated from the same three compartment fit with regional K1/k2 fixed to that of the cerebellum.

For group one in study I and for study II and III, the BP_{ND} was estimated using simplified reference tissue model (SRTM, see chapter 2.4.5). Cerebellum was used as a reference region. In this model BP_{ND} is defined as:

$$\frac{dC_T(t)}{dt} = R_1\frac{dC_R(t)}{dt} + k_2C_R(t) - \frac{k_2}{1+BP_{ND}}C_T(t)$$

And unknown variables R_1, k_2 and BP_{ND} were solved using nonlinear fitting (study I and II) and with basis function approach (study III).

4.8 Parametric BP_{ND} images

Parametric BP_{ND} images were calculated for statistical parametric mapping (SPM) analyses for study II and III using SRTM solved with basis function approach. In study II cerebellum TAC from individually drawn ROIs was used. In study III, subject-specific cerebellum ROIs were defined automatically for each subject using FreeSurfer software (version 6.0.0, http://surfer.nmr.mgh.harvard.edu/). Using these automatic ROIs, TACs were calculated for cerebellum and used as a reference region.

In study II, integral [18F]SPA-RQ images from 6-87 minutes were normalized using SPM2 to SPA-RQ template produced in-house using method described by (Meyer et al., 1999). Parameters derived were used to normalize parametric BP_{ND}-images. After normalization, the parametric images were smoothed using 12 mm Gaussian kernel. In study III, parametric BP_{ND} images were normalized to the MNI space and smoothed with 8mm FWHM kernel using SPM12.

4.9 Post-mortem human brain receptor autoradiography

For study I, the post-mortem human brain material was obtained from the University of Kuopio, Finland. Samples were from three males, age 35-50 years, post-mortem delay 9.5 –18.5 h, with no known history of neurological or psychiatric diseases. Thin 100 µm-thick horizontal left cerebral hemisphere slices were incubated for 60 min at room temperature with 50 pM [18F]SPA-RQ in Tris-HCl-buffer (pH 7.5) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Non-specific binding was estimated by competing the radiotracer binding with 1 µM excess of a cold highly specific SPA GR203040 (2-methoxy-5-tetrazol-1-yl-benzyl-(2-phenyl- piperidin-3-yl)-a mine). After washing and drying, the slices were placed to an imaging plate (Fuji Imaging Plate BAS-TR2025, Fuji Photo Film Co., Ltd., Japan) for digital autoradiography. The imaging plates were scanned after four hours exposure with the Fuji Analyzer BAS-5000.

4.10 Statistical analyses

4.10.1 ROI level data

In the study II, the ROI level data was normally distributed and parametric methods were used. Association between age, gender and NK1R-BP_{ND} was analysed using linear regression. In the study III, non-parametric methods were used when analysing the ROI level data due the small sample size. Between groups differences were analysed using Mann-Whitney *U* test and Bonferroni correction was done to correct for multiple comparisons. Correlation analysis were done using non-parametric Spearman correlation. These correlation effects were not corrected for multiple comparisons (26 ROIs and two scales included).

4.10.2 Statistical analysis of BP_{ND} images

In the study II and III, SPM analysis was performed to normalized BP_{ND} parametric images produced as defined before (chapter 4.9).

In the study II multiple regression analysis was used using SPM2. Association between NK1R-BP_{ND} and age was tested while controlling for gender and vice versa. Analysis was restricted to brain areas where significant association was found in the ROI based analysis using bitmap masks custom made based on ROI results. Age association analysis was restricted to frontal cortex, supramarginal gyrus, angular gyrus, superior temporal gyrus, parahippocampal gyrus. Gender difference analysis was restricted to striatum. Cortical masks was done using Pickatlas 1.04 (Maldjian et al., 2003) and striatal mask was done in-house. FWE corrected clusters $p < 0.05$ were considered statistically significant.

In the study III individual sample *T*-tests were done to analyse between groups differences and general linear model was used for correlation analysis. FWE corrected clusters $p < 0.05$ were considered statistically significant. SPM12 was used in this study.

5. RESULTS

5.1 Validation of [18F]SPA-RQ for imaging NK1Rs with PET

The radiotracer penetrated the blood-brain barrier into human brain in vivo. Highest uptake was observed in the putamen and in the caudate nucleus whereas lowest uptake was observed in cerebellum. In neocortex and thalamus uptake levels were moderate. Highest cortical binding was in the primary visual cortex and lowest uptake was in the medial prefrontal cortex. There was also a moderate uptake in the midbrain, medulla and pons. When the time-radioactivity curves from subjects in study I group I were combined, we observed that total radioactivity of putamen peaked on average at 215 min and in caudate at 225 min with putamen/cerebellum and caudate/cerebellum ratios between 5 and 6. The specific binding (cerebellum subtracted) peaked at 323 ± 33 min, 327 ± 44 min and 132 ± 17 min in caudate, putamen and occipital cortex, respectively (means ± SD).

In study I, the in vitro autoradiographic distribution of [18F]SPA-RQ binding followed the *in vivo* pattern with highest binding in striatum. Cortical regions and thalamus had moderate binding and there was no specific binding in cerebellum. The NK1R binding ratio between striatum and cortex was lower than that seen in young healthy male volunteers in vivo. The addition high concentration of cold SPA GR203040 did not affect cerebellar binding (57 ± 8 vs. 60 ± 7 PSL/mm^2, means ± SD, n=3) but prevented caudate,

putamen and occipital cortical specific binding of [18F]SPA-RQ almost completely (percentage blockade were 98± 2, 94 ± 2 and 95 ± 4, means ± SD, n= 3 respectively) .

In the study I group 2, the radioactivity measured in blood decreased rapidly and could not be measured reliably after 90 min. At 80 min about 30-40% of the tracer was unchanged. The radio-TLC analysis also showed an as yet unidentified hydrophilic metabolite and free fluoride. The kinetic modeling using a reference region and metabolite corrected arterial plasma was therefore done using the only the first 90 minutes. Cerebellar time activity curves were analyzed using two- and three-compartmental models. Even the two-compartment model described adequately the kinetics of [18F]SPA-RQ in the cerebellum although the three-compartment model fit was statistically better according to Akaike Information and Schwarz criteria (data not shown). ROI V_T derived indirectly from the unconstrained kinetic analysis indicated V_T values ranging from 5.6 in cerebellum to 26.6 in putamen. The methods provided reasonable estimates of BP_{ND} but with relatively high coefficients of variation and the binding potentials were unexpectedly low compared to the BP_{ND} estimates derived from the simple ratio data discussed above. In many of the smaller regions the full kinetic model was unstable and not suitable for quantification. Fixing the K_1/k_2 to that of cerebellum improved the stability of the method but the BP_{ND} underestimation was still apparent.

In study I group one, the SRTM described the data well and was used to find the optimum data acquisition times for [18F]SPA-RQ. At the earliest time intervals, the BP_{ND} estimates were highly variable and underestimated the but then stabilized with longer acquisition time. The 240 minute scanning time seemed to be sufficient for most of the structures but the BP_{ND} was still slightly underestimated in putamen. In general, the BP_{ND} estimates with the SRTM and longer data acquisition were clearly higher than those derived from plasma input models and 90 min data acquisition. The SRTM was robust enough for estimation of NK1R-BP_{ND} in discrete brain regions. As with all previously discussed models, the highest binding potentials was in the basal ganglia. Cortical regions and thalamus had moderate binding. There was also relatively high BP_{ND} in the midbrain/pons probably representing groups of nuclei such as the dorsal raphe, locus coeruleus and periaqueductal gray. BP_{ND} in the substantia nigra was relatively low.

It would be of interest to study NK1Rs in smaller nuclei and brain areas such as the area postrema related to emesis and vomiting. Thus, we also did a small feasibility study using [18F]SPA-RQ and high-resolution ECAT HRRT PET scanner in two healthy volunteers. These studies were one the first studies on this, at that time, a prototype scanner and a

more detailed quantification of the dynamic PET images and small brain stem nuclei was not optimal. This scanner has spatial resolution of about 2,5mm compared to 4,5mm in scanner used in studies I-III. The integral PET images produced in these studies show promise in delineating smaller brain nuclei in midbrain, pons and brainstem nuclei (Figure 5) which we were not able to clearly see using the older scanner. These methods need to be optimized and reliability and reproducibility studied in order to assess the usefulness of these methods in human studies.

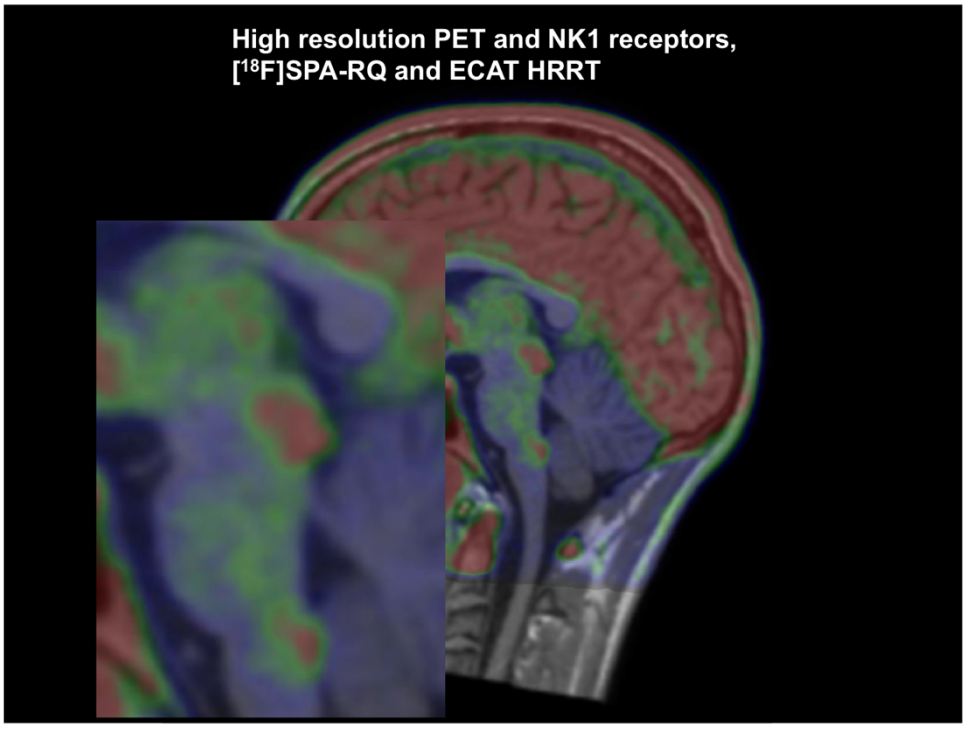

Figure 5. ECAT HRRT PET image delineating small structures in deep brain regions.

In all studies, radioactivity in the skull was noted in some subjects during the later scanning times. This uptake had accumulation-type kinetics and most likely represents free 18F from the in vivo metabolism of the tracer.

5.2 The effects of aging and gender to NK1Rs in human brain.

In study II age and gender together explained on average 26% of the total NK1R-BP_{ND} variance in the regression model. There was no co-linearity between age and gender in

any of the analysed brain region (tolerance 0.81). Results of all regression analyses are presented in Table 1.

The linear regression analysis indicated a robust association between age and NK1R-BP_{ND}. Based on the regression slopes the average decline of the regional NK1R-BP_{ND} in this data was 0.13 BP_{ND} units in a decade. This change corresponds to average 7% decline/decade from the average BP_{ND} value at 20 years of age. Females had lower NK1R-BP_{ND} values than males, reaching statistical significance in several regions. In putamen, females had 19 % lower BP_{ND} values than males. In cortical regions females had on average 17 % lower BP_{ND} values than males.

Independent SPM analyses were in line with the ROI based analyses. Statistically significant association between age and NK1R-BP_{ND} was seen in all but one analyzed areas (hippocampus). A difference between genders was replicated in putamen where significant clusters were observed in both sides (left Tmax 4.13, p 0.013; right Tmax 3.70, p=0.012).

Region	R^2	p_{model}	β^*_{age}	p_{age}	β^{**}_{gender}	p_{gender}
Medial Frontal Cortex	0.433	<0.001	-0.009	**0.031**	-0.342	**0.001**
Superior Temporal Gyrus	0.4	<0.001	-0.011	**0.01**	-0.291	**0.006**
Anterior Cingulate	0.398	<0.001	-0.007	0.113	-0.382	**<0.001**
Angular Gyrus	0.381	<0.001	-0.021	**0.001**	-0.225	0.11
Parahippocampal Gyrus	0.382	<0.001	-0.007	**0.03**	-0.24	**0.003**
Superior Colliculus	0.354	<0.001	-0.006	0.401	-0.619	**<0.001**
Putamen	0.353	<0.001	-0.006	0.543	-0.969	**<0.001**
Orbitofrontal Cortex	0.349	<0.001	-0.005	0.241	-0.376	**0.001**
Subgenual Cingulate Cortex	0.346	<0.001	-0.006	0.125	-0.32	**0.002**
Dorsolateral Prefrontal Cortex	0.345	<0.001	-0.018	**0.013**	-0.391	**0.022**
Supramarginal Gyrus	0.334	<0.001	-0.016	**0.003**	-0.197	0.112
Medial Temporal Gyrus	0.306	<0.001	-0.008	0.088	-0.31	**0.009**
Inferior Temporal Gyrus	0.297	0.001	-0.003	0.538	-0.368	**0.001**
Occipital cortex	0.275	0.001	-11	0.082	-0.345	**0.02**
Hippocampus	0.272	0.001	-0.009	**0.047**	-0.229	**0.039**
Amygdala	0.239	0.003	-0.012	0.102	-0.363	**0.036**
Posterior Cingulate Cortex	0.201	0.009	-0.006	0.169	-0.212	0.051
Thalamus	0.195	0.011	-0.006	0.272	-0.273	**0.034**
Caudatus	0.173	0.018	-0.017	0.143	-0.441	0.107
Raphe Nucleus	0.107	0.093	-0.006	0.473	-0.325	0.12
Globus Pallidus	0.079	-0.179	-0.003	0.751	-0.305	0.132

Table 1. Association of age and gender with NK1R-BP in all studied brain regions [R2, regression coefficient (b) and level of statistical significance]. Statistically significant ($p<0.05$) associations are indicated in bold

5.3 NK1Rs in patients with medication-naïve MDD compared to matched healthy volunteers.

In study III, we found no differences in NK1R-BP_{ND} between patients with MDD and healthy control subjects compared using non-parametric Mann-Whitney *U*-test. Patients had a trend towards increased hippocampal NK1R-BP_{ND}, but this did not survive Bonferroni correction for multiple comparisons. SPM analysis confirmed these results (higher in MDD: peak voxel at {-8 4 4} , t_{max} 4.04, cluster p_{FWE}= 1.00; lower in MDD: peak voxel at {-7 -76 16}, t_{max} 4.05, cluster p_{FWE}= 0.494).

Correlation analysis were done only to patients with MDD (n=9). Healthy controls depression or anxiety symptoms was not assessed in this study. In individual ROI based analysis no correlation was found between total HAM-D17 and NK1R-BP_{ND} using non-parametric Spearman correlation. However, a positive correlation was found between anxiety subscale and NK1R-BP_{ND} in putamen, hippocampus and parahippocampal gyrus. HAM-D17 total score correlated with anxiety subscale scores (Spearman correlation=0.688, p=0.041).

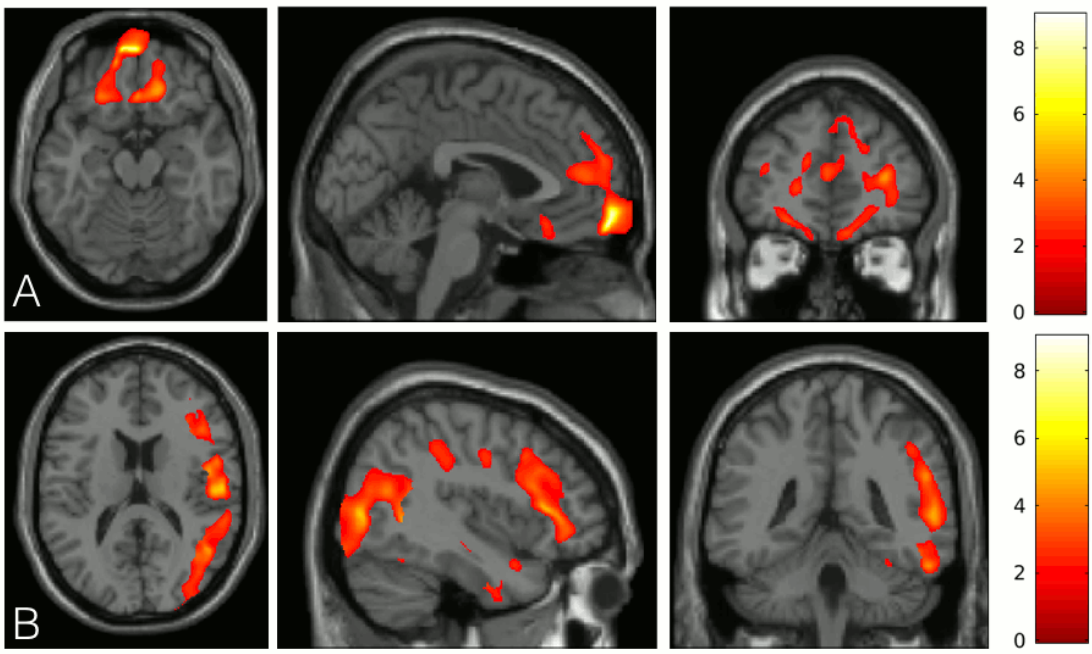

Figure. 6. (A) Frontal cluster where anxiety subscale score correlated with NK1R-BP_{ND} (cluster size 65,088 voxels, peak voxel at {36 18 15}, tmax 9.02, cluster pFWE = 0.020). (B)

Right cortical cluster where HAM-D17 score correlated with NK1R-BP_{ND} (cluster size 72,929 voxels, peak voxel at {52 −21 20}, tmax 9.02, pFWE = 0.003).

SPM analyses were done using parametric general linear model. Significant positive correlation was revealed between HAM-D17 and NK1R-BP_{ND} in right temporal, occipital and frontal regions. NK1R-BP_{ND} and anxiety subscale correlated positively in frontal cortical areas bilaterally (Figure 6). The effects in hippocampus and parahippocampal gyri did not survive multiple comparison correction.

Full NK1R-BP_{ND} data from this study III can be seen in Figure 7 below.

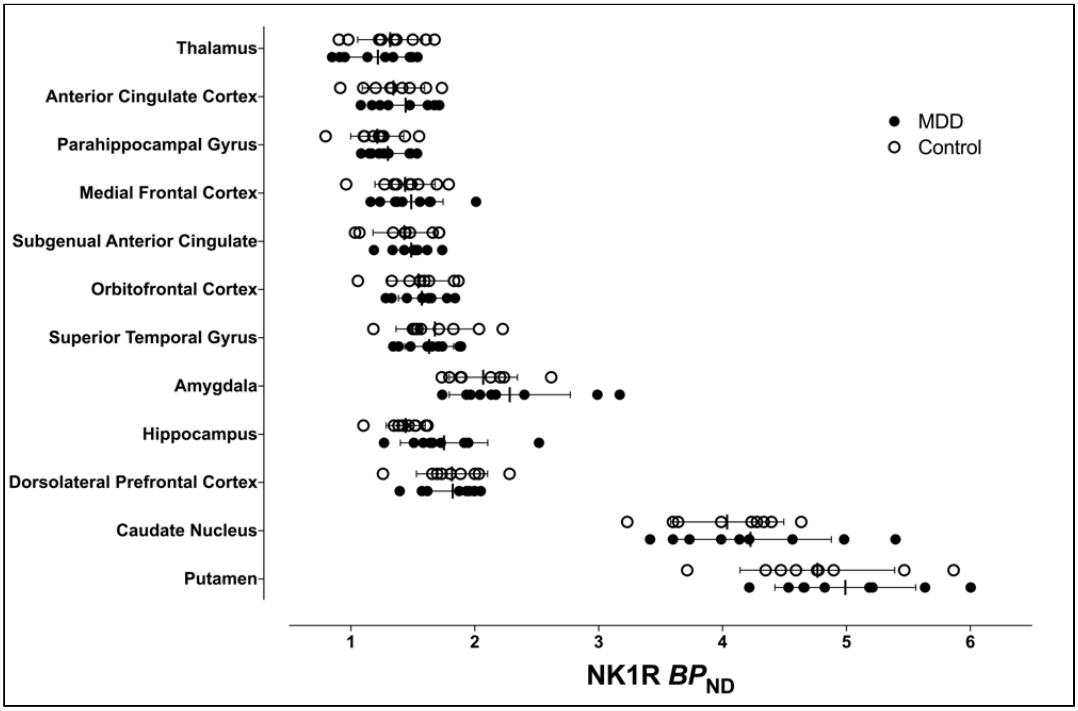

Figure 7. All data points, means and standard deviations of NK1R-BP_{ND} in patient and control groups.

6. DISCUSSION

In this study we have validated a novel method for imaging NK1Rs in vivo in human brain with PET. A novel NK1 receptor antagonist tracer, SPA-RQ was developed. Sufficient scan time for this radiotracer time was estimated to 240 minutes and we were able to model tissue TACs using SRTM. Later series of studies showed the usefulness of [18F]SPA-RQ in drug NK1R occupancy studies using a reference tissue model (Wallius et al., 2007). Healthy controls had decline in NK1R binding during aging and females were shown to have lower NK1R binding than males. Patients with MDD had no differences in NK1R availability compared to healthy controls, but overall depressive and anxiety symptoms were associated to higher NK1R availability in some brain regions.

6.1 Validity of [18F]SPA-RQ as a PET radiotracer

When developing a very high affinity receptor ligands it must be labeled to a very high specific activity to avoid significant receptor occupancy by the tracer itself. If the specific activity is low, more non-labeled molecules are injected which could interfere with receptor occupancy with labeled radiotracer. In this study, injected mass of SPA-RQ per subject was well below 800 ng and the possibility of significant NK1R occupancy (> 1%) with [18F]SPA-RQ is extremely low.

In study I group one, the brain radioactivity was followed up to six hours after injection due to the slow [18F]SPA-RQ kinetics. The total uptake was highest in the striatum followed by cortical regions, thalamus and brainstem. Uptake in the cerebellar cortex was low. The total binding peaked at about 210 min in the striatum and earlier in other regions indicating the reversibility of the ligand-receptor recognition process.

In study I group two, the measured blood radioactivity was relatively low and could not be measured reliably after 90 minutes. At 90 min, about 40 % of the radioactivity in plasma was unchanged [18F]SPA-RQ. One polar radiolabeled metabolite in was noted in the blood but we were not able to identify it with thin layer chromatography. It was hydrophilic in nature and it is unlikely to enter the brain significantly in comparison with the parent [18F]SPA-RQ.

The kinetics of the [18F]SPA-RQ was slow and the first 90 minutes was clearly not optimal for detailed quantification of NK1R-BP_{ND}. Modeling the data with full kinetic modeling

resulted in low BP_{ND} estimates and relatively high percentage of modeling failures in the estimation of small regions.

In the cerebellum, the tissue data could be modeled with a two or three-compartment model and the latter described the data statistically better. This has been observed for many valid tracers such as [^{11}C]raclopride and can be explained by rapid and slow components of the non-specific binding in the cerebellum (Oikonen et al., 2000). Specific binding can be excluded as there was no change in cerebellar activity when we added an excess concentration of SPA GR203040 in human post-mortem brain slices in vitro. Also in previous *in vivo* experiments in guinea pigs, cerebellar signal was also not altered by high doses of a competing cold SPA (Solin et al., 2004).

In human NK1R occupancy studies, there were no changes in cerebellar activity when very high occupancy (>95%) was achieved elsewhere using aprepitant (Bergström et al., 2004). Other possibilities still cannot be excluded entirely, such as a radioactive metabolite(s) of [18F]SPA-RQ. These cerebellar findings indicated that NK1R availability is negligible in human cerebellar cortex and it can be used as a reference region and as an estimate of free and nonspecific binding in PET studies with [18F]SPA-RQ in vivo (study I)

The reference tissue model using cerebellar input was robust giving data similar to the ratio method and is thus a good option for kinetic modeling and quantification of the NK1Rs. The time required for stable determination of binding potential was around 240 min. Even at this time, the specific binding in high density areas (e.g. putamen) may be slightly underestimated in some subjects. Reference tissue models were tested also in another study with [18F]SPA-RQ and they also found that SRTM is a robust method for regional BP_{ND} estimation using the 240 min scanning time (Yasuno et al., 2007). This study found no benefit for adding scanning time to 300 min. Two parameter multilinear reference tissue model (MRTM2) has also been used to model [18F]SPA-RQ data (Fujimura et al., 2009) and it seems to perform as well as SRTM (Yasuno et al., 2007). MRTM2 is linearized reference tissue model. As it is linearized, it is computationally fast. It has only two parameters to solve (R_1 and BP_{ND}), k_2 is fixed to value estimated from data from MRTM (a model with also k_2 estimation, like SRTM) (Ichise et al., 2003). [18F]SPA-RQ is not an ideal NK1 receptor tracer but modeling of the data is relatively straightforward and the method is suitable for measurement of NK1 receptor occupancy in human brain with sufficient accuracy.

There was activity also in the skull bone, most notable in later scans. NK1Rs have been reported to exist in bone (Goto et al., 2001) but the radioactivity is most likely due to

uptake of free fluoride by the bone since the uptake showed accumulation type kinetics for up to 6 hours and free fluoride was noted in the blood samples in study I group two.

6.1.1 Comparison with other NK1R PET tracers

Other commonly used NK1R radiotracer is [11C]GR205171. Compared to [18F]SPA-RQ the time frame of 11C decay limits the use of this tracer to shorter times. During the usual data collection of 60 minutes the tracer kinetics are basically irreversible and can be analyzed using Patlak's graphical analysis (Bergström et al., 2000; Michelgård et al., 2007). Nevertheless, this tracer is useful in drug occupancy studies but might be problematic if endogenous SP changes needs to be studied. [18F]SPA-RQ performs well in drug occupancy studies (Wallius et al., 2007). Bone radioactivity accumulation is obviously not a problem using carbon-11 GR205171 (Bergström et al., 2000; Danfors et al., 2011).

Fluoroethyl analog of [18F]SPA-RQ called [18F]-FE-SPA-RQ has also been developed. It was developed to have an advantage of slower defluorination of 18F and that should result in lower activity in bone and that was indeed reported (Okumura et al., 2008). [18F]-FE-SPA-RQ has higher affinity to NK1R (IC_{50} = 17 pM) that [18F]SPA-RQ (IC_{50} = 67 pM) and [18F]-FE-SPA-RQ TACs were almost identical with [18F]SPA-RQ. Okumura et al reported best results using indirect 3CM which includes blood sampling, but blood sampling failed in 3/7 subjects, due low radioactivity in blood, making this method very unreliable. SRTM was reliable, but underestimated BP_{ND} in high NK1R concentration areas even with 330 min scanning time. No other studies with [18F]-FE-SPA-RQ have been reported after the initial Okumura et al report in 2008.

Third available NK1R radiotracer is [11C]R116301. It was developed in hope to have faster kinetics and slower plasma clearance for reliable direct kinetic modeling (Wolfensberger et al., 2009). [11C]R116301 had quite fast plasma clearance and was also noticed to stick to the walls on plasma sampling tubing, and after attempts to model this sticking behavior, plasma curves were unreliable (Wolfensberger et al., 2011). SRTM was found to have good reproducibility and excellent reliability for quantifying [11C]R116301. There were also two subjects (out of 11) that had very low specific NK1R binding using this compound and reason for this phenomenon remains unknown (Wolfensberger et al., 2011). No further studies of [11C]R116301 after 2011 have been published.

NK1R research could also benefit from high resolution PET scanners like ECAT HRRT (see chapter 5.1 and figure 5). Imaging small midbrain, pons and brain stem nuclei might be

crucial in understanding SP and NK1R system involvement in different pathological conditions and e.g. the details of antiemetic actions of NK1R antagonists. Using this high resolution research scanner were able to see these smaller deep brain structures although quantification of was not optimal at that time.

6.2 The effects of aging and gender to NK1Rs in human brain.

In study II, we observed a negative association between NK1R-BP_{ND} and age in healthy volunteers. In addition, there was a gender difference in NK1R-BP_{ND}, females having lower levels of NK1R-BP_{ND} than males.

6.2.1 Age and NK1R

Previous data on NK1R-SP system and aging comes mainly from rodent studies and remains inconclusive (Fernández et al., 2002; Geraghty and Maguire, 2002; Mazzone and Geraghty, 1999; Pompei et al., 1999; Stoessl et al., 1993; Wang et al., 1993). Species differences between the rodent and human SP systems (Otsuka and Yoshioka, 1993; Solin et al., 2004) complicate the use of rodent data when interpreting human NK1R studies.

Early immunofluorescence and radioimmunoassay (Buck et al., 1981; Del Fiacco et al., 1984; Yates et al., 1983) suggested decreased SP concentrations in brain tissue during ageing. Our results indicate a robust negative association between NK1R-BP_{ND} and age in several brain regions. Most prominent age effects were seen in frontal, parietal and temporal cortical regions whereas the age effect was less marked in subcortical regions. In contrast with our results, a positive association between age and anterior cingulate cortex NK1R density was reported in a post mortem autoradiography study (Burnet and Harrison, 2000). The essentially different methodologies most likely contribute to the different results. However, in agreement with our findings, no association was found between age and NK1R densities in amygdala (Carletti et al., 2005)

Our study II was later replicated by (Engman et al., 2012) using [11C]GR205171 and PET. They based their ROI analyses on ROIs we had used and confirmed our results of age related decline in almost all of the regions we reported. Results differ in that they found a more widespread subcortical receptor decline than we and they did not find decline in hippocampus or in parietal regions. Different results in hippocampus might be related to different methods. Engman et al (2012) used normalized parametric BP_{ND} images and SPM for ROI analysis and this method might be less sensitive to changes in small structures like hippocampus. Reason for different results in other subcortical regions and

parietal cortex is not clear and might be related to different methods and differences in the studied volunteer samples. We found a decrease in cortical NK1R-BP_{ND} to be approximately 7% in decade and this average decrease of 7% was replicated (Engman et al., 2012).

NK1R-BP_{ND} decrease reported here is also similar in magnitude in D2 and D1 receptors as discussed in chapter 2.1.9. Availability of these receptors decrease by 5-10% and 7% per decade in striatal and extrastriatal regions respectively. As discussed in chapters 2.2.2-5 NK1R-SP system also interacts with serotonin, noradrenaline, glutamate and GABA neurotransmitter systems. Availability of alpha2-adrenoceptors, β-adrenoceptors and NMDA receptors also decline during aging in humans (Pascual et al., 1991; Sastre et al., 2001; Segovia et al., 2001; Villares and Stavale, 2001). In contrast, 5-HT_{1A} and GABA receptors do not appear to change with aging in humans (Parsey et al., 2002; Rabiner et al., 2002; Suhara et al., 1993).

Age related decline of NK1R and many other neurotransmitter receptors might be related to common age-related neuronal degeneration and cortical atrophy. However, gray matter decline varies regionally in the brain (Allen et al., 2005) with loss in the temporal lobes and hippocampus being observed predominantly only after 70 or 60 years of age. The decline reported here is between 19 and 55 years of age, so cortical tissue atrophy is not likely to explain the association we have seen between age and NK1R-BP_{ND}. Additionally, the heterogeneity of neuroreceptor loss is also counter to a common degenerative factor underlying our findings. Decline of NK1R-BP_{ND} with age could be related to age associated decline in other neurotransmitters systems although reason for it is not known.

6.2.2 Gender and NK1 receptors

Previous human studies report no gender differences in SP cerebrospinal fluid concentrations or in NK1R binding in post-mortem brain studies (Almay et al., 1988; Burnet and Harrison, 2000; Carletti et al., 2005). However, more recent experimental studies suggest that substance P is involved in reproductive functions as well as hormonal regulation. Our data on NK1R availability shows that on average women have generally lower brain NK1R-BP_{ND} compared to men. The gender effect was statistically significant in several regions and most marked in the putamen, where females had 19% lower NK1R-BP_{ND} when compared to males. Previous clinical and preclinical studies have found gender differences in the dopaminergic and serotonergic systems that may underlie or be

related to the observed gender differences in NK1R-BP_{ND} (Laakso et al., 2002; Parsey et al., 2002; Pohjalainen et al., 1998) since these neurotransmitter systems are interconnected in many brain regions as previously discussed.

Female hormonal status might also affect NK1R binding (Kukuvitis et al., 2003; Rance and Young, 1991; Rugarn et al., 2001). All females in our study were premenopausal but measurements of their exact hormonal status at the time of PET scanning was not done. Substance P and NK1R have a critical role in the control of reproductive functions in hypothalamus, pituitary and gonads (Lasaga and Debeljuk, 2011). SP and NK1R containing neurons are abundant in arcuate nucleus and there they seem to have a central role in control of gonadotropin-releasing hormone release along with other tachykinins (Navarro et al., 2015). NK1R agonist stimulate gonadotropin release and this effect is greater in females than in males (Navarro et al., 2015). SP release in arcuate nucleus and hypothalamus is inhibited by increased circulating estradiol (Navarro et al., 2015). In normally cycling women, increased plasma SP levels are associated with luteinizing hormone surge before ovulation and are associated also with increased levels of progesterone and it has been suggested that SP is involved in the ovulation process (Kerdelhué et al., 2006). SP seems to play an important role in the onset of puberty in female (Simavli et al., 2015) and male mouse (Maguire et al., 2017b). Early age related changes in the oestrous cycle has been associated with decreased hypothalamic levels of SP in senescence-accelerated mice (Yuan et al., 2005) but increased SP levels have been reported in postmenopausal women (Rance and Young, 1991). NK1Rs are even present in human spermatozoa where SP increases and SPAs decrease the motility of these cells (Pinto et al., 2010) and it has been suggested that altered SP concentrations in female genital tract might act as signal/map molecules for spermatozoa. These studies show a important role for tachykinins in the central feedback loop of reproduction and in gonadal level. Our observation of decreased NK1R-BP_{ND} in female subjects might be related to altered levels sex hormones during aging and menstrual cycle. These results are also important to note as they rise a possibility of sex hormone related and reproduction side effects when using SPAs.

Engman et al (2012) also studied gender differences in their study and only found a difference in right thalamus, where women had lower NK1R-BP_{ND} than males. Reason for this discrepancy is unclear. Our study population had more men (35) than Engman et al had (9 males and 9 females). The age ranges for males and females were similar but a difference in the mean age was noted in our sample. Thus, although age-adjusting did not change our results, group matching and statistical power may explain the differences between our data and the Engman et al data.

6.3 NK1Rs in patients with medication-naïve MDD compared to matched healthy volunteers

In study III, we found no overall differences in NK1R binding between medication-naïve patients with major depression and healthy controls, although there was a trend towards increased NKIR-BP_{ND} in both hippocampi in patients with MDD. However, in patients, NK1R-BP_{ND} in many cortical areas and areas including parts of limbic system was associated with symptom severity as measured with HAM-D17 clinical rating scores.

As discussed in chapter 2.4.3, in human autoradiographic studies, decreased binding to NK1R in patients with MDD has been found in orbitofrontal cortex and in superficial layer of anterior cingulate cortex in (Burnet and Harrison, 2000; Stockmeier et al., 2002). We did not observe significant differences between groups in these regions. Applied methods, illness duration and chronic antidepressant medication potentially contribute to differences between these autoradiographic and our results.

Previous studies in patients with MDD have reported decreased blood flow and glucose metabolism in neocortical areas and increased blood flow and glucose metabolism in limbic and paralimbic areas (Drevets et al., 2002; Goldapple et al., 2004; Kennedy et al., 2001; Mayberg et al., 1999). In this study, no differences in mean NK1R-BP_{ND} was found in these regions suggested to be related to depression neurocircuitry.

Effective treatments of major depression alter the function of the serotonergic and noradrenergic systems as discussed in chapter 2.4. When SPAs were first introduced as treatment of major depression, their functions were reported to be independent from these traditional monoamine systems (Kramer et al., 1998), but as discussed in chapters 2.2.2-4, NK1R-SP system is interacting with monoamine system. As previously discussed, disruption of NK1R-SP system modulates 5-HT and noradrenergic neurotransmission in animals. Also, NK1R agonists are associated with anxiogenic activity and SPAs are associated with anxiolytic activity in animals and human as discussed in chapters 2.4.1-3.

SPA monotherapies in patients with MDD have been positive in phase II trials but no positive phase III trial have been reported (Keller et al., 2006; Kramer et al., 2004, 1998; Ratti et al., 2011). Our results suggest that NK1R-SP system is modulating symptom severity in patients with MDD and therefore, adding SPA to conventional treatment might theoretically be beneficial. Combination therapies of SSRI and SPA in animal models of

MDD have been positive(Chenu et al., 2006; Lelas et al., 2013). However, a phase II trial combining SPA with a SSRI drug failed to show greater efficacy than SSRI monotherapy(Ball et al., 2014) and SSRI alone had even more anxiolytic effect than the combination therapy. Molecules combining NK1R antagonism and serotonin reuptake inhibition have been promising (Millan et al., 2010; Wu et al., 2014).

6.3.1 Relationship between NK1R availability and clinical ratings

Despite lack of group differences, NK1R-BP_{ND} correlated with clinical rating scores of the patient group in several brain regions. Variability in HAM-D17 depression symptoms was small (6.9 ±0.8, mead±SD), and other domains, such as anxiety, had more variability (5.6 ±1.4, mead±SD). Anxiety disorders and depression are strongly related (Hasin et al., 2005), and NK1R-SP system is associated also with anxiety related behavior in humans as discussed in chapters 2.4.1-3. Regions that showed a significant correlation between NK1R-BP_{ND} and HAM-D17 scores are involved in major depression (Biver et al., 1994; Goldapple et al., 2004; Kennedy et al., 2001; Mayberg et al., 1999).

NK1R-BP_{ND} correlated with anxiety (but not total HAMD-17 score) in hippocampus and putamen. Hippocampus and amygdala are involved in fear learning, pathophysiology of anxiety and various functions related to fear and anxiety such as social phobia, specific phobia, substance abuse, aversion, and emotional processing in general (Charney, 2003). Patients with SAD and PTSD have shown increased NK1R availability in right amygdala (Frick et al., 2016b, 2015). Treatment of SAD with a SPA is associated with reduced regional cerebral blood flow (rCBF) in amygdala and hippocampus (Furmark et al., 2005) and reduced serotonin production in amygdala and cortical areas (Frick et al., 2016a). In contrast to these results, SPA L759274 was not effective in treatment generalized anxiety disorder (Michelson et al., 2013) and SPA LY686017 lacked efficiency for the treatment of SAD (Tauscher et al., 2010). SPA GR205171 was also not effective in the treatment of PTSD (Mathew et al., 2011) and SPA aprepitant failed show efficiency in comorbid alcohol dependence and PTSD (Kwako et al., 2015).

Patients with panic disorder were found to have decreased NK1R availability in several receptor rich areas analysed (Fujimura et al., 2009). Patients with specific phobia have shown fewer available NK1R in right amygdala after fear provocation (Michelgård et al., 2007). These results may reflect the increased endogenous SP release whereas our results reflect the baseline state-dependent effect of anxiety on NK1R availability.

6.3.2 Hippocampal NK1R in major depression

As discussed in chapter 2.4, current hypothesis of depression have related MDD to changes in neuroplasticity and neurogenesis in prefrontal cortex, amygdala and hippocampus (Castrén, 2013; Duman and Monteggia, 2006). The hippocampus has an essential role in the pathophysiology of major depression (Campbell and Macqueen, 2004). Prolonged depression decreases hippocampal volume in vivo in humans (Bremner et al., 2000; Campbell et al., 2004; MacQueen et al., 2003; Sheline et al., 1999). Duration and number of episodes is associated with the level of hippocampal volume decrease (MacQueen et al., 2003; Sheline et al., 1999). Reduction of hippocampal volume in post-traumatic stress disorder patients is reversed with antidepressant medication (Vermetten et al., 2003). Patients with MMD using antidepressant medication have no reduction in hippocampal size (Sheline et al., 2003).

Hippocampal neurogenesis serves as the neural substrate underlying treatment efficacy (Malberg, 2004; Santarelli et al., 2003). In animal studies, current antidepressants and SPAs increase neurogenesis and prevent stress-induced cell proliferation decrease in the hippocampus (Manji et al., 2001; van der Hart et al., 2002). Hippocampal neurogenesis is requirement for antidepressant effect in mice (Santarelli et al., 2003). Neurogenesis occurs also in human hippocampi (Eriksson et al., 1998),

BDNF upregulation influences cell survival pathways (Duman et al., 1999). Transgenic BDNF signaling altered mice do not benefit from antidepressant treatment in stressful behavioral tests (Saarelainen et al., 2003). Antidepressive medication increases BDNF production in rats (Nibuya et al., 1995; Russo-Neustadt et al., 1999). Rat hippocampal BDNF and NK1R gene expression is decreased by acute and chronic pain and stress (Duric and McCarson, 2006a, 2005) and that decrease can be diminished by pretreatment with antidepressant (Duric and McCarson, 2006b). BDNF induces antidepressant-like effects in rats when infused to the hippocampal region (Shirayama et al., 2002; Siuciak et al., 1997). Increased levels of serotonin and norepinephrine might increase cell proliferation in dentate gyrus, and functional BDNF signaling is required for long term survival of these cells (Sairanen et al., 2005). NK1R knockout mice have increased neurogenesis and increased levels of BDNF in hippocampus (Morcuende et al., 2003). In contrast to wild type mice, neurogenesis was not increased by antidepressive medication in NK1R knockout mice (Morcuende et al., 2003). NK1R knockout mice genetic background influences the behavioral and molecular consequences of stress ((McCutcheon et al., 2008). Genetic background might influence NK1R-SP system and effects of SPAs also in

humans. Our results of increased NK1R availability associated with symptom severity might be related to altered neurogenesis in hippocampus in patients with MDD.

Increased vulnerability to major depression could be related to increased hippocampal NK1R-BP_{ND}. Anxiety score and hippocampal NK1R-BP_{ND} correlation show that increase might be state-dependent phenomenon and not solely related to MDD vulnerability. Studies in individuals at genetic risk for developing major depression might be able to discern vulnerability- and disease-specific effect on hippocampal NK1R availability in major depression.

6.4 Limitations

This study was a PET-study and it has a limited spatial resolution up to 4-5mm. With this resolution it is not possible to visualize small brainstem nuclei that are also important for understanding NK1R-SP function. These nuclei include for example locus coeruleus, ventral tegmental area and dorsal raphe nuclei.

The main outcome of the study (BP_{ND}) represents the product of receptor density (B_{max}), apparent affinity ($1/K_d$) and the free fraction of the non-displaceable tissue compartment (f_{ND}). The associations between NK1R-BP_{ND}, B_{max} and K_d have not been studied, but association between B_{max} and BP_{ND}, but not K_d in unchallenged studies in healthy volunteers using different radiotracer has been reported (Farde et al., 1995; Hietala et al., 1999). SP affinity to NK1R has been seems to be lower than [18F]SPA-RQ (SP K_d = 80 nM) (Scarrone et al., 2003). NK1R-BP_{ND} may thus preferentially reflect receptor density, but competition with endogenous SP and thus an effect on receptor Kd cannot be excluded.

Partial volume effect (PVE) could potentially introduce some error to our results given the relatively small size of the some of the regions. For example, as discussed earlier, reduced hippocampal size has been reported in patients with MDD. This could potentially influence our results in the hippocampus in study III, but PVE should underestimate rather than overestimate hippocampal BP_{ND} values. There were also no differences in the hippocampal ROI volumes between the patients and controls in the study III (left p=0.92, right p=0.96).

Extrapolation of our results from study II beyond the age range we have studied should be done with caution. Studies with other neurotransmitter systems support a general linear pattern of change even after 55-60 years (Meltzer et al., 2001; Rinne et al., 1990;

Wong et al., 1997). Engman et al also reported same kind of linear pattern of NK1R availability decline as we did, but their age range was also from 20 to 50.

Given the imbalance in the groups with respect to gender and the fact that the exact hormonal status of the females at the time of scanning was unknown, our results suggesting gender differences must be considered preliminary. Also, Engman et al did not fully confirm our findings in their study as they found difference only in right thalamus. Age was taken into account in the statistical analysis, but this could still be a factor in the gender differences we reported here.

In the study III, the main limitation was a small sample size, which may limit detection of small differences between groups. Correlation analyses were also based on only nine patients. Variability in the clinical ratings was also relatively small; for example, five patients had a same anxiety subscale score (6) and conclusions from these analyses should be done with caution.

6.5 Future plans

This thesis has shown that [18F]SPA-RQ is a suitable ligand for basic human NK1R research. Suitability for occupancy studies has been shown elsewhere (Wallius et al., 2007).

Main limitation for [18F]SPA-RQ was the slow kinetics and required scan time of 240 minutes. Tracers with faster kinetics would hold an advantage in e.g. pharmacological challenge studies. That would enable to plan studies with shorter scanner time requirements, reduce motion in dynamic images and make the examination easier for participants. On the other hand, occupancy studies using [18F]SPA-RQ has been done with good reliability using only time point 190-240 min after injection shortening the scanner time needed to 50 minutes. However, the long half-life of 18F does not allow two scans in the same subjects in the same day for challenge studies.

Substance P system acts as a first line of defense to acute stressor in animals and humans. Any pathological condition where chronic stress leads to undesired effects, SP and NK1R system might be activated. After the initial interest in affective disorders, this system has been studied in other areas of medicine and [18F]SPA-RQ may have applications for example in the field of oncology. NK1Rs are present in many CNS malignancies including gliomas, but no PET studies on NK1R in human brain gliomas have

been reported to date. This would be very interesting new field on NK1R studies and might even give us new tools for glioma imaging and therapy (Krolicki et al., 2018). Also, studies on reproduction, obesity, diabetes and inflammation related conditions in respiratory system and gastrointestinal tract have been reported. SP and NK1R seem to be involved in the control of body energy homeostasis and it might also be related to development of insulin resistance. PET study including obese patients would give us more information on this interesting new field. Effects of fasting and diabetes in brain NK1R binding could reveal new dynamics of SP and insulin in human body. A possibility to use NK1R imaging methods, such as [18F]SPA-RQ in vivo will most likely facilitate these research lines.

NK1R gender difference needs more studies. In our sample we found widespread differences in number of brain areas, the functional correlates of these differences remain to be elucidated but could e.g. relate to vulnerability to anxiety and mood disorders. These differences might also be related to sex hormone differences between genders and aging. Differences associated to menstrual cycle are possible.

Patients with MDD might have alterations in NK1R binding in smaller midbrain and brain stem nuclei which would require to use high resolution PET scanner. Enhanced imaging methods of the NK1 system may well shed light on the molecular neurobiology of aberrant neural circuits in affective disorder regardless of the usefulness of NK1R antagonists in treatment of major depression.

7. CONCLUSIONS

Based on the results from our studies reported here, we can conclude following:

1. [18F]SPA-RQ is a useful tracer for imaging NK1Rs in human brain with PET. Cerebellum is a valid reference tissue area and SRTM can be used to model TAC data.
2. There is a clear age-related decline in NK1R availability and widespread NK1R availability gender difference, females having lower NK1R availability than males. These factors should be considered when planning a novel NK1R study.
3. We found no significant differences in NK1R-BP_{ND} in patients with MDD compared to healthy controls. MDD symptom severity and anxiety were associated with NK1R-BP_{ND} in some brain regions. NK1R-SP system might be modulating symptom severity and profile in patients with MDD, but the modulatory effect of NK1R on anxiety and mood is probably too weak alone to induce significant antidepressant and anxiolytic effects in real-life clinical setting.

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