

## Research paper

Brain neurokinin-1 receptor availability in never-medicated patients with major depression – A pilot study<sup>☆</sup>

Mikko Nyman<sup>a,b</sup>, Olli Eskola<sup>c</sup>, Jaana Kajander<sup>a</sup>, Riitta Jokinen<sup>f</sup>, Jukka Penttinen<sup>f</sup>, Tomi Karjalainen<sup>a</sup>, Lauri Nummenmaa<sup>a</sup>, Jussi Hirvonen<sup>a,b</sup>, Donald Burns<sup>d</sup>, Richard Hargreaves<sup>d</sup>, Olof Solin<sup>c,e</sup>, Jarmo Hietala<sup>a,f,\*</sup>

<sup>a</sup> Turku PET Centre, Neuropsychiatric Imaging, Turku, Finland

<sup>b</sup> Department of Radiology, University of Turku and Turku University Hospital, Turku, Finland

<sup>c</sup> Turku PET Centre, Radiopharmaceutical Chemistry Laboratory, Turku, Finland

<sup>d</sup> Imaging Research, Merck Research Laboratories, West Point, PA, USA

<sup>e</sup> Department of Chemistry, University of Turku, Turku, Finland

<sup>f</sup> Department of Psychiatry, University of Turku and Turku University Hospital, Turku, Finland

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## ABSTRACT

**Background:** Neurotransmitter substance P (SP) and its preferred neurokinin-1 receptor (NK1R) have been implicated in the treatment of affective and addiction disorders. Despite promising preclinical data on antidepressant action, the clinical trials of NK1R antagonists in major depression have been disappointing. There are no direct *in vivo* imaging studies on NK1R characteristics in patients with a major depressive disorder (MDD). **Methods:** In this cross-sectional case-control study, we recruited nine never-medicated patients with moderate to severe MDD and nine matched healthy controls. NK1R availability (NK1R binding potential, BP<sub>ND</sub>) was measured with *in vivo* 3-D positron emission tomography and a specific NK1 receptor tracer [<sup>18</sup>F]SPA-RQ. Clinical symptoms were assessed with the 17-item Hamilton Rating Scale for Depression (HAM-D17). **Results:** NK1R-BP<sub>ND</sub> did not differ statistically significantly between patients with MDD and healthy controls. HAM-D17 total scores (range 21–32) correlated positively with NK1R-BP<sub>ND</sub> in cortical and limbic areas. HAM-D17 subscale score for anxiety symptoms correlated positively with NK1R-BP<sub>ND</sub> in specific brain areas implicated in fear and anxiety. **Limitations:** Small sample size. Low variability in the clinical HAM-D subscale ratings may affect the observed correlations. **Conclusions:** Our preliminary results do not support a different baseline expression of NK1Rs in a representative sample of never-medicated patients with MDD during a current moderate/severe depressive episode. The modulatory effect of NK1Rs on affective symptoms is in line with early positive results on antidepressant action of NK1 antagonists. However, the effect is likely to be too weak for treatment of MDD with NK1R antagonists alone in clinical practice.

## 1. Introduction

Neurotransmitter substance P (SP) acts as general neuromodulator that maintains brain homeostasis and regulates stress responses, emesis, pain, mood, and anxiety. The majority of these actions are transmitted via the neurokinin-1 receptor (NK1R) subtype that has also emerged as a target for treatment of affective disorders (Chandra et al., 2010; Gobbi and Blier, 2005; Hesketh et al., 2003; Kramer et al., 1998; McCabe

et al., 2009; Rupniak et al., 2000; Santarelli et al., 2001; Teixeira et al., 2004). Intravenous infusion of SP in healthy males lowers mood, induces anxiety, causes sleep disturbances, and worsens short-term memory (Herpfer et al., 2007; Lieb et al., 2002). Conversely, substance P antagonists (SPAs) increase neural processing of positive emotional information in healthy volunteers (Chandra et al., 2010; McCabe et al., 2009).

NK1R-SP system is also implicated in major depressive disorder

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\* Corresponding author.

E-mail address: [jahi@utu.fi](mailto:jahi@utu.fi) (J. Hietala).

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(MDD) and in affective disorders in general (Burnet and Harrison, 2000; Carletti et al., 2005; Stockmeier et al., 2002). In MDD, neuronal activity is altered in cortico-thalamic-limbic areas that also are abundant with SP and NK1Rs (Engman et al., 2012; Hietala et al., 2005; Nyman et al., 2007; Okumura et al., 2008). Patients with MDD have elevated cerebrospinal fluid (CSF) SP concentrations, and patients developing MDD after stroke have elevated substance P concentrations in plasma and CSF (Geraciotti et al., 2006; Li et al., 2009). Although treatment response to SPAs in patients with MDD was positive in phase II trials, no positive phase III trials have been reported (Keller et al., 2006; Kramer et al., 2004, 1998; Ratti et al., 2011). MDD is known to be highly comorbid with anxiety disorders, and early reports suggested that SPA L-597,274 also triggers anxiolytic activity in patients with MDD (Kramer et al., 2004). In addition, social anxiety disorder (SAD) was reported to respond to SPA (Furmark et al., 2005). These findings were indirectly supported by imaging studies suggesting changes in NK1R availability in SAD as well as in post-traumatic stress disorder (PTSD; Frick et al., 2015, 2016a, 2016b). However, later larger clinical trials did not support efficacy of SPA in anxiety disorders with direct studies in generalized anxiety disorder (Michelson et al., 2013).

Clinical trials do not support the NK1R hypothesis of antidepressant drug action. Yet, the NK1R hypothesis of major depression assumes increased NK1R function as a part of aberrant neurocircuitry in patients with MDD. Here, we tested this hypothesis by studying whether NK1R availability is changed *in vivo* in a representative sample of never-medicated patients with MDD. Nine patients with MDD and nine age and sex-matched healthy controls were studied using 3-D positron emission tomography (PET) and a specific NK1R antagonist [<sup>18</sup>F]SPA-RQ as a radiotracer. Based on previous data, we hypothesized that patients with MDD would have increased NK1R availability in brain circuits regulating mood and anxiety, including regions such as amygdala, prefrontal cortex, and hippocampus (Etkin et al., 2013).

## 2. Methods

The study was conducted in accordance with the Declaration of Helsinki. The ethical board of the Hospital District of Southwest Finland reviewed and approved the study protocol. All participants gave written informed consent prior to inclusion in the study.

### 2.1. Sample ascertainment

We recruited nine patients with MDD and nine healthy age and sex-

matched controls (Table 1). Inclusion criteria for patients with MDD 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. Structured clinical interview (SCID-I) was carried out by an experienced clinician to exclude other axis I disorders. Both patients and healthy controls were screened by a physician, and they were tested with laboratory tests of blood and urine. Substance abuse was excluded by blood and urine tests. To exclude abnormalities in the brain and to provide anatomical reference, T1-weighted MR images with 1 mm<sup>3</sup> isotropic voxels were acquired with 1.5 T Siemens Magnetom (Iselin, NJ) device.

### 2.2. PET data acquisition

The radiosynthesis of [<sup>18</sup>F]SPA-RQ has been previously described in detail (Solin et al., 2004). PET imaging was performed using the GE Advance whole-body PET scanner (Milwaukee, WI, USA) in 3-D mode as described previously (Nyman et al., 2007). Mean molar activity of [<sup>18</sup>F]SPA-RQ was 1206 ± 892 (mean ± SD) GBq/μmol (range 72–2825 GBq/μmol) at the time of the injection, and the average mass of the injected tracer was 124 ± 178 ng (mean ± SD, range 22–772 ng). Injected radioactivity or the injected mass/weight of the subject ratio (tracer mass/kg) did not differ between the groups ( $t = 0.141$ ,  $p = 0.890$ ; and  $t = -1.333$ ,  $p = 0.201$ , respectively). PET scanning was started after [<sup>18</sup>F]SPA-RQ injection, and three consecutive dynamic scans were done (0–87 min, 120–160 min, and 190–240 min).

A summed PET image was calculated for every subject using part of the first dynamic scan (3–87 min). All dynamic images were frame-to-frame motion corrected to the integral image (3–87 min) using normalized mutual information coregistration. Magnetic resonance images (MRI) were next coregistered to the integral image. Regions of interests (ROIs) (see Table 2), were drawn individually to the coregistered MR-images, and ROI total time-activity curves (TACs) were calculated from PET-images. There were no statistically significant differences in ROI volumes between the groups, except for left caudate ( $p = 0.048$ ) and right superior temporal gyrus ( $p = 0.023$ ), which were larger in patients versus controls. ROI volumes and NK1R-BP<sub>ND</sub> were not correlated in any region ( $p > 0.05$ ). To control for unequal sampling over scan time, TACs were weighted according to frame duration and true counts in each frame (Gunn et al., 1998) using in-house software. TACs were analyzed using a linear simplified reference tissue model (SRTM; Lammertsma and Hume, 1996) and solved using the basis function

**Table 1**  
Demographics of the studied sample.

Patients	Age	Gender	Weight	Height	BMI	Number of depressive episodes	Length of current depressive episode (months)	HAM-D17 total score	Depression (1 + 2 + 3)	Anxiety (9 + 10 + 11)
1	36	Female	79	163	30	3	0.5	23	7	6
2	28	Female	52	159	31	3	3	27	7	6
3	28	Male	73	178	25	5	3	31	8	6
4	42	Female	77	170	27	0	0.5	24	7	6
5	46	Male	76	176	26	40	6	21	8	3
6	28	Female	68	165	29	0	3	28	7	5
7	42	Female	59	160	31	–	–	21	6	4
8	48	Male	76	176	26	0	18	32	6	8
9	39	Female	71	164	29	2	3	22	6	6
Controls										
1	28	Male	60	170	27					
2	25	Female	63	167	28					
3	35	Female	63	169	28					
4	40	Female	64	161	30					
5	47	Male	86	191	22					
6	42	Female	64	158	32					
7	44	Male	67	174	26					
8	39	Female	55	159	31					
9	22	Female	70	170	27					

**Table 2**  
Correlations between regional NK1R-BP<sub>ND</sub> and clinical total HAM-D17 score and HAM-D17 anxiety subscale. Correlation coefficient and p-values are reported.

Cortical areas	HAM-D17 total score Spearman correlation	p	HAM-D17 anxiety score Spearman correlation	p
Dorsolateral prefrontal cortex sn	0.326	0.256	0.391	0.507
Dorsolateral prefrontal cortex dx	0.527	0.584	0.145	0.099
Medial frontal cortex sn	0.561	0.602	0.116	0.086
Medial frontal cortex dx	0.502	0.621	0.168	0.074
Orbitofrontal cortex sn	0.536	0.310	0.137	0.416
Orbitofrontal cortex dx	0.594	0.548	0.092	0.127
Superior temporal gyrus sn	0.728	0.329	0.026	0.388
Superior temporal gyrus dx	0.569	0.292	0.110	0.446
<b>Basal ganglia and thalamus</b>				
Caudate nucleus sn	0.577	0.164	0.104	0.673
Caudate nucleus dx	0.594	0.329	0.092	0.388
Putamen sn	0.402	0.749	0.284	0.020
Putamen dx	0.301	0.749	0.431	0.020
Thalamus sn	0.159	0.073	0.683	0.852
Thalamus dx	-0.050	-0.146	0.898	0.708
<b>Limbic system</b>				
Amygdala sn	0.594	0.548	0.092	0.127
Amygdala dx	0.310	0.420	0.417	0.260
Hippocampus sn	0.335	0.694	0.379	0.038
Hippocampus dx	0.427	0.767	0.252	0.016
Parahippocampal gyrus sn	0.887	0.676	0.001	0.046
Parahippocampal gyrus dx	0.444	0.183	0.232	0.638
Anterior Cingulate gyrus sn	0.510	0.621	0.160	0.074
Anterior Cingulate gyrus dx	0.435	0.529	0.242	0.143
Subgenual anterior cingulate sn	0.310	0.347	0.417	0.360
Subgenual anterior cingulate dx	0.828	0.529	0.006	0.143

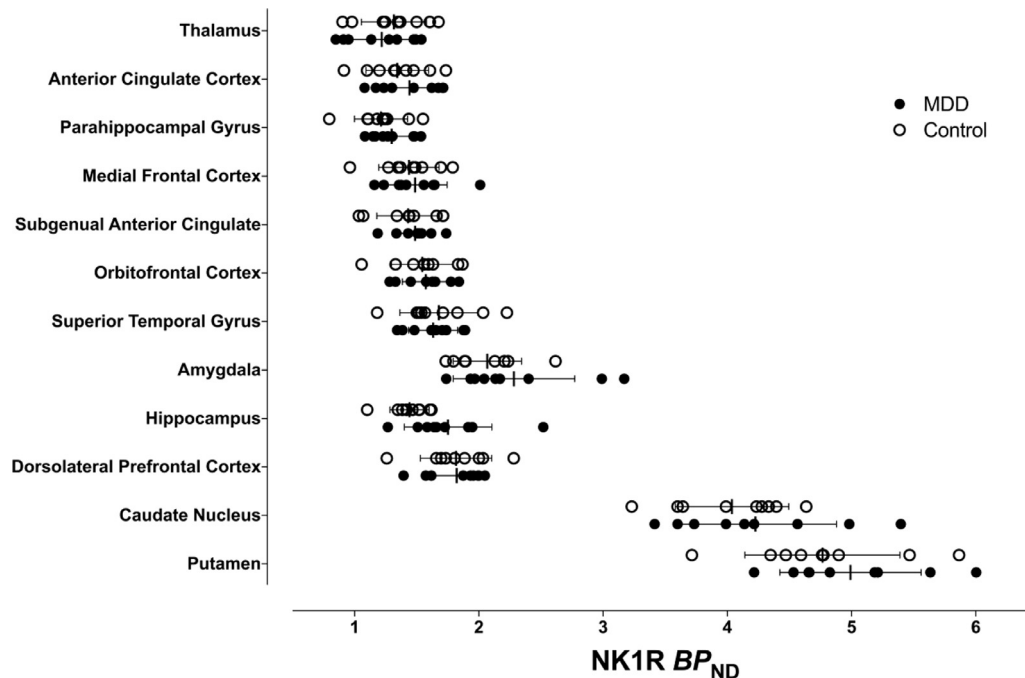
approach (Gunn et al., 1997) using cerebellum as a reference tissue (Hietala et al., 2005). This method provides the parameters  $R_1$ ,  $k_2$ , and  $BP_{ND}$  (binding potential), where  $BP_{ND} = k_3/k_4 = f_{ND} * B_{max}/K_D$  ( $B_{max}$  = maximum number of receptor binding sites,  $K_D$  = equilibrium dissociation constant,  $f_{ND}$  = free fraction of the nondisplaceable tissue compartment). Our main parameter of interest,  $BP_{ND}$ , can also be conceptualized as the ratio of specific to nondisplaceable radiotracer binding, and it is proportional to receptor density (Innis et al., 2007).

Parametric  $BP_{ND}$  images were calculated for confirmatory statistical parametric mapping (SPM) analyses. Voxel size in these parametric images was 2 mm<sup>3</sup>. To provide reference tissue TACs, cerebellar ROIs were defined separately for each subject using FreeSurfer software (<http://surfer.nmr.mgh.harvard.edu/>). Subject-specific regional TACs were next calculated for each subject. An SRTM was then used to model tracer kinetics (Lammertsma and Hume, 1996). Voxel-level fitting was done using the basis-functions implementation of SRTM (Gunn et al., 1997). Subsequently, parametric images were normalized to the MNI space using SPM12 and smoothed with an 8 mm FWHM kernel.

**2.3. Statistical analyses**

The ROI level statistical analyses were performed using SPSS version 23. Due the small sample size, between-group differences in regional radiotracer binding were analyzed using non-parametric test and distributions across groups was compared using Mann-Whitney U test. Results were corrected for multiple comparisons using Bonferroni correction. Spearman correlations were computed between NK1R-BP<sub>ND</sub> and total HAM-D17 score as well as HAM-D17 anxiety subscale (including factors for agitation, psychic anxiety, and somatic anxiety). Correlations between NK1R-BP<sub>ND</sub> and core depression symptoms were not analyzed because of the lack of variability in the depression scores in this sample that was due to the inclusion criteria (see Table 1).

Confirmatory SPM analyses were performed using SPM12. Between-group differences were analyzed using individual sample T-tests. Correlation analyses were calculated using a general linear model. Statistical threshold was set at  $p < 0.05$ , FWE corrected at cluster level.



**Fig. 1.** All data points, means and standard deviations of NK1R-BP<sub>ND</sub> in patient and control groups.

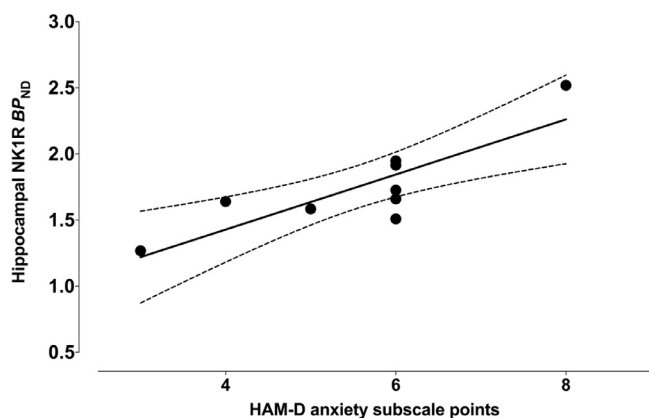


Fig. 2. A scatter plot of patients hippocampal NK1R-BP<sub>ND</sub> and HAM-D17 anxiety subscale scores.

### 3. Results

#### 3.1. Group comparison of NK1R-BP<sub>ND</sub>

Full data of the nine patients and controls analyzed can be seen in Table 1 and Fig. 1. We found no differences in NK1R-BP<sub>ND</sub> between patients with MDD and healthy control subjects using a non-parametric Mann–Whitney *U* test. Patients had a trend towards increased hippocampal NK1R-BP<sub>ND</sub>, but this did not survive correction for multiple comparisons. Lack of significant differences in ROI-based analyses was consistent with the SPM analysis, which revealed no significant differences (higher in MDD: peak voxel at  $\{-8\ 4\ 4\}$ ,  $t_{\max}$  4.04, cluster  $p_{\text{FWE}} = 1.00$ ; lower in MDD: peak voxel at  $\{-7\ -76\ 16\}$ ,  $t_{\max}$  4.05, cluster  $p_{\text{FWE}} = 0.494$ ).

#### 3.2. Correlations between NK1R-BP and clinical variables

In individual ROI based analysis, the anxiety subscale correlated positively with NK1R-BP<sub>ND</sub> in putamen, hippocampus and parahippocampal gyrus (see Fig. 2). Total HAM-D17 scores did not correlate with any brain regions studied (Table 2). HAM-D17 total scores correlated with anxiety subscale scores (Spearman correlation = 0.688,  $p = 0.041$ ).

In SPM analyses, the anxiety subscale correlated positively with NK1R-BP<sub>ND</sub> in frontal cortical areas bilaterally (Fig. 3). The effects in hippocampus and parahippocampal gyri were replicated using the SPM analyses, although they did not survive multiple comparison corrections. Total HAM-D17 scores correlated positively with NK1R-BP<sub>ND</sub> in right temporal, occipital, and frontal regions.

### 4. Discussion

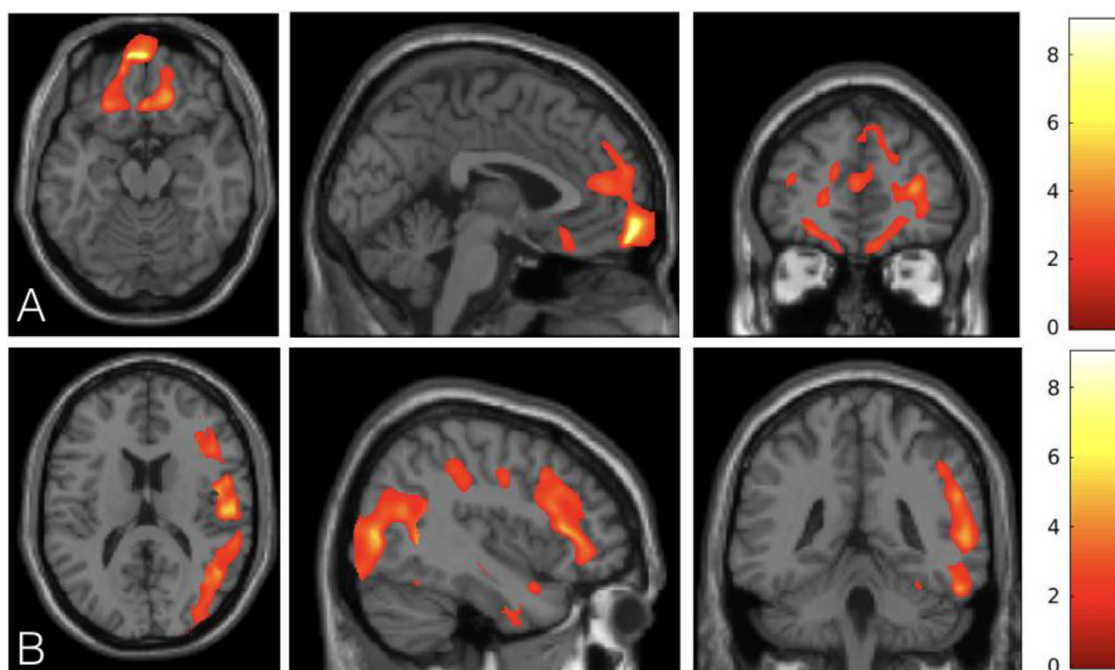
Our main finding was that there were no overall differences in *in vivo* NK1R availability between medication-naïve patients with MDD and healthy controls as quantified with PET and an NK1R antagonist tracer. The main outcome of the study (BP<sub>ND</sub>) represents the product of receptor density ( $B_{\max}$ ), apparent affinity ( $1/K_D$ ), and the free fraction of the non-displaceable tissue compartment ( $f_{\text{ND}}$ ). The associations between NK1R-BP<sub>ND</sub>,  $B_{\max}$  and  $K_D$  have not been studied. Association between  $B_{\max}$  and BP<sub>ND</sub> but not  $K_D$  has been reported in unchallenged studies with healthy volunteers using a different radiotracer (Farde et al., 1995; Hietala et al., 1999). Therefore, we assume that NK1R-BP<sub>ND</sub> preferentially reflect NK1 receptor density, but interference with endogenous SP in receptor availability measures cannot be fully excluded. Accordingly, our results do not support the hypothesis of increased NK1 receptor density in MDD. This is in line with clinical trial data on SPA monotherapies in patients with MDD that were negative

despite early promising results (Keller et al., 2006; Kramer et al., 2004, 1998; Ratti et al., 2011).

The patient sample was representative and carefully screened. All patients had a current depressive episode with moderate to severe symptomatology as indicated by HAM-D scores of up to 32 and no history of medication. 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<sub>ND</sub> was found in these regions known to be related to depression neuro-circuitry despite a non-significant trend for increased NK1R-BP<sub>ND</sub> in both hippocampi in MDD. The hippocampus has a critical role in the pathophysiology of major depression (Campbell and Macqueen, 2004), and hippocampal neurogenesis serves as the neural substrate underlying treatment efficacy (Malberg, 2004; Santarelli et al., 2003). Brain-derived neurotrophic factor (BDNF) upregulation influences cell survival pathways (Duman et al., 1999). NK1R knockout mice have increased neurogenesis and increased levels of BDNF in hippocampus (Morcuende et al., 2003). In NK1R knock-out mice, the behavioral and molecular consequences of stress is also altered (McCutcheon et al., 2008). In line with this, we found preliminary evidence for a state-dependent NK1 receptor availability in MDD.

NK1R-BP<sub>ND</sub> in the limbic system and frontal cortex associated with symptom severity as measured with HAM-D17 scores is in line with a neuromodulatory action of substance P. Regions showing a significant association between BP<sub>ND</sub> and HAM-D17 scores are also involved in major depression (Biver et al., 1994; Goldapple et al., 2004; Kennedy et al., 2001; Mayberg et al., 1999). NK1R-BP<sub>ND</sub> correlated with anxiety (but not total HAM-D-17 scores) in hippocampus, parahippocampal gyrus 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). In animals, NK1R agonists are associated with anxiogenic activity, and SPAs are associated with anxiolytic activity (Dableh et al., 2005; Ebner et al., 2004; Heldt et al., 2009; Kramer et al., 1998; Santarelli et al., 2001; Teixeira et al., 1996; Wallace-Boone et al., 2008). Anxiety disorders and depression are strongly comorbid (Hasin et al., 2005), and NK1R-SP systems are also associated with anxiety-related behavior in humans. Patients with SAD and PTSD have increased NK1R availability in the right amygdala (Frick et al., 2015, 2016b). Treatment of SAD with an SPA is associated with reduced regional cerebral blood flow (rCBF) in the amygdala and hippocampus (Furmark et al., 2005) and reduced serotonin production in amygdala and cortical areas (Frick et al., 2016a). Patients with panic disorder were found to have altered NK1R availability in all receptor-rich areas analyzed (Fujimura et al., 2009). Patients with specific phobias have shown fewer available NK1R in the right amygdala after fear provocation (Michelgård et al., 2007). However, clinical trials do not support efficacy of NK1 antagonists in anxiety disorders. SPA L759,274 was not effective in treatment of generalized anxiety disorder (Michelson et al., 2013), and SPA LY686017 lacked efficiency for the treatment of SAD (Tauscher et al., 2010). In addition, SPA GR205171 was not effective in the treatment of PTSD (Mathew et al., 2011) and SPA aprepitant failed to show efficiency in comorbid alcohol dependence and PTSD (Kwako et al., 2015).

Taken together, 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 settings. This conclusion was recently contradicted by Rupniak and Kramer (2017) who suggesting that lack of understanding of details in NK1R occupancy-clinical effect relationships lead to premature abandoning of NK1R-class antidepressant development. This concern, however, seems to be unjustified. According to the law of mass action, receptor occupancy is not related to baseline receptor density. This study additionally shows that there is no



**Fig. 3.** (A) Frontal cluster where anxiety subscale score correlated with NK1R-BP<sub>ND</sub> (cluster size 65,088 voxels, peak voxel at {36 18 15},  $t_{\max}$  9.02, cluster  $p_{\text{FWE}} = 0.020$ ). (B) Right cortical cluster where HAM-D17 score correlated with NK1R-BP<sub>ND</sub> (cluster size 72,929 voxels, peak voxel at {52 -21 20},  $t_{\max}$  9.02,  $p_{\text{FWE}} = 0.003$ ).

marked difference in baseline NK1R density in MDD. As the NK1R occupancy-dose (and concentration) relationship was well documented for clinically-developed SPAs, it is clear that adequate NK1R occupancies were obtained in clinical trials for testing the NK1R hypothesis of antidepressant drug action.

Current effective treatments of major depression alter the function of the serotonergic and noradrenergic systems. 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). Serotonergic projections originate mainly from the nucleus of dorsal raphe (DRN). NK1R are present in DRN (Léger et al., 2002), and disruption of the NK1R-SP system modulates 5-HT neurotransmission in mouse DR (Commons and Valentino, 2002; Lacoste et al., 2006; Liu et al., 2002; Santarelli et al., 2001; Valentino et al., 2003). Ascending noradrenergic neurons in locus coeruleus are innervated by SP and NK1R-containing neurons (Caberloto 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). As our results suggest that the NK1R-SP system is modulating symptom severity in patients with MDD, it can be speculated that adding SPA to conventional antidepressant treatment might be beneficial. Combination therapies of SSRI and SPA in animal models of MDD have been positive (Chenu et al., 2006; Lelas et al., 2013). In addition, molecules combining NK1R antagonism and serotonin reuptake inhibition have been promising in this context (Millan et al., 2010; Wu et al., 2014). However, a phase II trial combining SPA with SSRI failed to show superior efficacy over SSRI monotherapy. In fact, SSRI alone had even greater anxiolytic effect than the SPA-SSRI combination therapy (Ball et al., 2014).

## 5. Limitations

The main limitation of this study is small sample size, which limits detection of small differences between groups. However, all the subjects were currently suffering from an MDD episode and were never

medicated, thus the sample was clinically representative of typical MDD. Variability in the clinical subscale ratings was also relatively small, and conclusions from these analyses should be made with caution. Healthy controls were not screened for subclinical depression symptoms, and this might also interfere with our results.

Larger sample studies using higher resolution PET cameras might clarify the role of NK1R in MDD. Partial volume effect (PVE) could potentially introduce some error to our results given the relatively small size of some of the regions, particularly because reduced hippocampal size has been reported in patients with major depression (Bremner et al., 2000; Campbell et al., 2004). There were no differences in the hippocampal ROI volumes between the patients and controls in the present study (left  $p = 0.92$ , right  $p = 0.96$ ). PVE could potentially influence our results in the hippocampus, but it should underestimate rather than overestimate hippocampal BP<sub>ND</sub> values thus making this concern unwarranted.

## 6. Conclusion

In our preliminary study, we found no significant differences in NK1R availability in patients with MDD compared to healthy controls. However, MDD symptom severity and anxiety were associated with NK1R-BP<sub>ND</sub> in several brain regions. NK1R-SP system may be modulating the affective symptomatology in MDD patients but randomized clinical trials in patients with MDD suggest that this effect is not sufficient to produce an antidepressant effect *per se*.

## Author disclosure

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

## Role of the funding source

This study was funded by Merck Research Laboratories, USA as a part of the NK1 receptor project. Turku University CRO; Clinical

Research Services Turku (CRST) subcontracted and coordinated the whole study including clinical as well as imaging studies. PET and MRI studies were done in Turku PET Centre (Hietala, Nyman, Kajander, Eskola, Solin, Nummenmaa, Karjalainen, Hirvonen). Study subjects were recruited and assessed by private sector psychiatrist (Penttinen and Jokinen) from Turku area. All analyses were performed independently in Turku PET Centre and CRST and reported to Merck Research Laboratories. Funding source had no influence on decision to submit this manuscript for publication. Final manuscript was reviewed before submission by the authors from the funding source.

We wish to confirm that there are no other known conflicts of interest associated with this publication and there has been no other significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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Electronic approval has been given to first author by all authors in April 2018.

Mikko Nyman, first author  
27.4.2018, Turku, Finland

#### Conflict of interest statement

Funding from Merck Research Laboratories, USA was used to support the work of Drs Nyman, Kajander, Huttunen and Jokinen.

All other authors declare that they have no conflicts of interest.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jad.2018.08.084](https://doi.org/10.1016/j.jad.2018.08.084).

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