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Evidence of fatigue, disordered sleep and peripheral inflammation, but not increased brain TSPO expression, in seasonal allergy: A [^{11}C]PBR28 PET study

Sandra Tamm^{a,b,*}, Simon Cervenka^c, Anton Forsberg^c, Johanna Estelius^d, Johan Grunewald^d, Pär Gyllfors^e, Bianka Karshikoff^b, Eva Kosek^{a,f}, Jon Lampa^d, Catarina Lensmar^a, Victoria Strand^e, Torbjörn Åkerstedt^{a,b}, Christer Halldin^c, Martin Ingvar^a, Caroline Olgart Höglund^{a,d,g,1}, Mats Lekander^{a,b,1}

^a Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden^b Stress Research Institute, Stockholm University, Stockholm, Sweden^c Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet & Stockholm Health Care Services, Stockholm County Council, Stockholm, Sweden^d Department of Medicine and Center for Molecular Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden^e Asthma & Allergy Clinic, S:t Görans Hospital, Stockholm, Sweden^f Stockholm Spine Center, Stockholm, Sweden^g Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

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ABSTRACT

Allergy is associated with non-specific symptoms such as fatigue, sleep problems and impaired cognition. One explanation could be that the allergic inflammatory state includes activation of immune cells in the brain, but this hypothesis has not been tested in humans. The aim of the present study was therefore to investigate seasonal changes in the glial cell marker translocator protein (TSPO), and to relate this to peripheral inflammation, fatigue and sleep, in allergy. We examined 18 patients with severe seasonal allergy, and 13 healthy subjects in and out-of pollen season using positron emission tomography ($n = 15/13$) and the TSPO radioligand [^{11}C]PBR28. In addition, TNF- α , IL-5, IL-6, IL-8 and IFN- γ were measured in peripheral blood, and subjective ratings of fatigue and sleepiness as well as objective and subjective sleep were investigated. No difference in levels of TSPO was seen between patients and healthy subjects, nor in relation to pollen season. However, allergic subjects displayed both increased fatigue, sleepiness and increased percentage of deep sleep, as well as increased levels of IL-5 and TNF- α during pollen season, compared to healthy subjects. Allergic subjects also had shorter total sleep time, regardless of season. In conclusion, allergic subjects are indicated to respond to allergen exposure during pollen season with a clear pattern of behavioral disruption and peripheral inflammatory activation, but not with changes in brain TSPO levels. This underscores a need for development and use of more specific markers to understand brain consequences of peripheral inflammation that will be applicable in human subjects.

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1. Introduction

Seasonal allergy is a highly prevalent condition, affecting 10–40% of the general population (Bernstein et al., 2016). Beyond disease-specific symptoms (e.g. rhinoconjunctivitis), patients commonly suffer from fatigue (Benninger and Benninger, 2009; Marshall et al., 2002), increased risk of depression (Hurwitz and Morgenstern, 1999; Sanna et al., 2014), sleep problems (Jernelöv

et al., 2009; Mullol et al., 2008; Santos et al., 2006) and impaired cognition (Marshall et al., 2000; Marshall and Colon, 1993; Trikojat et al., 2014). Such problems exacerbate during pollen season (Marshall et al., 2002, 2000; Trikojat et al., 2017), and both disturbed sleep and fatigue are major contributors to the reported lower quality of life seen in patients with allergy compared to the general population (Camelo-Nunes and Solé, 2010; Meltzer et al., 2012; Small et al., 2013). One hypothetical mechanism behind the listed comorbid problems could be inflammatory activity in the brain, activated through immune-to-brain pathways similar to the chain of events that occur after detection of infectious stimuli in the periphery (Dantzer et al., 2008; Hart, 1988; Watkins and Maier, 1999). As shown in experimental models and during

* Corresponding author at: Department of Clinical Neuroscience, Karolinska Institutet, Nobels Väg 9, 171 77 Stockholm, Sweden.

E-mail address: sandra.tamm@ki.se (S. Tamm).

¹ These authors contributed equally to this work.

infections, triggering of the immune system leads to behavioral changes such as weakness, tiredness, hyperalgesia, and changed mood, motivation and appetite (Dantzer, 2001; Dantzer et al., 2008). This set of behavioral adaptations (“sickness behavior”) is caused by pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and TNF- α (Dantzer et al., 2008; Maier, 2003; Watkins et al., 2007). Topographically, symptoms of sickness resemble non-specific symptoms associated with allergy, symptoms which should aggravate after exposure to antigen during pollen season.

When measured in peripheral blood, the pathogenesis of seasonal allergy involves an interplay of type 2 helper (Th2) T-cells and regulatory T-cells and production of immunoglobulin (Ig) E in response to an allergen, mediating effects on mucous tissue (Osguthorpe, 2012). Th2-cells orchestrate the inflammatory process through cytokines such as IL-4, IL-5, IL-12 and IL-13, which together with IL-10 from regulatory T-cells and lowered Th1 activity are typical markers of allergic inflammation (Barnes, 2011; Osguthorpe, 2012). Compared to inflammatory responses to bacterial or viral stimuli, influences on brain and behavior from these specific allergic inflammatory mediators are sparsely investigated. However, pro-inflammatory cytokines such as IL-1, IL-6 and TNF- α that mediate sickness behavior are to some extent also implicated in allergy (Broide et al., 1992; Townley and Horiba, 2003; Trikojati et al., 2017) and could be part of an immune-to-brain signaling mechanism. Thus, both allergy-specific and classic pro-inflammatory mediators might underpin non-specific symptoms connected to allergy.

In support for this notion, animal studies indicate that the peripheral allergic inflammation is reflected also in the brain (Basso et al., 2004; Costa-Pinto et al., 2006; Mirotti et al., 2010; Sarlus et al., 2013, 2012; Tonelli et al., 2009). For example, experimental mice models of chronic ovalbumin-induced airway allergy demonstrated increased IgE and IgG in the brain as well as increased tau phosphorylation (Sarlus et al., 2012) and induction of changes in brain gene expression toward insulin resistance and inflammatory responses (Sarlus et al., 2013). To our knowledge, no study has investigated inflammatory activation in the CNS in human allergy.

Using positron emission tomography (PET), and radioligands targeting the 18 kDa translocator protein (TSPO), CNS inflammation can be assessed *in vivo*. TSPO is a transmembrane protein that is located mainly in the outer mitochondrial membrane in microglia, and has shown increased expression as a result of immune cell activation (Venneti et al., 2013, 2006). The second generation TSPO radioligand [^{11}C]PBR28 has been utilized in clinical and experimental models to measure immune activation in the brain in various disorders, such as stroke (Tóth et al., 2016), virus infection (Dimber et al., 2016; Parente et al., 2016), Alzheimer's disease (Kreisl et al., 2013), and depression (Hannestad et al., 2013). Moreover, injection of lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, caused significant increases in [^{11}C]PBR28 binding across the brain in baboons, verified as immune cell activation by post-mortem analysis (Hannestad et al., 2012). The same pattern was subsequently shown in a small human study (Sandiego et al., 2015), with increased TSPO levels in grey matter 3 h after injection, accompanied by sickness symptoms. If allergic inflammation in the periphery leads to inflammatory changes in the brain, potentially contributing to fatigue, depressed mood and disturbed sleep, it could be expected that allergic subjects show higher binding of [^{11}C]PBR28 compared to healthy subjects, and that this difference would further increase during pollen season.

As noted, fatigue is a major contributor to lower quality of life in patients with allergy (Camelo-Nunes and Solé, 2010), and is in general an important determinant of health care consumption (Watt, 2000), sick-leave (Akerstedt et al., 2007) and poor self-rated health

(Lekander et al., 2013). In the context of allergy, both inflammatory activation and suboptimal sleep might contribute to increased fatigue. Indeed, patients with allergy often present with sleep problems, demonstrated as worse sleep quality (Santos et al., 2006), longer sleep latencies and less restorative sleep (Jernelöv et al., 2009), and microarousals and sleep-disordered breathing (snoring, obstructive sleep apnea) related to nasal congestion (Kim et al., 2017; Storms, 2008). Beyond the direct effects of for example a swollen nose in rhinoconjunctivitis, both fatigue and sleep are interrelated with systemic as well as local inflammatory processes (Hsu et al., 2003; Irwin, 2015; Karshikoff et al., 2017; Wisor et al., 2011; Zhu et al., 2012). Thus, both animal and human data speak for a need to include measure of sleep when studying sequelae of allergic inflammation.

In the present study, TSPO PET, peripheral inflammatory markers and measures of sleep and fatigue were combined to study seasonal changes and differences between subjects with severe seasonal allergy and healthy subjects. The overarching aim was to investigate if seasonal allergy is reflected in immune cell activation in the brain, as a possible mechanism behind fatigue and other non-specific symptoms. Specifically, we hypothesized that allergic subjects would display increased binding of the TSPO ligand [^{11}C]PBR28 in the grey matter across the brain during pollen season compared to outside pollen season and compared to healthy subjects. We also hypothesized that the degree of [^{11}C]PBR28 binding would be related to increased fatigue as well as worse subjective and objective sleep quality in subjects with allergy, specifically during pollen season. Both allergy specific (IL-5) and pro-inflammatory cytokines (TNF- α , IL-6 and IL-8) were hypothesized to be higher in allergic subjects during pollen season, and the Th2-driven inflammation was thought to be reflected in a decreased IFN- γ /IL-5 ratio. We also hypothesized that the degree of peripheral immune activation, i.e. levels of circulating cytokines, would be associated with the extent of [^{11}C]PBR28 grey matter binding in the brain, as indicating immune-to-brain signaling.

2. Method

2.1. Overall design

We investigated 18 (15 with PET) patients with severe allergic rhinoconjunctivitis induced by birch or grass pollen and 13 healthy subjects twice, once in and once out of pollen season (Fig. 1) between 2012 and 2014. Pollen levels were controlled based on data from the Swedish palynological laboratory at the Stockholm Natural History Museum (www.pollenrapporten.se), see supplement for details. Birch pollen peak levels were confirmed to be very high during 2012 and 2014, and moderate during 2013. Grass pollen peak levels were high both 2012, 2013 and 2014. At each test occasion (2 study days), participants underwent PET examinations, filled in questionnaires and provided blood samples. Sleep was recorded during the preceding week. The full protocol is described in supplement. The study was performed in accordance with the Helsinki declaration and was approved by the regional ethical committee of Stockholm (no: 2011/1846-31/1).

2.2. Participants and screening procedure

To access individuals with severe seasonal allergy to pollen, patients were recruited from allergy immunotherapy waiting lists at allergy clinics in Stockholm, and healthy subjects were recruited through advertisements. PET data from a subgroup of the healthy control subjects have been reported previously in a methodological paper, but not addressing the research question investigated in this paper (Kanegawa et al., 2016). All participants were given written

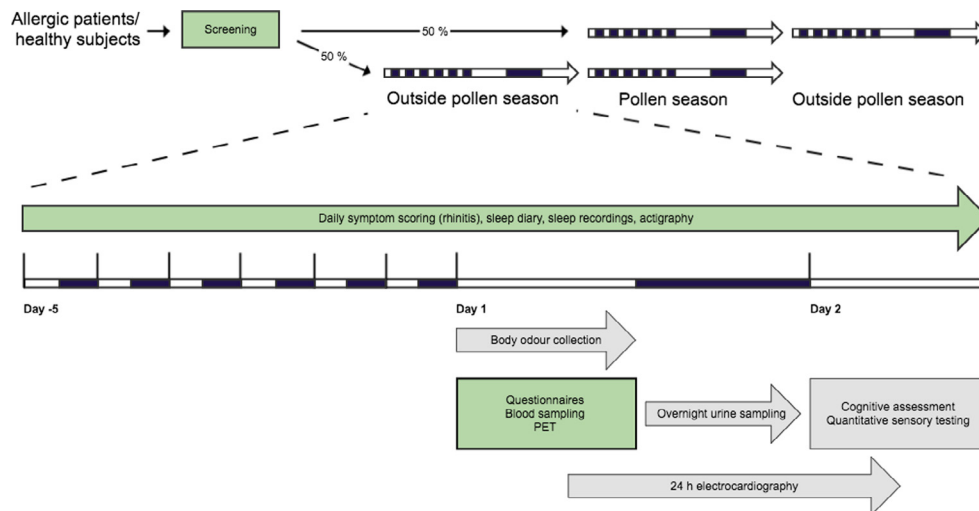


Fig. 1. Overall study design. At each participation (2 study days), participants underwent PET examinations, filled in questionnaires and provided blood samples. Sleep and allergy symptoms were monitored the week preceding study participation. As part of the data collection, several other variables were measured (in grey), see supplement for details. The intention to counterbalance the order and include 50% of subjects during and 50% outside pollen season was partly fulfilled: 23 participants started the experiment during pollen season and 8 started outside pollen season.

and oral information regarding the study design. If willing to participate, inclusion criteria were checked through a telephone interview. The screening process also contained a physical examination (including heart, lung, abdomen, ears, noses, lymph nodes and blood pressure) and a detailed medical history, including nicotine use and specific allergic symptoms. Phadiatop tests and a skin prick tests were performed to confirm or rule out allergy. Spirometry was carried out and routine blood samples (B cells, C-reactive protein (CRP) and erythrocyte sedimentation rate) analyzed. During the screening period, a structural MRI examination was performed. T1- and T2-weighted MR images were obtained for each individual, using the 1.5 Tesla Siemens Avanto scanner (at Medicinsk Röntgen, Odenplan). T2 images were evaluated for pathology by a neuroradiologist. T1 images were used for subsequent co-registration to PET and definition of regions of interest (ROIs), see below. During the course of the study, we also added collection of saliva samples at screening, for analysis of polymorphisms of the TSPO gene which is known to affect [^{11}C]PBR28 binding (Kreisl et al., 2013; Owen et al., 2012). Genotyping was performed using a Taqman SNP genotyping assay and individuals were classified as high affinity binders (HAB), mixed affinity binders (MAB) and low affinity binders (LAB). LAB individuals were excluded from further participation.

Allergy-related inclusion criteria for the present study were set identical with those for initiation of allergy immunotherapy (Jutel et al., 2015; Theodoropoulos and Lockey, 2000). In short, this encompasses verified allergy, perseverance of severe allergy-symptoms despite full anti-allergic medication and no severe asthma. Furthermore, to fulfill inclusion criteria all participants (both in the allergy and the comparison group) had to be healthy with no chronic disease (except allergy, well-controlled hypothyroidism or hypertension), no regular medication (except for allergy, hypothyroidism or hypertension), no chronic pain or migraine and no mental illness, BMI < 29, fluent in Swedish, right-handed and 20–70 years of age. Participants were excluded if pregnant, reporting use of estrogen containing hormonal contraceptives or having a current infection, based on symptoms and CRP ≥ 10 . Allergy patients were excluded if having a comorbid pet allergy and regular contact with animals, or if recently treated with cortisone injection. Mild and moderate comorbid asthma was allowed. If asthma exacerbations occurred, patients were instructed to contact the study co-ordinator. All participants gave

written informed consent and were compensated with 4000 SEK. Patients and healthy subjects were matched, based on age, sex and genotype. 18 allergy patients and 13 healthy subjects were included in any analyses (15 and 13 for PET). The study was interrupted after planned interim analyses, explaining the relatively limited sample size (however including a total of 55 PET registrations) and not complete balance between patients and healthy subjects.

Participants were instructed to avoid alcohol and hard physical exercise 2 days before PET examinations and through the study days, to drink coffee as usual and to sleep regularly 2 days before participation. The allergy patients were specifically instructed not to use any steroids (inhalation, nasal or systemic) or leukotriene antagonists 10 days before the participation and to avoid any anti-histamines 5 days before participation. No other change in medication was allowed the week before participation. Bronchodilators and decongestant nasal spray were allowed. In addition, participants were instructed not to travel across more than 2 time zones or to engage in shift work (nights) 3 weeks before study participation. Any deviation from the instructions were to be reported to the study coordinator.

BMI was based on data from self-reported weight and length or data from screening visit or patient records. Educational level was obtained in conjunction to the cognitive assessment (not reported here), where participants rated their highest level of education (elementary/high school, some college, college graduate, some university, university graduate and other).

2.3. PET data acquisition

PET examinations were performed at the PET center at Karolinska Institutet, Stockholm, using a High Resolution Research Tomograph (Siemens Molecular Imaging, Knoxville, TN). The time of examinations was standardized (mornings) in order to avoid potential diurnal effects on TSPO binding (Collste et al., 2016). A plaster helmet was made to fixate the subject's head in order to prevent head motion and differences in positioning between examinations. [^{11}C]PBR28 was prepared as described previously (Collste et al., 2016) and was administered intravenously as a bolus injection. PET images were acquired in list mode for 93 min except for one individual where data acquisition was limited to 63 min. The radioactivity level in arterial blood was measured through an

automatic blood sampling system (ABSS; Allogg Technology, Mariefred, Sweden) for the first 5 min, followed by manual sampling. Blood sampling time was at 0 to 5, 6, 8, 10, 15, 20, 25, 30, 40, 50, 60, 70, and 90 min. Radioactivity level in arterial plasma was measured after centrifugation of the blood sample. To calculate plasma radioactivity for the first 5 min, measurements from manual blood samples (2 and 4 min) were linearly interpolated to create a time curve for plasma-to-blood ratio, which was then multiplied with the blood curve from the automated sampling. Radiometabolite analysis was performed at 4, 10, 20, 30, 40, 50, 70, and 90 min as described previously (Kanegawa et al., 2016). To assess the effect of protein binding, the free fraction of radioligand was analyzed using an ultrafiltration method (Collste et al., 2016).

2.4. Quantification of TSPO binding

Regions of interest (ROIs) were defined based on the Automated Anatomical Labelling system in Statistical Parametric Mapping (<http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>). The primary ROI was brain grey matter (GM), since the main hypothesis concerned general brain changes. Regional differences were explored visually, see [Supplementary Fig. 5](#).

PET images were reconstructed, corrected for head motion and coregistered to T1 MR images as described previously (Collste et al., 2016; Kanegawa et al., 2016). Regional time-activity data was extracted from the dynamic PET images from the ROIs described above. Based on the estimated increase between 63 and 93 min, we imputed by interpolation a value for 93 min for the subject with only 63 min data collection. [^{11}C]PBR28 binding was quantified using the two-tissue compartment model (2TCM) with a metabolite-corrected arterial plasma curve as input function using PMOD v3.2 software. The regional [^{11}C]PBR28 binding was expressed as the total volume of distribution (V_T), which is the sum of both specific and non-displaceable binding. For additional details on quantification of binding see (Collste et al., 2016). Estimated values of kinetic parameters (K_1 , k_2 , k_3 and k_4) and details regarding radiochemistry as well as protein binding are presented in [Supplementary Table 2](#). Average curves for metabolite-corrected plasma input function for both groups, in and out of pollen season, is plotted in [Supplementary Fig. 6](#). There were no statistical differences between groups for plasma input function.

2.5. Blood sampling and cytokine analyses

Venous blood was collected directly before bringing the participants into the PET scanner. For one patient and one control, arterial blood had to be used instead for one of the sessions. After 1–1.5 h, samples were centrifuged for 10 min at 3200g or 20 min at 1500g in room temperature and serum was aliquoted and put in -80°C until analysis. Serum concentration of cytokines were analyzed by sandwich immunoassays using multiplex V-PLEX Proinflammatory panel 1, human, K15049D (IFN- γ , IL-10, IL-12p70, IL-13, IL-1 β , IL-2, IL-4, IL-6, IL-8, TNF- α), single spot V-PLEX Human IL-5 Kit, K151QSD (IL-5) and single spot V-PLEX Human MCP-1 K151NND (MCP-1) from Meso Scale Diagnostics (www.mesoscale.com), following the manufacturer's instruction, with overnight incubation at 4°C . All cytokines were analyzed as duplicates and a mean was calculated of the two. Two sets of plates were used to analyze data from this project, with slightly differing detection limits for each analyte and plate. If the values were under the detection limit, the value was imputed with the detection limit value for the respective plate. For the allergy patients and their comparison group, we only used TNF- α , IL-1-beta, IL-6, IL-8, IL-5 and IFN- γ for statistical analyses. This was based on sensitivity for detection, and being of interest a priori for immune-to-brain

communication (TNF- α , IL-1-beta, IL-6, IL-8) or Th1/Th2 proportions (IL-5 and IFN- γ). IL-6, IL-5, IL-8 and IFN- γ were log transformed before any further analyses to better approximate a normal distribution, see [Supplementary materials](#) for histograms. Ratio of log(IFN- γ) and log(IL-5) was calculated to reflect Th1/Th2 balance (Eisenbarth, 2003).

2.6. Rating scales

Validated questionnaires were used to measure level of fatigue (The Multidimensional Fatigue Inventory, MFI-20 (Ericsson and Mannerkorpi, 2007; Smets et al., 1995)), asthma (Asthma Control Questionnaire, ACQ (Juniper et al., 1999)) and allergic symptoms (i.e. daily symptom scoring). The MFI-20 is a 20-item self-report measuring 5 dimensions of fatigue, i.e. general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity, with 4 items corresponding to each dimension. Scores on each dimension can range from 4 to 20, where higher scores correspond to higher fatigue. The ACQ is a validated asthma questionnaire with 7 questions, from which a mean is calculated. Scores can reach from 0 (no symptoms) to 6. The original scale requires an objective measure of forced expiratory volume in 1 s (FEV1), but we only used the 6 self-reported items. Nine common allergic symptoms (runny nose, itching nose, sneezing, nasal congestion, loss of smell, runny eyes, itching eyes, eye redness and swollen eyes) were scored daily during the week preceding the PET investigation, with 0 corresponding to no symptoms and 10 corresponding to maximal symptoms.

2.7. Sleep measures

The week before the PET examination, participants filled out sleep diaries every morning (Åkerstedt et al., 1994) and used a single frontal EEG electrode (MyZeo, Zeo Inc., Boston MA, USA) to measure sleep length and percentage of sleep stages (Shambroom et al., 2012) in their homes. A subset of the participants also used actigraphs (Actiwatch, Philips Respironics), a wrist-watch measuring movement, rest and sleep, not reported here. Participants received the sleep equipment 6 days before the PET examination, but recorded sleep in a mean of 5.8 days (range 1–11), based on practical circumstances such as rescheduled participation.

Data from sleep diaries was used to obtain a measure of subjective sleep quality (Åkerstedt et al., 1994). An index based on 4 questions ("Did you have difficulties falling asleep?" (1 = very much to 5 = not at all), "How did you sleep?" (1 = very poorly to 5 = very well), "Did you have a restless sleep?" (1 = very much to 5 = not at all), and "Did you wake up very early without being able to fall asleep?" (1 = very much too early to 5 = no)) was calculated. Self-reported bedtime and rise time was used to define time in bed. Karolinska Sleepiness Scale (KSS) was used as a measure of sleepiness (1 = alert to 9 = very sleepy, great effort keeping awake, fighting sleep).

Data from MyZeo was inspected manually and exported through Zeo Sleep Viewer (version 0.2.9). Recordings where participants reported that the electrode fell off during the night were removed, as well as recordings where most of the recording was missing. We also defined 2 criteria to remove data with poor recording quality. First, we only used data where less than 5% of the recording for one night was missing. Second, we removed data where the total duration was less than 75% of the time in bed estimated from sleep diaries for the specific night. The second criterion was applied because the first criterion would not reflect if the electrode fell off completely during a period of the night. We analyzed total sleep time and percentage of deep sleep.

2.8. Data loss

One patient did not undergo PET examinations, because of technical problems with the cyclotron. Two patients were excluded from PET scanning/analyses because of being low affinity binders, in one case after PET had been performed (i.e. before genotype was included in the screening procedure). One further patient only underwent PET once (during pollen season), since the subject had pet allergy and initiated regular contact with a dog during the study period. Hence, for main analyses of PET data, data from 15 (29 scans) patients and 13 healthy subjects (26 scans), with equal sex distribution, were used. For other variables, data loss is reported in supplement.

2.9. Statistical analyses

The effects of group (patient vs healthy subjects) and pollen season on TSPO binding, cytokine levels, questionnaires and sleep parameters were investigated using mixed effects models, with a random intercept for each subject and a second random intercept for each matched pair. TSPO polymorphism and sex were added as a covariate in all analyses regarding effects on [¹¹C]PBR28 binding. All mixed models are presented as effect estimates in original units with 95% CI. Other predictors of [¹¹C]PBR28 binding (peripheral cytokine levels, ratings of fatigue and sleep) were investigated using similar mixed models with group, pollen season and genotype, and other predictors added subsequently to the model. Deviation coding was used for all contrasts, so that main effects represent overall main effects. All scripts can be found at: <https://doi.org/10.5281/zenodo.1015473>.

3. Results

3.1. Demographic measures and allergic symptom severity

Demographic variables are presented in Table 1. Additional data on clinical variables (spirometry, skin prick test and Phadiatop/IgE) can be found in Supplementary Table 1. Outside the study period, all subjects with allergy were medicated with oral antihistamines. Before the temporary withdrawal of medication, all except one allergic subjects also used nasal spray (steroids and/or antihistamines) and all except 6 allergic subjects used eye drops (cromoglicic acid and/or antihistamines). Also before withdrawal, eight of the allergic subjects received inhalation treatment for asthma (steroids and/or beta-2 agonists). Four subjects reported a history of intermittent use of oral steroids. One allergic subject was medicated with progesterone for endometriosis and one allergic subject was treated with an ACE-inhibitor and a calcium antagonist due to hypertension, levothyroxine due to hypothyroidism and a potassium supplement. None of the control subjects reported any regular medication. One allergic subject reported night work (on call) during the week before study participation. Allergic symptom ratings are presented in Table 2, confirming higher symptom scores in allergic patients during pollen season.

3.2. [¹¹C]PBR28 binding

All results are described as effect estimates with 95% confidence intervals in hard brackets. As expected, HAB genotype was associated with increased [¹¹C]PBR28 binding in the total grey matter in the brain (1.35 [0.84, 1.85], $p < .0001$), compared to MAB. There was no group*pollen season interaction (0.49 [−0.48, 1.46], $p = .30$, Figs. 2 and 3A) for [¹¹C]PBR28 binding in the brain. Further, there was no main effect of group (allergic subjects vs healthy subjects) (0.29 [−0.21, 0.80], $p = .24$, Fig. 3) or pollen season (in vs out

Table 1

Demographic variables. Continuous values are reported as means with standard deviations, unless otherwise indicated). Categorical data are reported with percentages.

Variables	Allergy	Healthy subjects
<i>Sample</i>		
Number of subjects	18	13
<i>Genotype</i>		
Low affinity binders	2 (11.1%)	0 (0.0%)
Mixed affinity binders	9 (50.0%)	5 (38.5%)
High affinity binders	7 (38.9%)	8 (61.5%)
<i>Demographics</i>		
Age (median, interquartile range)	34.0 (30.2–44.2)	34.0 (27.0–46.0)
Sex (females)	8 (44.4%)	5 (38.5%)
BMI	25.3 (±4.4)	22.4 (±3.0)
<i>Education</i>		
College graduate	1 (5.6%)	0 (0.0%)
Elementary/High School	0 (0.0%)	1 (10.0%)
Other	1 (5.6%)	0 (0.0%)
Some college	5 (27.8%)	1 (10.0%)
Some university	2 (11.1%)	5 (50.0%)
University graduate	9 (50.0%)	3 (30.0%)

of season) (0.01 [−0.47, 0.50], $p = .96$, Fig. 3A) on [¹¹C]PBR28 binding. Male sex was significantly associated with lower [¹¹C]PBR28 binding (−0.69 [−1.16, −0.23], $p = .005$), but adjusting the model for sex did not appreciably change results. Because this is the first TSPO PET study in allergy, we additionally provide effects for each ROI in patients and healthy subjects across seasons, plotted in Supplementary Fig. 5, for descriptive purposes only.

3.3. Plasma cytokines

For IFN- γ and IL-8, 60/60 of the observations were above the detection limit. For TNF- α and IL-6 the corresponding numbers were 59/60 and 42/60. As expected, IL-5 was more readily detectable in allergic subjects (28/34) than in healthy subjects (7/26). Detection limits are presented in supplement. Cytokines were analyzed using mixed effects models and results are presented as effect estimates with 95% CI intervals in Table 3. Group and pollen season interacted significantly for TNF- α and log-IL-5. When decomposed, allergic subjects had higher levels of TNF- α during pollen season compared to outside of pollen season (0.37 [0.00, 0.73], $p = .048$, Fig. 3B), whereas healthy subjects did not (−0.06 [−0.22, 0.10], $p = .40$, Fig. 3B). Outside pollen season, there was no difference between allergic subjects and healthy subjects (−0.04 [−0.47, 0.38], $p = .84$, Fig. 3B). Outside pollen season, allergic subjects had higher levels of log-IL-5 compared to healthy subjects (0.20 [0.02, 0.38], $p = .03$, Fig. 3D) and were even higher during pollen season (0.14 [0.00, 0.27], $p = .048$, Fig. 3D). In healthy subjects, no difference was observed between seasons (−0.05 [−0.14, 0.04], $p = .29$, Fig. 3D). For neither log-IL-6, log-IFN- γ nor log-IL-8, there was any significant group*season interaction or any main effect of group or pollen season (Fig. 3C, E, F, Table 3). No significant interaction or main effect for group or pollen season was found for the log-IFN- γ /log-IL5 ratio (Table 3).

3.4. Ratings of fatigue

Group and pollen season interacted significantly for *general fatigue*, *physical fatigue*, *reduced motivation* and *reduced activity*, but not for *mental fatigue*, see Fig. 4 and Table 4. For all factors, allergic patients had higher ratings of fatigue during pollen season, compared to outside of pollen season, whereas no difference between seasons was seen for healthy subjects. The main effect of group was significant for *general fatigue*, *physical fatigue*, and *reduced*

Table 2
Rhinitis symptoms scored daily during the week preceding participation. Asthma symptoms scored on the day of participation. Means and 95% confidence intervals and *p* values for pairwise comparisons (in vs out of season for each group and allergic subjects vs healthy subjects outside pollen season).

Group	Allergic			Healthy subjects			
Pollen season	In	Out	<i>p</i> (allergic: in vs out)	In	Out	<i>p</i> (healthy subjects: in vs out)	<i>p</i> (allergic vs healthy subjects (out))
<i>Symptoms</i>							
Runny nose	4.13 (2.12–6.15)	0.35 (−0.11 to 0.8)	<.001	0.01 (−0.03 to 0.05)	0.22 (−0.13 to 0.58)	<.001	.51
Itching nose	4.85 (2.89–6.81)	0.49 (0.01–0.97)	<.001	0.01 (−0.03 to 0.05)	0.07 (−0.1 to 0.24)	.03	.03
Sneezing	4.69 (2.64–6.74)	0.61 (0.04–1.18)	<.001	0.04 (−0.05 to 0.13)	0.04 (−0.06 to 0.14)	.62	.01
Nasal congestion	5.27 (3.03–7.51)	0.21 (−0.14 to 0.57)	<.001	0.14 (−0.16 to 0.44)	0.35 (−0.26 to 0.96)	<.001	.56
Loss of smell	4.29 (2.04–6.55)	0.24 (−0.08 to 0.56)	<.001	0 (0–0)	0 (0–0)	1	.05
Runny eyes	4.39 (2.17–6.61)	0.09 (−0.08 to 0.27)	<.001	0 (0–0)	0 (0–0)	1	.20
Itching eyes	5.24 (3.05–7.42)	0.3 (−0.06 to 0.66)	<.001	0.04 (−0.03 to 0.11)	0.02 (−0.02 to 0.07)	.49	.04
Eye redness	4.3 (2.37–6.22)	0.16 (−0.03 to 0.36)	<.001	0 (0–0)	0 (0–0)	1	.03
Swollen eyes	3.97 (2.06–5.87)	0.2 (−0.15 to 0.55)	<.001	0 (0–0)	0.08 (−0.16 to 0.31)	<.001	.40
Asthma Control Questionnaire	0.96 (0.23–1.69)	0.22 (−0.13 to 0.57)	.005	0 (0–0)	0 (0–0)	1	.13

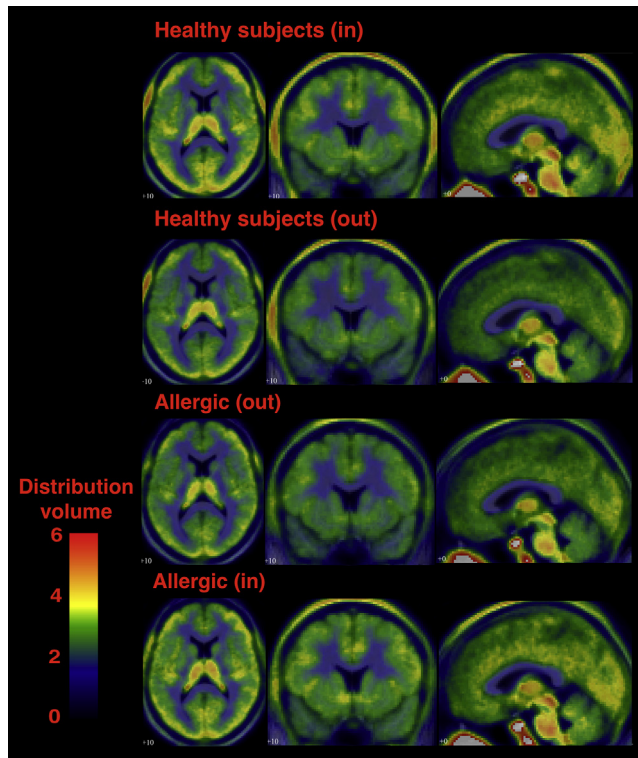


Fig. 2. No difference in distribution volume of [^{11}C]PBR28 was found between allergic subjects and healthy subjects nor between pollen season (=in) and non-pollen season (=out). The figure shows mean distribution volume across all subjects per group and season.

activity, and was mainly driven by higher ratings during pollen season. [^{11}C]PBR28 binding did not predict fatigue scores on any of the subscales of MFI-20, *general fatigue* (0.03 [−0.87, 0.92], $p = .95$), *physical fatigue* (0.21 [−0.69, 1.12], $p = .63$), *mental fatigue* (−0.14 [−0.94, 0.66], $p = .72$), *reduced motivation* (0.11 [−0.67, 0.89], $p = .77$), *reduced activity* (−0.23 [−1.03, 0.57], $p = .56$), results expressed as effect estimates with 95% CI.

3.5. Sleep

3.5.1. Sleep diary data

A group*season interaction was seen for subjective sleep quality (−0.28 [−0.55, −0.00], $p = .047$, Fig. 5B). Subjects with allergy

reported worse sleep quality during pollen season (−0.30 [−0.49, −0.10], $p = .003$), whereas no seasonal change was observed in healthy subjects (−0.01 [−0.19, 0.16], $p = .88$). Outside pollen season, the difference between allergic subjects and healthy subjects was not significant (−0.21 [−0.54, 0.12], $p = .21$).

Group and season interacted in their effects on morning sleepiness (0.76 [0.14, 1.38], $p = .02$, Fig. 5A). Allergic subjects rated higher levels of morning sleepiness during pollen season compared to outside pollen season (0.90 [0.48, 1.32], $p < .0001$), whereas no difference was seen in healthy subjects (0.13 [−0.31, 0.58], $p = .55$). Outside pollen season, there was no difference between allergic subjects and healthy subjects (−0.20 [−0.91, 1.30], $p = .72$).

3.5.2. Objective sleep measures

No group*season interaction was seen for total sleep time (10.81 min [−26.45, 48.08], $p = .57$, Fig. 5C). The main effect of group was significant, with allergic subjects having shorter total sleep time (−37.77 min [−72.80, −2.75], $p = .04$, Fig. 5C) across seasons. No main effect of season was seen (−10.00 min [−26.45, 48.08], $p = .44$).

For percentage of deep sleep, group and season interacted (2.08 [0.06, 4.11], $p = .04$, Fig. 5D). Pairwise comparisons showed that in allergic subjects, pollen season was associated with a higher percentage of deep sleep (1.99 [0.31, 3.67], $p = .02$), whereas no seasonal change was seen in healthy subjects (−0.12 [−1.35, 1.11], $p = .84$). Outside pollen season, no difference was observed between allergic subjects and healthy subjects (0.29 [−4.56, 5.13], $p = .90$).

For number of awakenings, there was no group*season interaction (−0.19 [−1.18, 0.80], $p = .70$). Further, there was no effect of group (0.40 [−1.02, 1.83], $p = .57$) or season (0.12 [−0.56, 0.80], $p = .73$).

Mean total sleep time was not associated with [^{11}C]PBR28 binding (0.16 [−0.32, 0.65], $p = .48$) or to *general fatigue* (−0.54 [−2.21, 1.14], $p = .50$).

3.6. Associations between plasma cytokines and TSPO binding, fatigue and sleep

Neither TNF- α , log-IL-6, log-IL-5, log-IL-8 nor log-IFN- γ significantly predicted [^{11}C]PBR28 binding in the brain (Supplementary Table 3). Neither was any of the measured cytokines significantly predicting *general fatigue* scores (Supplementary Table 3) or total sleep time (Supplementary Table 3).

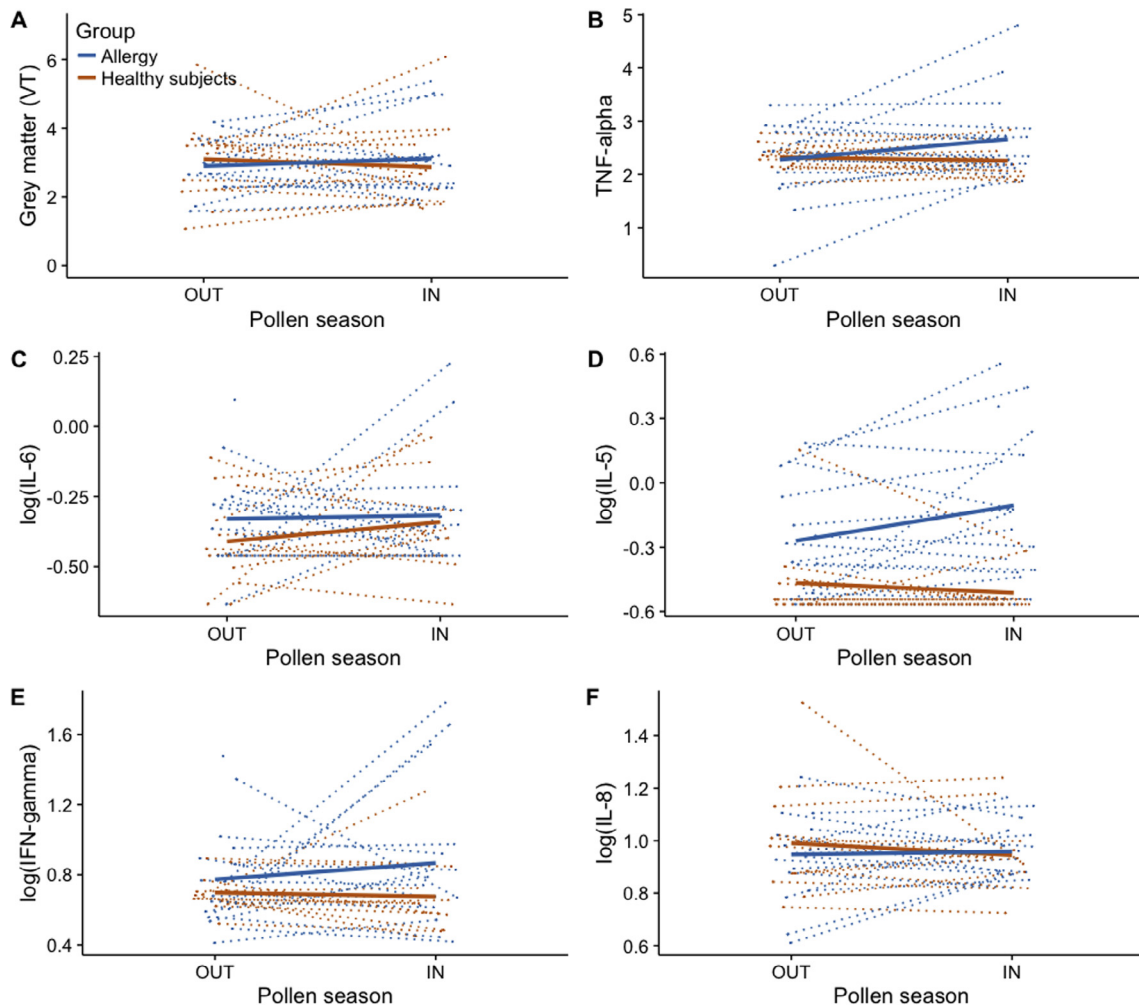


Fig. 3. TSPO binding and cytokine levels in patients and healthy subjects. Dotted lines represent individual observations and solid lines represent group means. A: [^{11}C]PBR28 in grey matter in patients and healthy subjects. No difference was observed between patients and healthy subjects or between pollen season (=in) and non-pollen season (=out). B–E: Cytokine levels in patients and healthy subjects. B: TNF- α concentration. Allergic subjects had higher values during pollen season, whereas healthy subjects did not. C: Log(IL-6) concentration. No difference between patients and healthy subjects was observed. D: Log(IL-5) concentration. Patients with allergy had higher concentrations compared to healthy subjects, and levels were even higher during pollen season. E: Log(IFN- γ) concentration. No difference between patients and healthy subjects was observed. F: Log(IL-8) concentration. No difference between patients and healthy subjects was observed.

Table 3

Effects of group and pollen season on plasma cytokines. Table showing effect estimates + 95% CI and *p* values obtained through mixed effects models.

	Intercept	Group (allergic vs healthy subjects)		Season (in vs out)		Group*season	
	Estimate + CI	Estimate + CI	<i>p</i>	Estimate + CI	<i>p</i>	Estimate + CI	<i>p</i>
<i>Cytokine</i>							
TNF- α	2.38 (2.19–2.58)	0.18 (–0.21 to 0.57)	.347	0.15 (–0.06 to 0.36)	.146	0.43 (0.01–0.85)	.043
log(IL-6)	–0.35 (–0.4–0.3)	0.05 (–0.04 to 0.15)	.269	0.04 (–0.05 to 0.14)	.378	–0.06 (–0.25 to 0.13)	.545
log(IL-5)	–0.34 (–0.42–0.25)	0.31 (0.13–0.48)	.001	0.05 (–0.04 to 0.13)	.267	0.18 (0.02–0.35)	.031
log(IFN- γ)	0.75 (0.67–0.83)	0.13 (–0.03 to 0.29)	.098	0.04 (–0.11 to 0.19)	.618	0.12 (–0.18 to 0.42)	.426
log(IL-8)	0.96 (0.91–1.01)	–0.01 (–0.11 to 0.09)	.822	–0.01 (–0.07 to 0.05)	.659	0.06 (–0.06 to 0.19)	.285
log(IFN- γ)/log(IL-5)	–0.88 (–1.98 to 0.23)	0.63 (–1.57 to 2.84)	.561	0 (–2.22 to 2.21)	.997	0.63 (–3.79 to 5.06)	.771

5. Discussion

To investigate a possible mechanism behind fatigue and other comorbid problems in allergy, the present study sought to examine if seasonal allergy is reflected in immune cell activation in the brain. Compared to healthy subjects, allergic subjects displayed both increased fatigue and impaired sleep, as well as increased levels of TNF- α and IL-5, during pollen season. However, no increase in grey matter TSPO binding as measured with [^{11}C]

PBR28 was observed in patients with allergy, neither in relation to pollen season nor in relation to healthy subjects.

The hypothesis of immune cell activation in the brain, operationalized as increased TSPO binding, in patients with seasonal allergy was partly motivated by previous animal data. For example, brain consequences of systemic allergic inflammation in rodents encompass increased intracerebral levels of IgG and IgE, enhanced tau phosphorylation and modified gene expression toward inflammatory responses (Sarlus et al., 2013, 2012). Further, in mice and

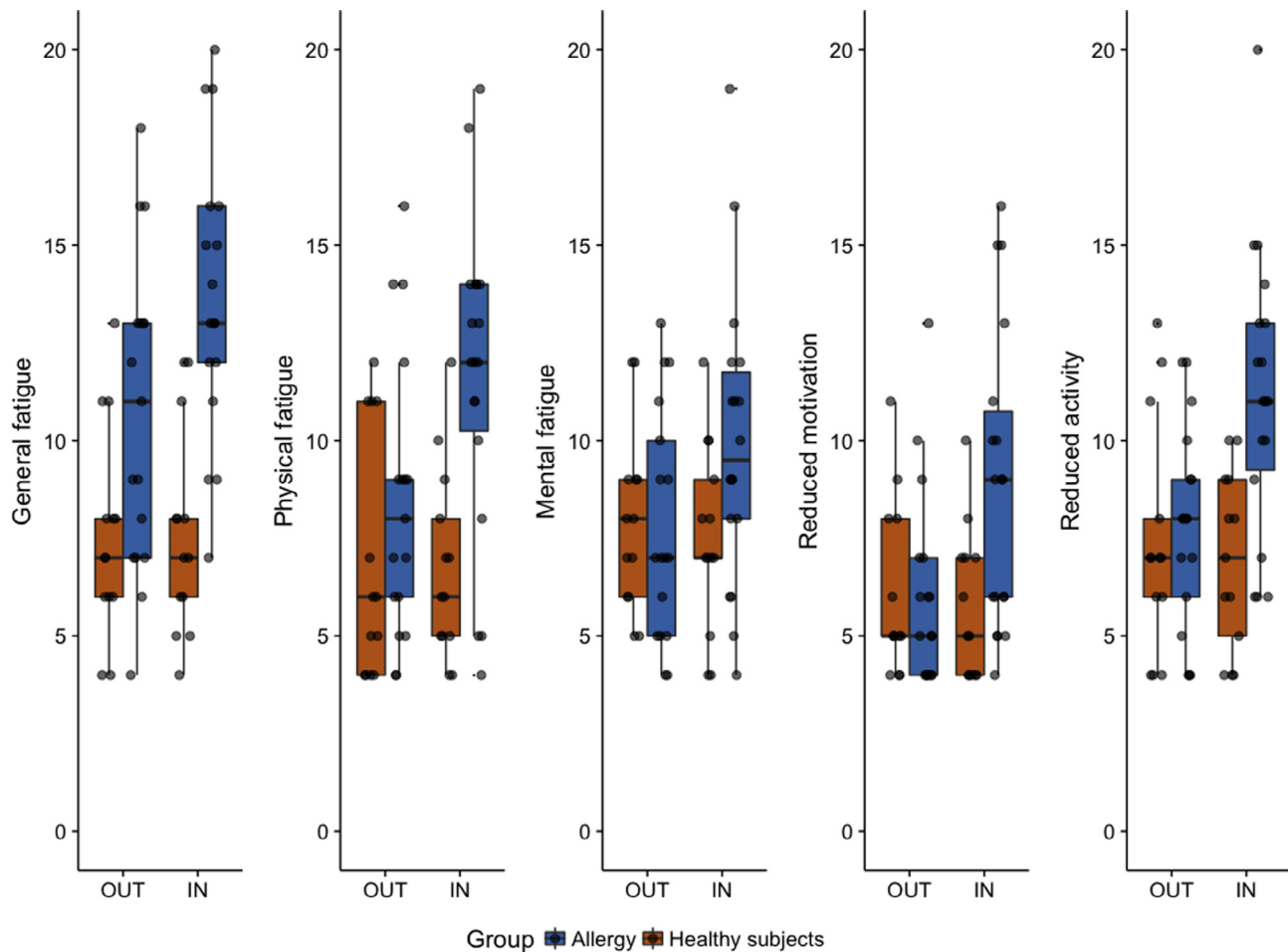


Fig. 4. Rated fatigue on The Multidimensional Fatigue Inventory 20. Dots represent individual observations. Boxplots represent medians, interquartile range and ± 2 SD.

rat models of allergic rhinitis, a Th2-biased profile of cytokine mRNA (IL-4, IL-5, IL-13) in the olfactory bulb and the prefrontal cortex was found (Tonelli et al., 2009). In this first study of the human CNS immune system in allergy, the hypothesis of increased TSPO levels was not confirmed. This lack of effect could be explained both by conceptual differences in human and animal pathophysiology, by differences between animal models and human conditions, by differences between acute and chronic conditions, and/or by differences in methods used to gauge immune activity in the brain compared to the experimental studies. Regarding the latter, the literature so far indicates that TSPO imaging can reflect inflammatory changes in stroke (Tóth et al., 2016), trauma (Folkersma et al., 2011; Israel et al., 2016), virus infection (Dimber et al., 2016), Alzheimer's disease (Knezevic and Mizrahi, 2017) and depression (Setiawan et al., 2015), but less consistently in other conditions where immune activation is hypothesized, such as addiction (Auvity et al., 2017; Hillmer et al., 2017; Kalk et al., 2017), post-surgery (Forsberg et al., 2017) and schizophrenia (Collste et al., 2017; Notter et al., 2017). Importantly, TSPO has been shown to be expressed not only in microglia, but also astrocytes, macrophages, vascular and perivascular cells (Cosenza-Nashat et al., 2009; Notter et al., 2017; Venneti et al., 2006), and the specificity for microglia can differ between animal models and human tissue (Venneti et al., 2013). In addition, TSPO cannot differentiate between pro-inflammatory vs anti-inflammatory microglial or astrocytic phenotypes (Liddelow et al., 2017; Tronel et al., 2017). Therefore, compensatory down-regulation (Forsberg et al., 2017), including hypo-active tolerant phenotypes of glial

cells (Eggen et al., 2013) in allergy could possibly explain the present results. In fact, some data indicate that allergic reactions cause pulmonary macrophages to transform to an immature state (Lensmar et al., 2006) and if the same is true for corresponding cells in the brain, microglia, this might not be reflected in TSPO binding.

A recent mouse study investigating microglia in allergy found fewer Iba1+ microglia in the granular layer and subgranular zone of the hippocampal dentate gyrus and a lower number of Iba1+MHCII+ cells in the same areas, indicating a reduced microglial surveillance and activation in the hippocampus (Klein et al., 2016). In the study by Sarlus et al. (2012), no difference between healthy and allergic mice was seen in F4/80 antibody staining, another microglial marker. Recently, a decrease in brain [^{11}C]PBR28 binding was observed after abdominal surgery concomitant with increases in peripheral pro-inflammatory cytokines (Forsberg et al., 2017). It is also possible that the present lack of effects in TSPO binding reflects that immune cells in the human brain are not activated in response to peripheral allergic inflammatory processes. This would speak for other mechanisms through which allergy could affect the brain. For example, serotonin pathways have been suggested as one such mechanism (Trikojat et al., 2017).

In the present study, patients with seasonal allergy displayed somewhat increased levels of TNF- α during pollen season compared to healthy subjects, partly suggesting a pro-inflammatory state. This increase in TNF- α is in concordance with some observations in patients with asthma (Broide et al., 1992; Townley and Horiba, 2003) and the late phase response to allergen in rhinitis

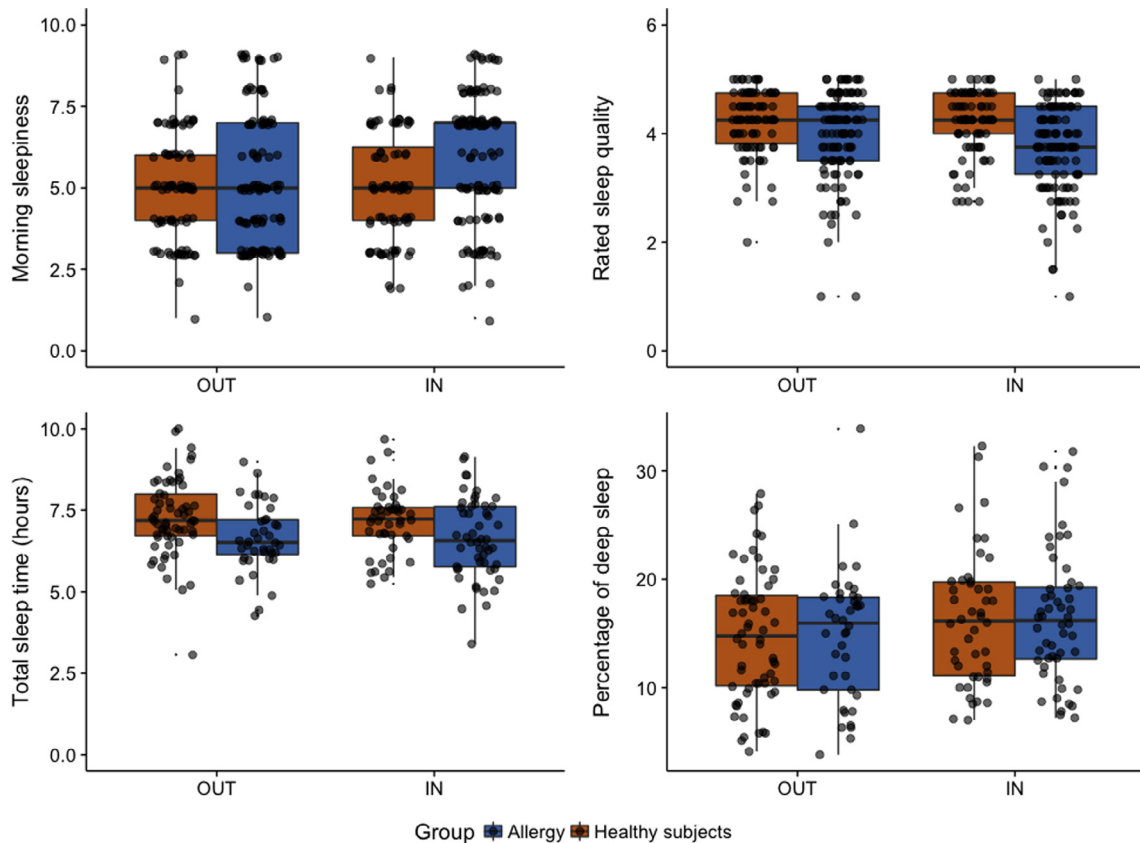


Fig. 5. Subjective and objective sleep measures during pollen season (=in) and non-pollen season (=out). Dots represent individual observations. Boxplots represent medians, interquartile range and ± 2 SD. A: Rated morning sleepiness using Karolinska Sleepiness Scale. Data points have been vertically jittered for visualization. Group and pollen season interacted, so that allergic subjects had higher ratings of sleepiness during pollen season, whereas healthy subjects did not. B: Rated sleep quality based on 4 questions in The Karolinska Sleep Diary. During pollen season, subjects with allergy reported lower subjective sleep quality, whereas healthy subjects did not. C: EEG-recorded total sleep time (MyZeo). Subjects with allergy had shorter total sleep time, regardless of pollen season. D: Percentage of deep sleep (MyZeo). In allergic subjects, pollen season was associated with a higher percentage of deep sleep, whereas no seasonal change was seen in healthy subjects.

Table 4

Effects of group and pollen season on ratings of fatigue. Table showing effect estimates + 95% CI and *p* values obtained through mixed effects models.

	Intercept	Group (allergic vs healthy subjects)		Season (in vs out)		Group*season	
	Estimate + CI	Estimate + CI	<i>p</i>	Estimate + CI	<i>p</i>	Estimate + CI	<i>p</i>
<i>MFI-20</i>							
General fatigue	9.89 (8.83–10.95)	4.55 (2.43–6.67)	<.001	1.5 (0.12–2.88)	.035	3 (0.23–5.77)	.035
Physical fatigue	8.43 (7.3–9.56)	3.17 (0.92–5.42)	.007	1.52 (0.24–2.8)	.022	3.34 (0.79–5.9)	.012
Mental fatigue	8.31 (7.4–9.23)	1.16 (–0.66 to 2.99)	.203	0.86 (–0.43 to 2.15)	.185	2.48 (–0.1 to 5.07)	.059
Reduced motivation	6.71 (5.88–7.55)	1.58 (–0.09 to 3.25)	.062	1.23 (–0.09 to 2.55)	.066	3.08 (0.45–5.72)	.024
Reduced activity	8.31 (7.38–9.23)	2.31 (0.46–4.16)	.016	1.47 (0.24–2.71)	.021	3.87 (1.4–6.34)	.003

(see Trikojat et al., 2017), and indicates a less clear dissociation between allergy-related and bacterial-type inflammation that could be relevant for sickness-reminiscent symptoms in allergy. As indicated, pro-inflammatory cytokines released after detection of bacterial stimuli activate microglia (Hannestad et al., 2012; Watkins et al., 2007), modulate activity in emotion-regulatory brain circuits (Harrison et al., 2009; Lekander et al., 2016) and cause fatigue (Harrison et al., 2009), as well as a general expression of sickness (Lekander et al., 2016). In the present study, the increase in TNF- α was not consistent across the patient group and clearly not as pronounced as in response to LPS (Lasselin et al., 2016; Sandiego et al., 2015). Also, no increase in IL-6 or IL-8 was seen, perhaps making a contained increase in TNF- α less likely to drive an activation of TSPO at the time of observation. It should be pointed out that studies investigating seasonal changes in inflammatory markers have used widely different methods

and that results are mixed (see e.g. (Djukanović et al., 1996; Kurt et al., 2010; Winther et al., 1999)). The observed increased IL-5 in allergic subjects, especially during pollen season, reflects Th-2 cell driven peripheral inflammatory activity, with a less well-described role in immune-to-brain-communication. Notably, the present data did not provide evidence for an association between either TNF- α or IL-5 on the one hand and brain grey matter TSPO levels on the other.

Increased fatigue, or being less well-rested after sleep, has previously been reported in subjects with allergy (Benninger and Benninger, 2009; Jernelöv et al., 2009; Léger et al., 2015; Ozdoganoglu et al., 2012). Fatigue was increased in allergic subjects compared to healthy subjects, especially during pollen season, similar to the findings by Marshall et al. (2002), who also used the MFI-20, a validated fatigue questionnaire with 5 dimensions. The pattern of results was similar across dimensions, but

most prominent for general fatigue and less for mental fatigue. In the Marshall study, it was observed that allergy was associated mostly with general, but also mental fatigue (Marshall et al., 2002). In relation to inflammation, the different aspects/sub-components of fatigue may be affected differently by immune activation (Karshikoff et al., 2017). The literature on immune-to-brain effects suggests that inflammation affects motivation and cognition (Harrison et al., 2009; Karshikoff et al., 2017; Lasselin et al., 2017), but the subdimensions of fatigue specifically have not been sufficiently investigated at this point in time.

In relation to fatigue, it is interesting to note that allergic subjects reported decreased subjective sleep quality and increased morning sleepiness during pollen season compared to healthy subjects. A pattern with increased fatigue and decreased sleep quality has previously been reported in patients with allergic rhinitis in epidemiological studies (Léger et al., 2015; Santos et al., 2006), and sleep quality to decrease during pollen as compared to non-pollen season (Trikojot et al., 2017). Perhaps surprisingly, total sleep time was decreased in allergic subjects independent of pollen season. Short sleep in allergic patients could be caused by several factors, including allergic symptoms (nasal congestion etc.), but our data indicate shorter sleep also during seasons when specific symptoms are not as prevalent. This indicates a more general effect, and speaks against effects merely from the airways. In spite of the lack of increases in TSPO binding, the shortened sleep could possibly be caused by inflammation, since inflammation is known to be associated to sleep length in infection (Krueger and Opp, 2016) and in experimental animal studies, although the direction of the effect differs with time (Krueger et al., 1986). Also, no difference was seen in number of awakenings, which would have been expected in an airway dependent pathology. It should be noted that the single electrode sleep recorder used in the present study does not capture all aspects of sleep, but has been validated in comparison to polysomnography and actigraphy, in particular showing high agreement with total sleep time and amount of deep sleep (Shambroom et al., 2012). For percentage of deep sleep, regarded as an objective measure of relevance for sleep quality (Dijk, 2010), there was a significant group*season interaction. The increase in deep sleep during pollen season in allergic subjects might possibly reflect a homeostatic response to the increased fatigue and sleepiness (Akerstedt et al., 2009) or be a consequence of inflammation. For example, it is previously shown that TNF- α and IL-1 increase non rapid eye movement sleep (Imeri and Opp, 2009) and that infections lead to altered sleep (Krueger and Opp, 2016). However, no association between cytokine levels and sleep length was seen in the present study. It should also be noted that total sleep time was not significantly associated with general fatigue. Therefore, it is unlikely that fatigue seen in allergy can be explained merely by worse sleep.

One limitation in the present study is sample size. Although clear effects were found for both fatigue, sleepiness, objective measures of sleep and peripheral inflammation (TNF- α and IL-5), the high inter-individual variability of [^{11}C]PBR28 binding (Collste et al., 2016; Fujita et al., 2008) means that larger sample sizes may be needed to detect putative subtle changes in TSPO. This also applies to analyses of correlations between CNS and peripheral immune measures and behavioral outcomes, which should be considered exploratory given variability also in measures of peripheral inflammation. Likely, stronger effects and lower variability in psychological measurements (fatigue, sleep quality and sleepiness) in a pattern of deterioration during pollen season in allergic but not healthy subjects, suggest that power is less restricted for these analyses. In sum, further studies of neuroinflammation – or other plausible mechanisms behind non-specific symptoms – with complementary methods and optimized sample sizes are warranted in the context of allergy.

6. Conclusion

During pollen season, allergic subjects showed increased fatigue and sleepiness, reported lower sleep quality and had elevated levels of TNF- α and IL-5 compared to healthy subjects. Yet, these effects were not paralleled by changed TSPO levels in the brain. Further studies should investigate possible immune mechanisms behind the disabling problems with fatigue, disturbed sleep and other non-specific symptoms that are common in individuals suffering from allergy. There is a clear need for development and use of more specific markers to identify neural allergy-related immune activation in humans.

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Designed the study: SC, PG, EK, JL, CL, VS, CH, MI, COH, ML. Collected data: ST, JE. Analyzed PET data: ST, SC, AF. Analyzed cytokines: JE. Performed statistical analyses: ST. Interpreted results: all authors. Drafted manuscript: ST, ML. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bbi.2017.10.013>.

References

- Akerstedt, T., Hume, K., Minors, D., Waterhouse, J., 1994. The subjective meaning of good sleep, an intraindividual approach using the Karolinska Sleep Diary. *Percept. Mot. Skills* 79, 287–296. <https://doi.org/10.2466/pms.1994.79.1.287>.
- Akerstedt, T., Kecklund, G., Alfredsson, L., Selen, J., 2007. Predicting long-term sickness absence from sleep and fatigue. *J. Sleep Res.* 16, 341–345. <https://doi.org/10.1111/j.1365-2869.2007.00609.x>.
- Akerstedt, T., Kecklund, G., Ingre, M., Lekander, M., Axelsson, J., 2009. Sleep homeostasis during repeated sleep restriction and recovery: support from EEG dynamics. *Sleep* 32, 217–222.
- Auvity, S., Goutal, S., Thézé, B., Chaves, C., Hosten, B., Kuhnast, B., Saba, W., Boisgard, R., Buvat, I., Cisternino, S., Tournier, N., 2017. Evaluation of TSPO PET imaging, a marker of glial activation, to study the neuroimmune footprints of morphine exposure and withdrawal. *Drug Alcohol Depend.* 170, 43–50. <https://doi.org/10.1016/j.drugalcdep.2016.10.037>.

- Barnes, P.J., 2011. Pathophysiology of allergic inflammation. *Immunol. Rev.* 242, 31–50. <https://doi.org/10.1111/j.1600-065X.2011.01020.x>.
- Basso, A.S., Costa-Pinto, F.A., Giorgetti Britto, L.R., De Sá-Rocha, L.C., Palermo-Neto, J., 2004. Neural pathways involved in food allergy signaling in the mouse brain: role of capsaicin-sensitive afferents. *Brain Res.* 1009, 181–188. <https://doi.org/10.1016/j.brainres.2004.02.062>.
- Benninger, M.S., Benninger, R.M., 2009. The impact of allergic rhinitis on sexual activity, sleep, and fatigue. *Allergy Asthma Proc.* 30, 358–365. <https://doi.org/10.2500/aap.2009.30.3244>.
- Bernstein, D.I., Schwartz, G., Bernstein, J.A., 2016. Allergic rhinitis. *Immunol. Allergy Clin. North Am.* 36, 261–278. <https://doi.org/10.1016/j.iac.2015.12.004>.
- Broide, D.H., Lotz, M., Cuomo, A.J., Coburn, D.A., Federman, E.C., Wasserman, S.I., 1992. Cytokines in symptomatic asthma airways. *J. Allergy Clin. Immunol.* 89, 958–967. [https://doi.org/10.1016/0091-6749\(92\)90218-Q](https://doi.org/10.1016/0091-6749(92)90218-Q).
- Camelo-Nunes, I.C., Solé, D., 2010. Allergic rhinitis: indicators of quality of life. *J. Bras. Pneumol.* 36, 124–133. <https://doi.org/10.1111/j.1742-6723.2011.01411.x>.
- Collste, K., Forsberg, A., Varrone, A., Amini, N., Aeneband, S., Yakushev, I., Halldin, C., Farde, L., Cervenka, S., 2016. Test-retest reproducibility of [¹¹C]PBR28 binding to TSPO in healthy control subjects. *Eur. J. Nucl. Med. Mol. Imaging* 43, 173–183. <https://doi.org/10.1007/s00259-015-3149-8>.
- Collste, K., Sigra, P.P., Fatouros-bergman, H., Ikonen, P., Schain, M., Forsberg, A., Amini, N., Aeneband, S., Erhardt, S., Halldin, C., Flyckt, L., Farde, L., Cervenka, S., 2017. Lower levels of the glial cell marker TSPO in drug-naïve first episode psychosis patients as measured using PET and [¹¹C]PBR28. *Revis. Mol. Psychiatry* 1–7. <https://doi.org/10.1038/mp.2016.247>.
- Cosenza-Nashat, M., Zhao, M.-L., Suh, H.-S., Morgan, J., Natividad, R., Morgello, S., Lee, S.C., 2009. Expression of the translocator protein of 18 kDa by microglia, macrophages and astrocytes based on immunohistochemical localization in abnormal human brain. *Neuropathol. Appl. Neurobiol.* 35, 306–328. <https://doi.org/10.1111/j.1365-2990.2008.01006.x>.
- Costa-Pinto, F.A., Basso, A.S., De Sá-Rocha, L.C., Britto, L.R.G., Russo, M., Palermo-Neto, J., 2006. Neural correlates of IgE-mediated allergy. *Ann. N. Y. Acad. Sci.* 1116–131. <https://doi.org/10.1196/annals.1366.028>.
- Dantzer, R., 2001. Cytokine-induced sickness behavior: mechanisms and implications. *Ann. N. Y. Acad. Sci.* 933, 222–234.
- Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. <https://doi.org/10.1038/nrn2297>.
- Dijk, D.-J., 2010. Slow-wave sleep deficiency and enhancement: implications for insomnia and its management. *World J. Biol. Psychiatry* 11, 22–28. <https://doi.org/10.3109/15622971003637645>.
- Dimber, R., Guo, Q., Bishop, C., Adonis, A., Buckley, A., Kocsis, A., Owen, D., Kalk, N., Newbould, R., Gunn, R.N., Rabiner, E.A., Taylor, G.P., 2016. Evidence of brain inflammation in patients with human T-lymphotropic virus type 1-associated myelopathy (HAM): a pilot, multimodal imaging study using [¹¹C]-PBR28 PET, MR T1-weighted, and diffusion-weighted imaging. *J. Nucl. Med.* 57, 1905–1912. <https://doi.org/10.2967/jnumed.116.175083>.
- Djukanović, R., Feather, I., Gratzou, C., Walls, A., Peroni, D., Bradding, P., Judd, M., Howarth, P.H., Holgate, S.T., 1996. Effect of natural allergen exposure during the grass pollen season on airways inflammatory cells and asthma symptoms. *Thorax* 51, 575–581.
- Eggen, B.J.L., Raj, D., Hanisch, U.-K., Boddeke, H.W.G.M., 2013. Microglial phenotype and adaptation. *J. Neuroimmune Pharmacol.* 8, 807–823. <https://doi.org/10.1007/s11481-013-9490-4>.
- Eisenbarth, S., 2003. The master regulators of allergic inflammation: dendritic cells in Th2 sensitization. *Curr. Opin. Immunol.* 15, 620–626. <https://doi.org/10.1016/j.coi.2003.09.003>.
- Ericsson, A., Mannerkorpi, K., 2007. Assessment of fatigue in patients with fibromyalgia and chronic widespread pain. Reliability and validity of the Swedish version of the MFI-20. *Disabil. Rehabil.* 29, 1665–1670. <https://doi.org/10.1080/09638280601055782>.
- Folkersma, H., Boellaard, R., Yaqub, M., Kloet, R.W., Windhorst, A.D., Lammertsma, A. A., Vandertop, W.P., van Berckel, B.N.M., 2011. Widespread and prolonged increase in (R)-11C-PK11195 binding after traumatic brain injury. *J. Nucl. Med.* 52, 1235–1239. <https://doi.org/10.2967/jnumed.110.084061>.
- Forsberg, A., Cervenka, S., Jonsson Fagerlund, M., Rasmussen, L.S., Zetterberg, H., Erlandsson Harris, H., Stridh, P., Christensson, E., Granström, A., Schening, A., Dymmel, K., Knave, N., Terrando, N., Maze, M., Borg, J., Varrone, A., Halldin, C., Blennow, K., Farde, L., Eriksson, L.L., 2017. The immune response of the human brain to abdominal surgery. *Ann. Neurol.* 81, 572–582. <https://doi.org/10.1002/ana.24909>.
- Fujita, M., Imaizumi, M., Zoghbi, S.S., Fujimura, Y., Farris, A.G., Suhara, T., Hong, J., Pike, V.W., Innis, R.B., 2008. Kinetic analysis in healthy humans of a novel positron emission tomography radioligand to image the peripheral benzodiazepine receptor, a potential biomarker for inflammation. *Neuroimage* 40, 43–52. <https://doi.org/10.1016/j.neuroimage.2007.11.011>.
- Hannestad, J., DellaGioia, N., Gallezot, J.-D., Lim, K., Nabulsi, N., Esterlis, I., Pittman, B., Lee, J.-Y., O'Connor, K.C., Pelletier, D., Carson, R.E., 2013. The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: a [¹¹C]PBR28 PET study. *Brain Behav. Immun.* 33, 131–138. <https://doi.org/10.1016/j.bbi.2013.06.010>.
- Hannestad, J., Gallezot, J.D., Schafbauer, T., Lim, K., Kloczynski, T., Morris, E.D., Carson, R.E., Ding, Y.S., Cosgrove, K.P., 2012. Endotoxin-induced systemic inflammation activates microglia: [¹¹C]PBR28 positron emission tomography in nonhuman primates. *Neuroimage* 63, 232–239. <https://doi.org/10.1016/j.neuroimage.2012.06.055>.
- Harrison, N.A., Brydon, L., Walker, C., Gray, M.A., Steptoe, A., Dolan, R.J., Critchley, H. D., 2009. Neural origins of human sickness in interoceptive responses to inflammation. *Biol. Psychiatry* 66, 415–422. <https://doi.org/10.1016/j.biopsych.2009.03.007>.
- Hart, B.L., 1988. Biological basis of the behavior of sick animals. *Neurosci. Biobehav. Rev.* 12, 123–137. [https://doi.org/10.1016/S0149-7634\(88\)80004-6](https://doi.org/10.1016/S0149-7634(88)80004-6).
- Hillmer, A.T., Sandiego, C.M., Hannestad, J., Angarita, G.A., Kumar, A., McGovern, E. M., Huang, Y., O'Connor, K.C., Carson, R.E., O'Malley, S.S., Cosgrove, K.P., 2017. In vivo imaging of translocator protein, a marker of activated microglia, in alcohol dependence. *Mol. Psychiatry*. <https://doi.org/10.1038/mp.2017.10>.
- Hsu, J.C., Lee, Y.S., Chang, C.N., Chuang, H.L., Ling, E.A., Lan, C.T., 2003. Sleep deprivation inhibits expression of NADPH-d and NOS while activating microglia and astroglia in the rat hippocampus. *Cells Tissues Organs* 173, 242–254. <https://doi.org/10.1159/000070380>.
- Hurwitz, E.L., Morgenstern, H., 1999. Cross-sectional associations of asthma, hay fever, and other allergies with major depression and low-back pain among adults aged 20–39 years in the United States. *Am. J. Epidemiol.* 150, 1107–1116.
- Imeri, L., Opp, M.R., 2009. How (and why) the immune system makes us sleep. *Nat. Rev. Neurosci.* 10, 199–210. <https://doi.org/10.1038/nrn2576>.
- Irwin, M.R., 2015. Why sleep is important for health: a psychoneuroimmunology perspective. *Annu. Rev. Psychol.* 66, 143–172. <https://doi.org/10.1146/annurev-psych-010213-115205>.
- I. Israel A. Ohsiek E. Al-Momani C. Albert-Weissenberger C. Stetter S. Menci A.K. Buck C. Kleinschmitt S. Samnick A.L. Sirén, Combined [18F]DPA-714 micro-positron emission tomography and autoradiography imaging of microglia activation after closed head injury in mice. *J. Neuroinflamm.* 13 2016 140 10.1186/s12974-016-0604-9
- Jernelöv, S., Olgart Höglund, C., Axelsson, J., Axén, J., Grönneberg, R., Grunewald, J., Stierna, P., Lekander, M., 2009. Effects of examination stress on psychological responses, sleep and allergic symptoms in atopic and non-atopic students. *Int. J. Behav. Med.* 16, 305–310. <https://doi.org/10.1007/s12529-008-9020-6>.
- Juniper, E.F., O'Byrne, P.M., Guyatt, G.H., Ferrie, P.J., King, D.R., 1999. Development and validation of a questionnaire to measure asthma control. *Eur. Respir. J.* 14, 902–907.
- Jutel, M., Agache, I., Bonini, S., Burks, A.W., Calderon, M., Canonica, W., Cox, L., Demoly, P., Frew, A.J., O'Hehir, R., Kleine-Tebbe, J., Muraro, A., Lack, G., Larenas, D., Levin, M., Nelson, H., Pawankar, R., Pfaar, O., van Ree, R., Sampson, H., Santos, A.F., Du Toit, G., Werfel, T., Gerth van Wijk, R., Zhang, L., Akdis, C.A., 2015. International consensus on allergy immunotherapy. *J. Allergy Clin. Immunol.* 136, 556–568. <https://doi.org/10.1016/j.jaci.2015.04.047>.
- Kalk, N.J., Guo, Q., Owen, D., Cherian, R., Erritzoe, D., Gilmour, A., Ribeiro, A.S., McGonigle, J., Waldman, A., Matthews, P., Cavanagh, J., McInnes, I., Dar, K., Gunn, R., Rabiner, E.A., Lingford-Hughes, A.R., 2017. Decreased hippocampal translocator protein (18 kDa) expression in alcohol dependence: a [¹¹C]PBR28 PET study. *Transl. Psychiatry* 7, e996. <https://doi.org/10.1038/tp.2016.264>.
- Kanegawa, N., Collste, K., Forsberg, A., Schain, M., Arakawa, R., Jucaite, A., Lekander, M., Olgart Höglund, C., Kosek, E., Lampa, J., Halldin, C., Farde, L., Varrone, A., Cervenka, S., 2016. In vivo evidence of a functional association between immune cells in blood and brain in healthy human subjects. *Brain Behav. Immun.* 54, 149–157. <https://doi.org/10.1016/j.bbi.2016.01.019>.
- Karshikoff, B., Sundelin, T., Lasselén, J., 2017. Role of inflammation in human fatigue: relevance of multidimensional assessments and potential neuronal mechanisms. *Front. Immunol.* 8, 21. <https://doi.org/10.3389/fimmu.2017.00021>.
- Kim, S.-H., Won, H.-K., Moon, S.-D., Kim, B.-K., Chang, Y.-S., Kim, K.-W., Yoon, I.-Y., 2017. Impact of self-reported symptoms of allergic rhinitis and asthma on sleep disordered breathing and sleep disturbances in the elderly with polysomnography study. *PLoS One* 12, e0173075. <https://doi.org/10.1371/journal.pone.0173075>.
- Klein, B., Mrowetz, H., Thalhammer, J., Scheibhofer, S., Weiss, R., Aigner, L., 2016. Allergy enhances neurogenesis and modulates microglial activation in the hippocampus. *Front. Cell. Neurosci.* 10, 1–15. <https://doi.org/10.3389/fncel.2016.00169>.
- Knezevic, D., Mizrahi, R., 2017. Molecular imaging of neuroinflammation in Alzheimer's disease and mild cognitive impairment. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. <https://doi.org/10.1016/j.pnpbp.2017.05.007>.
- Kreisl, W., Lyoo, C.H., McGwier, M., Snow, J., Jenko, K.J., Kimura, N., Corona, W., Morse, C.L., Zoghbi, S.S., Pike, V.W., McMahon, F.J., Turner, R.S., Innis, R.B., 2013a. In vivo radioligand binding to translocator protein correlates with severity of Alzheimer's disease. *Brain* 136, 2228–2238. <https://doi.org/10.1093/brain/awt145>.
- Kreisl, W.C., Jenko, K.J., Hines, C.S., Lyoo, C.H., Corona, W., Morse, C.L., Zoghbi, S.S., Hyde, T., Kleinman, J.E., Pike, V.W., McMahon, F.J., Innis, R.B., Biomarkers Consortium PET Radioligand Project Team, 2013b. A genetic polymorphism for translocator protein 18 kDa affects both in vitro and in vivo radioligand binding in human brain to this putative biomarker of neuroinflammation. *J. Cereb. Blood Flow Metab.* 33, 53–58. <https://doi.org/10.1038/jcbfm.2012.131>.
- Krueger, J.M., Kubillus, S., Shoham, S., Davenne, D., 1986. Enhancement of slow-wave sleep by endotoxin and lipid A. *Am. J. Physiol.* 251, 591–597.
- Krueger, J.M., Opp, M.R., 2016. Sleep and microbes. *Int. Rev. Neurobiol.*, 207–225. <https://doi.org/10.1016/bs.im.2016.07.003>.
- Kurt, E., Aktas, A., Gulbas, Z., Erginel, S., Arslan, S., 2010. The effects of natural pollen exposure on inflammatory cytokines and their relationship with nonspecific bronchial hyperresponsiveness in seasonal allergic rhinitis. *Allergy Asthma Proc.* 31, 126–131. <https://doi.org/10.2500/aap.2010.31.3325>.

- Lasselín, J., Elsenbruch, S., Lekander, M., Axelsson, J., Karshikoff, B., Grigoleit, J.-S., Engler, H., Schedlowski, M., Benson, S., 2016. Mood disturbance during experimental endotoxemia: predictors of state anxiety as a psychological component of sickness behavior. *Brain Behav. Immun.* 57, 30–37. <https://doi.org/10.1016/j.bbi.2016.01.003>.
- Lasselín, J., Treadway, M.T., Lacourt, T.E., Soop, A., Olsson, M.J., Karshikoff, B., Paues-Goranson, S., Axelsson, J., Dantzer, R., Lekander, M., 2017. Lipopolysaccharide alters motivated behavior in a monetary reward task: a randomized trial. *Neuropsychopharmacol.* 42 (4), 801–810.
- Léger, D., Annesi-Maesano, I., Carat, F., Rugina, M., Chanal, I., Pribil, C., El Hasnaoui, A., Bousquet, J., 2015. Allergic rhinitis and its consequences on quality of sleep. *Arch. Intern. Med.* 166, 1744–1748. <https://doi.org/10.1001/archinte.166.16.1744>.
- Lekander, M., Andreasson, A.N., Kecklund, G., Ekman, R., Ingre, M., Akerstedt, T., Axelsson, J., 2013. Subjective health perception in healthy young men changes in response to experimentally restricted sleep and subsequent recovery sleep. *Brain Behav. Immun.* 34, 43–46. <https://doi.org/10.1016/j.bbi.2013.06.005>.
- Lekander, M., Karshikoff, B., Johansson, E., Soop, A., Fransson, P., Lundström, J.N., Andreasson, A., Ingvar, M., Petrovic, P., Axelsson, J., Nilsson, G., 2016. Intrinsic functional connectivity of insular cortex and symptoms of sickness during acute experimental inflammation. *Brain Behav. Immun.* 56, 34–41. <https://doi.org/10.1016/j.bbi.2015.12.018>.
- Lensmar, C., Katchar, K., Eklund, A., Grunewald, J., Wahlström, J., 2006. Phenotypic analysis of alveolar macrophages and lymphocytes following allergen inhalation by atopic subjects with mild asthma. *Respir. Med.* 100, 918–925. <https://doi.org/10.1016/j.rmed.2005.08.014>.
- Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Wilson, C.J., Schirmer, L., Bennett, M.L., Münch, A.E., Chung, W.-S., Peterson, T.C., Wiltan, D.K., Froin, A., Napier, B.A., Panicker, N., Kumar, M., Buckwalter, M.S., Rowitch, D.H., Dawson, V.L., Dawson, T.M., Stevens, B., Barres, B.A., 2017. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481–487. <https://doi.org/10.1038/nature21029>.
- Maier, S.F., 2003. Bi-directional immune-brain communication: implications for understanding stress, pain, and cognition. *Brain Behav. Immun.* 17, 69–85.
- Marshall, P.S., Colon, E.A., 1993. Effects of allergy season on mood and cognitive function. *Ann. Allergy* 71, 251–258.
- Marshall, P.S., O'Hara, C., Steinberg, P., 2002. Effects of seasonal allergic rhinitis on fatigue levels and mood. *Psychosom. Med.* 64, 684–691. <https://doi.org/10.1097/01.PSY.0000021944.35402.44>.
- Marshall, P.S., O'Hara, C., Steinberg, P., 2000. Effects of seasonal allergic rhinitis on selected cognitive abilities. *Ann. Allergy Asthma Immunol.* 84, 403–410. [https://doi.org/10.1016/S1081-1206\(10\)62273-9](https://doi.org/10.1016/S1081-1206(10)62273-9).
- Meltzer, E.O., Gross, G.N., Kattal, R., Storms, W.W., 2012. Allergic rhinitis substantially impacts patient quality of life: findings from the Nasal Allergy Survey Assessing Limitations. *J. Fam. Pract.* 61, S5–S10.
- Mirotti, L., Castro, J., Costa-Pinto, F.A., Russo, M., 2010. Neural pathways in allergic inflammation. *J. Allergy* 2010, 1–11. <https://doi.org/10.1155/2010/491928>.
- Mullol, J., Maurer, M., Bousquet, J., 2008. Sleep and allergic rhinitis. *J. Invest. Allergol. Clin. Immunol.*
- Notter, T., Coughlin, J.M., Gschwind, T., Wang, Y., Kassiou, M., Vernon, A.C., Benke, D., Pomper, M.G., 2017. Translational evaluation of translocator protein as a marker of neuroinflammation in schizophrenia. *Nat. Publ. Gr.* 1–12. <https://doi.org/10.1038/mp.2016.248>.
- Osguthorpe, J.D., 2012. Pathophysiology of and potential new therapies for allergic rhinitis. *Int. Forum Allergy Rhinol.* 1–9. <https://doi.org/10.1002/alf.21120>.
- Owen, D.R., Yeo, A.J., Gunn, R.N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D.J., Bennacef, I., Parker, C.A., Stejan, P.L., Cardon, L.R., Mooser, V.E., Matthews, P.M., Rabiner, E.A., Rubio, J.P., 2012. An 18-kDa Translocator Protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J. Cereb. Blood Flow Metab.* 32147, 1–5. <https://doi.org/10.1038/jcbfm.2011.147>.
- Ozdoganoglu, T., Songu, M., Inancil, H.M., 2012. Quality of life in allergic rhinitis. *Ther. Adv. Respir. Dis.* 6, 25–39. <https://doi.org/10.1177/1753465811424425>.
- Parente, A., Feltes, P.K., Vallez Garcia, D., Sijbesma, J.W.A., Moriguchi Jeckel, C.M., Dierckx, R.A.J.O., de Vries, E.F.J., Doorduyn, J., 2016. Pharmacokinetic analysis of 11C-PBR28 in the rat model of herpes encephalitis: comparison with (R)-11C-PK11195. *J. Nucl. Med.* 57, 785–791. <https://doi.org/10.2967/jnumed.115.165019>.
- Sandiego, C.M., Gallezot, J.-D., Pittman, B., Nabulsi, N., Lim, K., Lin, S.-F., Matuskey, D., Lee, J.-Y., O'Connor, K.C., Huang, Y., Carson, R.E., Hannestad, J., Cosgrove, K.P., 2015. Imaging robust microglial activation after lipopolysaccharide administration in humans with PET. *Proc. Natl. Acad. Sci. U.S.A.* 112, 12468–12473. <https://doi.org/10.1073/pnas.1511003112>.
- Sanna, L., Stuart, A.L., Pasco, J.A., Jacka, F.N., Berk, M., Maes, M., O'Neil, A., Girardi, P., Williams, L.J., 2014. Atopic disorders and depression: findings from a large, population-based study. *J. Affect. Disord.* 155, 261–265. <https://doi.org/10.1016/j.jad.2013.11.009>.
- Santos, C.B., Pratt, E.L., Hanks, C., McCann, J., Craig, T.J., 2006. Allergic rhinitis and its effect on sleep, fatigue, and daytime somnolence (quiz 586–9, 671) *Ann. Allergy. Asthma Immunol.* 97, 579–586. [https://doi.org/10.1016/S1081-1206\(10\)61084-8](https://doi.org/10.1016/S1081-1206(10)61084-8).
- Sarlus, H., Höglund, C.O., Karshikoff, B., Wang, X., Lekander, M., Schultzberg, M., Oprica, M., 2012. Allergy influences the inflammatory status of the brain and enhances tau-phosphorylation. *J. Cell. Mol. Med.* 16, 2401–2412. <https://doi.org/10.1111/j.1582-4934.2012.01556.x>.
- Sarlus, H., Wang, X., Cedazo-Minguez, A., Schultzberg, M., Oprica, M., 2013. Chronic airway-induced allergy in mice modifies gene expression in the brain toward insulin resistance and inflammatory responses. *J. Neuroinflamm.* 10, 866. <https://doi.org/10.1186/1742-2094-10-99>.
- Setiawan, E., Wilson, A.A., Mizrahi, R., Rusjan, P.M., Miler, L., Rajkowska, G., Suridjan, I., Kennedy, J.L., Rekkas, P.V., Houle, S., Meyer, J.H., 2015. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry* 72, 268. <https://doi.org/10.1001/jamapsychiatry.2014.2427>.
- Shambroom, J.R., Fábregas, S.E., Johnstone, J., 2012. Validation of an automated wireless system to monitor sleep in healthy adults. *J. Sleep Res.* 21, 221–230. <https://doi.org/10.1111/j.1365-2869.2011.00944.x>.
- Small, M., Piercy, J., Demoly, P., Marsden, H., 2013. Burden of illness and quality of life in patients being treated for seasonal allergic rhinitis: a cohort survey. *Clin. Transl. Allergy* 3, 33. <https://doi.org/10.1186/2045-7022-3-33>.
- Smets, E.M., Garssen, B., Bonke, B., De Haes, J.C., 1995. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J. Psychosom. Res.* 39, 315–325.
- Storms, W., 2008. Allergic rhinitis-induced nasal congestion: its impact on sleep quality. *Prim. Care Respir. J.* <https://doi.org/10.3132/pcrj.2008.00001>.
- Theodoropoulos, D.S., Lockey, R.F., 2000. Allergen immunotherapy: guidelines, update, and recommendations of the World Health Organization. *Allergy Asthma Proc.* 21, 159–166.
- Tonelli, L.H., Katz, M., Kovacsics, C.E., Gould, T.D., Joppy, B., Hoshino, A., Hoffman, G., Komarow, H., Postolache, T.T., 2009. Allergic rhinitis induces anxiety-like behavior and altered social interaction in rodents. *Brain Behav. Immun.* 23, 784–793. <https://doi.org/10.1016/j.bbi.2009.02.017>.
- Tóth, M., Little, P., Arnberg, F., Häggkvist, J., Mulder, J., Halldin, C., Gulyás, B., Holmin, S., 2016. Acute neuroinflammation in a clinically relevant focal cortical ischemic stroke model in rat: longitudinal positron emission tomography and immunofluorescent tracking. *Brain Struct. Funct.* 221, 1279–1290. <https://doi.org/10.1007/s00429-014-0970-y>.
- Townley, R.G., Horiba, M., 2003. Airway hyperresponsiveness: a story of mice and men and cytokines. *Clin. Rev. Allergy Immunol.* 24, 85–109. <https://doi.org/10.1385/CRIAI.24:1:85>.
- Trikojatz, K., Buske-Kirschbaum, A., Schmitt, J., Plessow, F., 2014. Altered performance in attention tasks in patients with seasonal allergic rhinitis: seasonal dependency and association with disease characteristics. *Psychol. Med.* 45, 1–11. <https://doi.org/10.1017/S0033291714002384>.
- Trikojatz, K., Luksch, H., Rösen-Wolff, A., Plessow, F., Schmitt, J., Buske-Kirschbaum, A., 2017. “Allergic mood” – depressive and anxiety symptoms in patients with seasonal allergic rhinitis (SAR) and their association to inflammatory, endocrine, and allergic markers. *Brain Behav. Immun.* <https://doi.org/10.1016/j.bbi.2017.05.005>.
- Tronel, C., Largeau, B., Santiago Ribeiro, M., Guilloteau, D., Dupont, A.-C., Arlicot, N., 2017. Molecular targets for PET imaging of activated microglia: the current situation and future expectations. *Int. J. Mol. Sci.* 18, 802. <https://doi.org/10.3390/ijms18040802>.
- Venneti, S., Lopresti, B.J., Wiley, C.A., 2013. Molecular imaging of microglia/macrophages in the brain. *Glia* 61, 10–23. <https://doi.org/10.1002/glia.22357>.
- Venneti, S., Lopresti, B.J., Wiley, C.A., 2006. The peripheral benzodiazepine receptor (translocator protein 18 kDa) in microglia: from pathology to imaging. *Prog. Neurobiol.* 80, 308–322. <https://doi.org/10.1016/j.pneurobio.2006.10.002>.
- Watkins, L.R., Hutchinson, M.R., Ledebor, A., Wieseler-Frank, J., Milligan, E.D., Maier, S.F., 2007. Glia as the “bad guys”: implications for improving clinical pain control and the clinical utility of opioids. *Brain Behav. Immun.* 21, 131–146. <https://doi.org/10.1016/j.bbi.2006.10.011>.
- Watkins, L.R., Maier, S.F., 1999. Implications of immune-to-brain communication for sickness and pain. *Proc. Natl. Acad. Sci. U.S.A.* 96, 7710–7713. <https://doi.org/10.1073/pnas.96.14.7710>.
- Watt, T., 2000. Fatigue in the Danish general population. Influence of sociodemographic factors and disease. *J. Epidemiol. Community Heal.* 54, 827–833. <https://doi.org/10.1136/jech.54.11.827>.
- Winther, L., Moseholm, L., Reimert, C.M., Stahl Skov, P., Kaergaard Poulsen, L., 1999. Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis. *Allergy* 54, 436–445.
- Wisor, J.P., Schmidt, M.A., Clegern, W.C., 2011. Evidence for neuroinflammatory and microglial changes in the cerebral response to sleep loss. *Sleep* 34, 261–272.
- Zhu, B., Dong, Y., Xu, Z., Gompf, H.S., Ward, S.A.P., Xue, Z., Miao, C., Zhang, Y., Chamberlin, N.L., Xie, Z., 2012. Sleep disturbance induces neuroinflammation and impairment of learning and memory. *Neurobiol. Dis.* 48, 348–355. <https://doi.org/10.1016/j.nbd.2012.06.022>.

Evidence of fatigue, disordered sleep and peripheral inflammation, but not increased brain TSPO expression, in seasonal allergy: A [^{11}C]PBR28 PET study

Sandra Tamm, Simon Cervenka, Anton Forsberg, Johanna Estelius, Johan Grunewald, Pär Gyllfors, Bianka Karshikoff, Eva Kosek, Jon Lampa, Catarina Lensmar, Victoria Strand, Torbjörn Åkerstedt, Christer Halldin, Martin Ingvar, Caroline Olgart Höglund, Mats Lekander

Supplement

Method

The present study was part of a larger project. In parallel with the reported study, patients with rheumatoid arthritis (RA) and matched healthy subjects were studied at one occasion, but in an otherwise corresponding design. These and other data will be presented elsewhere.

As shown in fig 1 in the main text, cognitive assessments and quantitative sensory testing (pain tests) were performed, heart rate variability (ECG) was measured and samples of urine and body odor collected.

In conjunction to the PET-examinations, validated questionnaires were used to measure level of anxiety (*State-Trait Anxiety Inventory*), depression (*Hospital Anxiety and Depression Scale*), stress (*Perceived Stress Scale*, a visual analogue scale (VAS)), tiredness/fatigue (*Swedish Occupational Fatigue Inventory*, *Fatigue Severity Scale*, *The Multidimensional Fatigue Inventory*, VAS), sleepiness (*Karolinska Sleepiness Scale*), sleep (*Pittsburgh Sleep Quality Index*, *Karolinska Sleep Diary*), subjective health (*The Short Form (36) Health Survey*, *Self-Rated Health*) and sickness behavior (*Sickness Q*). Allergic symptoms were measured with questionnaires for rhinitis and asthma (i.e. daily symptom scoring and scoring

of symptoms for the 2 last weeks, *The Rhinoconjunctivitis Quality of Life Questionnaire*, *The Asthma Quality of Life Questionnaire*, *The Asthma Control Questionnaire*). We also measured RA symptoms (*Health Assessment Questionnaire*), pain (*Pain Disability Index*, VAS, “pain drawing”) and quality of life (*EuroQol five dimensions questionnaire*). To be able to collapse PET data across studies, participants filled in scales of personality (*The Revised NEO Personality Inventory*, *The Temperament and Character Inventory*, *Swedish Universities Scales of Personality*) used in other studies at the PET center.

Data loss

MFI-20 was available for 18 patients and 13 healthy subjects, but one patient only had data from one measurement (during pollen season). Symptom ratings were available for 18 patients and 13 healthy subjects, but two patients only filled in the symptom reports during pollen season and 1 healthy subject only filled in reports outside pollen season. Spirometry data was available for 16 patients and 13 healthy subjects, skin prick tests for 17 patients and 13 healthy subjects, and phadiatop for 18 patients and 12 healthy subjects. Data on educational level was missing for three healthy subjects, BMI for seven patients. Cytokine data were available for 18 patients and 13 healthy subjects, but two patients only had data for one of the seasons (one from during pollen season and one from outside pollen season).

Sleep diaries were available for 18 patients and 13 healthy subjects. Two patients and two healthy subjects only filled in the diary for the first participation. Five patients and one healthy subject had missing MyZeo recordings from one or both seasons. After quality check, data from 15 patients (5 with only data from one season) and 13 healthy subjects (5 had only data from one season) were included in analyses. This resulted in 200 successfully recorded nights.

ACQ was available for 18 patients and 13 healthy subjects, but one patient only had data from during pollen season and one healthy subject only had data from outside pollen season.

Cytokine analyses

Two sets of plates were used to analyze cytokine data from this project, with slightly differing detection limits for each analyte and plate. If the values were under the detection limit, the value was imputed with the detection limit value for the respective plate. Detection limits for cytokines were as following: TNF- α : 0.145/0.145 pg/mL; IL-6: 0.116/0.173 pg/mL; IFN- γ : 0.562/0.676 pg/mL; IL-8: 0.0873/0.112 pg/mL; and IL-5: 0.136/0.143 pg/mL.

Before analyzing the cytokines, data were plotted as histograms. Based on visual inspection, IFN- γ , IL-5, IL-6 and IL-8 were log transformed to better approximate a normal distribution. Histograms are presented in supplementary figure 1.

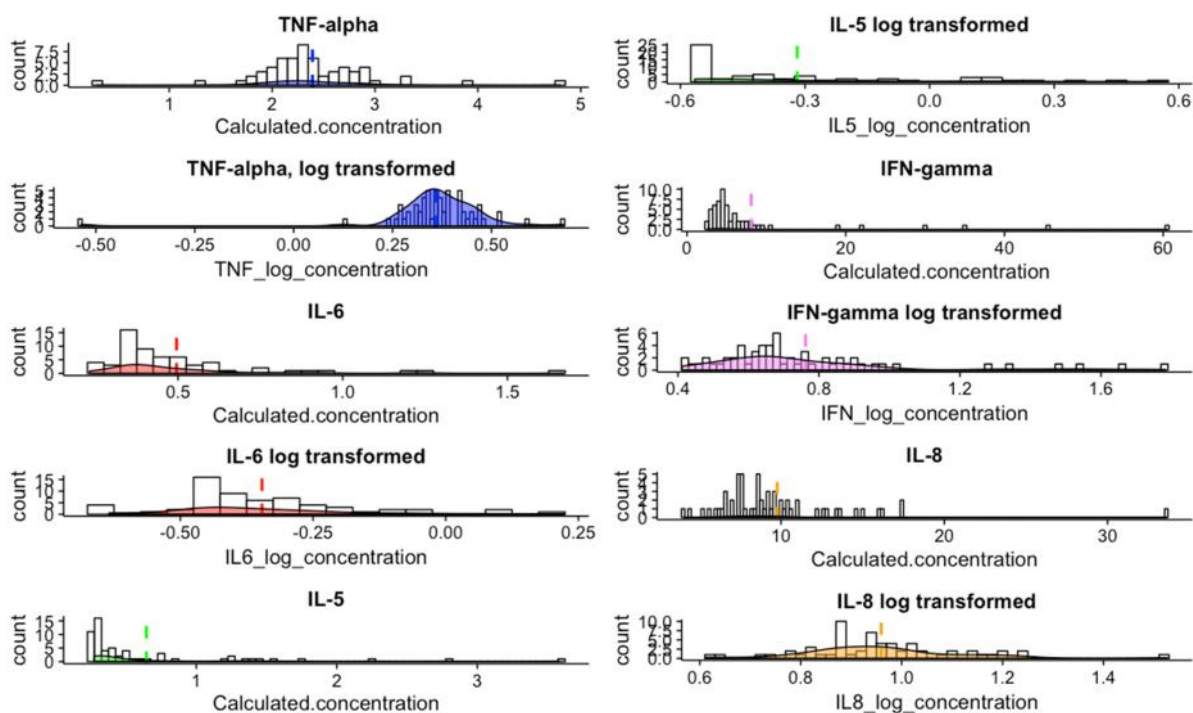


Figure S1. Histograms of cytokines.

Additional demographical data

Additional clinical data for patients and healthy subjects are presented in supplementary table 1. Age is described as intervals, to prevent identification of individual participants.

Table S1. Clinical data describing the full sample.

[illegible]

[illegible]

Pollen data

Data on pollen levels in the Stockholm area were obtained from the Swedish palynological laboratory at the Stockholm Natural History Museum (www.pollenrapporten.se) and are described in supplementary fig 2. Notably, the birch pollen levels were only moderate during 2013. However, only 2 allergic subjects were scanned during pollen season this year and only one of these subjects had an isolated birch allergy.

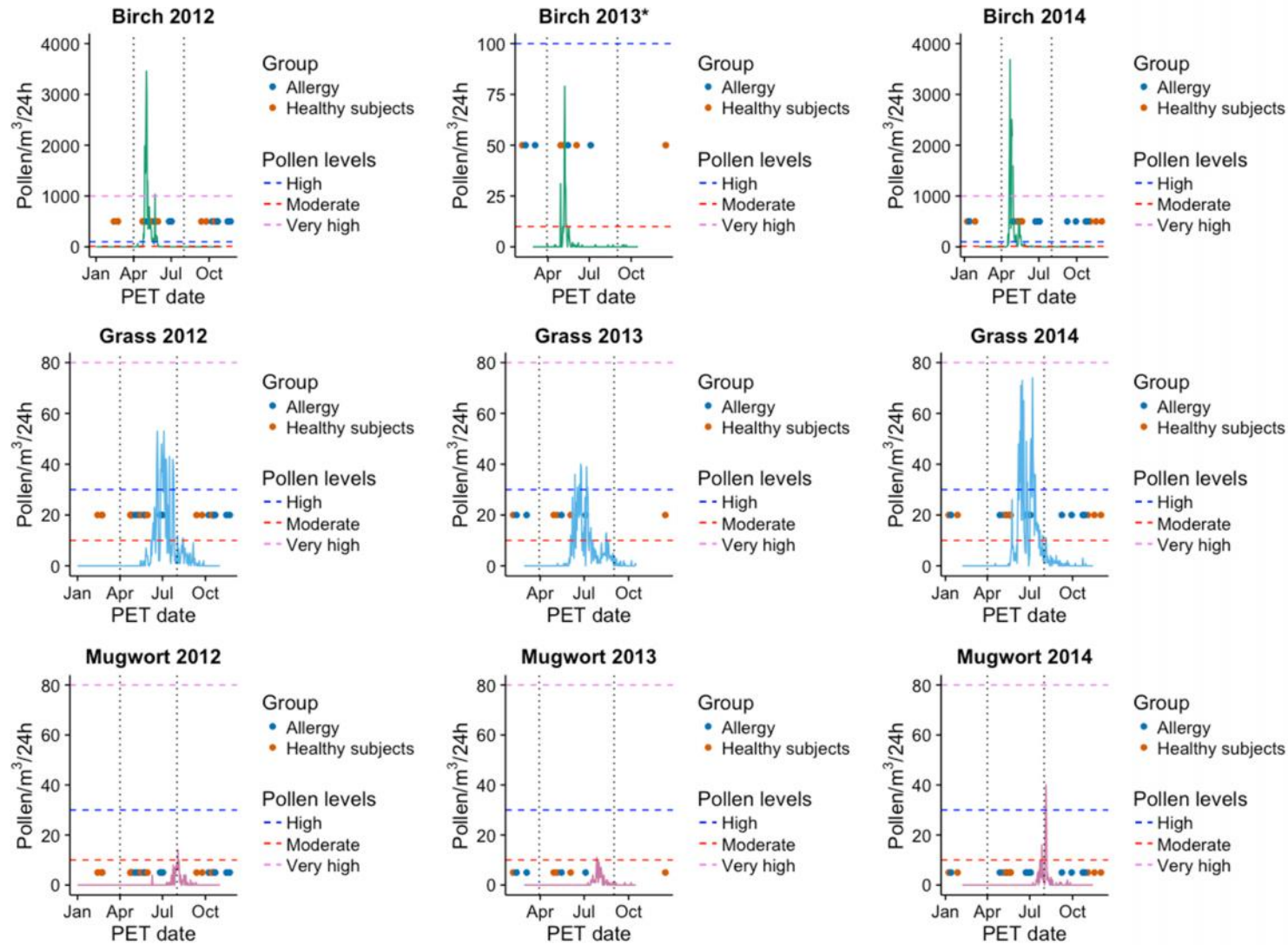


Figure S2. Pollen levels in the Stockholm are during 2012, 2013 and 2014. Vertical lines indicate between which time points scanning was considered in season and dots indicate specific time points for each subject. *Note that the y axis is on a different scale.

PET data

Table S2. Kinetic parameters and radiochemistry details for the PET examination. Values represent means and SE in brackets. At a significance level of 0.05, there was no difference between groups or seasons for the free fraction or the input function.

Group	Pollen season	K1	k2	k3	k4	Specific radioactivity (GBq μmol^{-1})	Input function (calculated as AUC)	Injected mass of tracer (μg)	Free fraction (%)
Allergy	In	0.16 (0.02)	0.18 (0.02)	0.1 (0.02)	0.04 (0)	314 (65)	35940 (2985)	0.58 (0.1)	9.66 (0.92)
Allergy	Out	0.15 (0.01)	0.17 (0.02)	0.08 (0.02)	0.04 (0)	267 (42)	31787 (4111)	0.61 (0.13)	8.4 (1.2)
Healthy subjects	In	0.13 (0.02)	0.16 (0.02)	0.09 (0.02)	0.03 (0)	362 (73)	36647 (3910)	0.49 (0.08)	8.76 (0.96)
Healthy subjects	Out	0.15 (0.02)	0.19 (0.02)	0.12 (0.03)	0.04 (0.01)	310 (73)	34856 (4561)	0.62 (0.13)	9.15 (1.42)

TSPO genotype and sex were strong predictors of [^{11}C]PBR28 binding. Data plotted in supplementary fig 3 and 4.

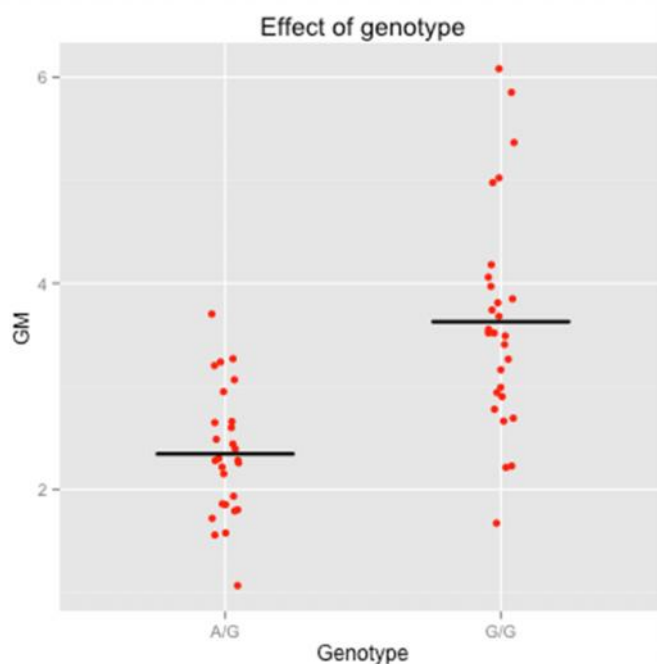


Figure S3. Effect of genotype. Lines indicate means across all subjects and dots indicate individual scans (2 per subject).

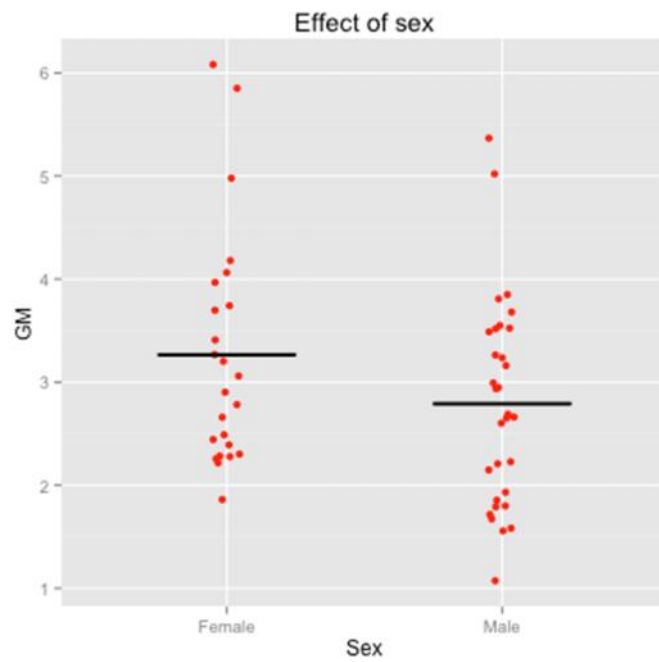


Figure S4. Effect of sex. Lines indicate means across all subjects and dots indicate individual scans (2 per subject).

Because this is the first imaging study to examine TSPO-binding in allergy, we plotted effects for each ROI in patients and healthy subjects across seasons, supplementary fig 5, for descriptive purposes only. Based on visual inspection, no regional differences were observed.

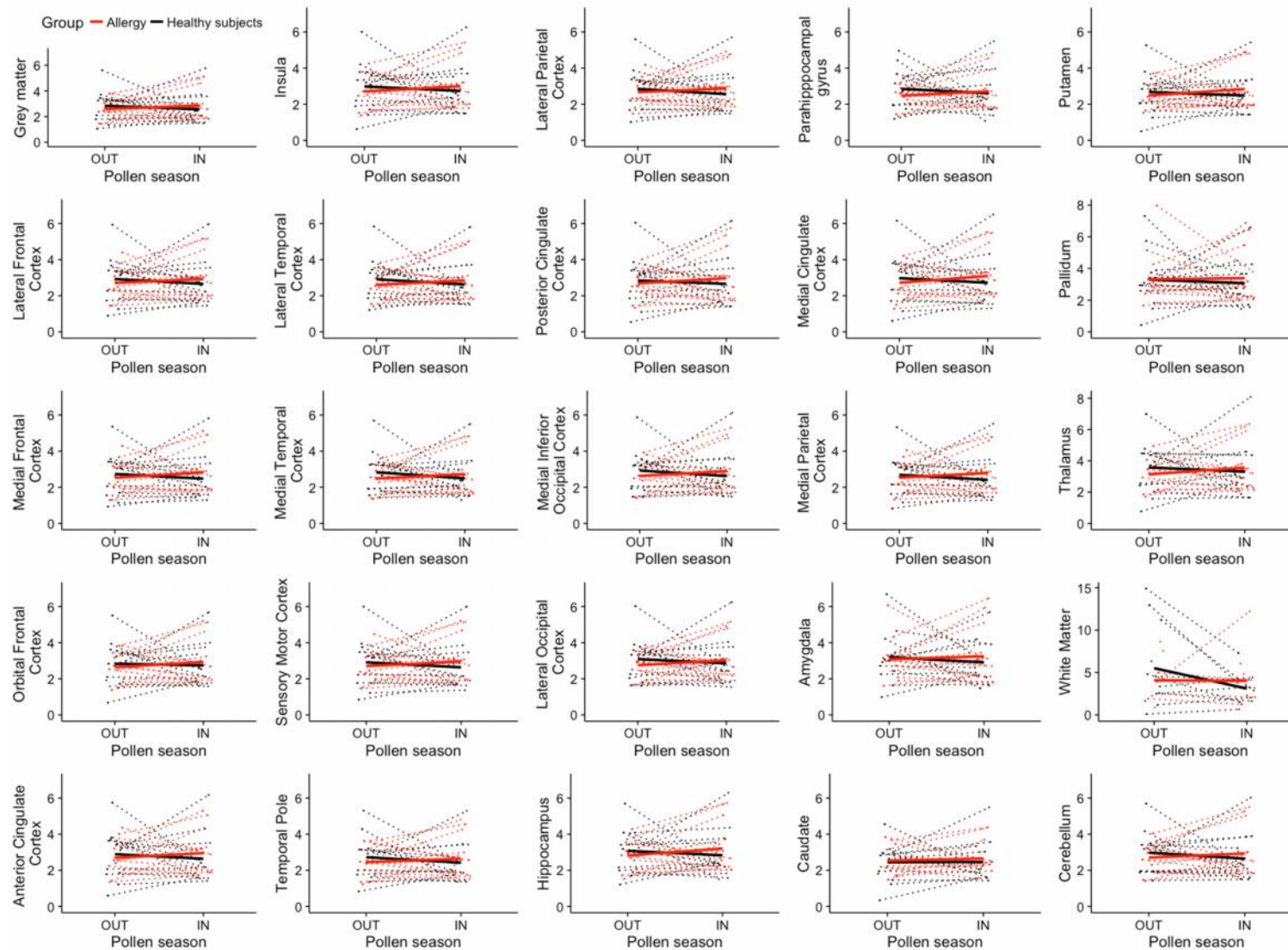


Figure S5. Regional differences in $[^{11}\text{C}]\text{PBR28}$ binding in and out of pollen season plotted per ROI. Dotted lines indicate individual subjects and solid lines indicate means across groups

Supplementary table 3. Cytokines as predictors of TSPO binding, general fatigue and total sleep time. Table showing effect estimates + 95 % CI and p values obtained through mixed effects models.

	^[11C] PBR28		General fatigue		Total sleep time	
	Estimate + CI	<i>p</i>	Estimate + CI	<i>p</i>	Estimate + CI	<i>p</i>
Cytokines						
TNF- α	0.18 (-0.34-0.69)	0.482	-0.06 (-1.72-1.61)	0.946	-0.01 (-0.43-0.41)	0.954
log(IL-6)	-0.69 (-2.21-0.82)	0.352	2.29 (-2.52-7.09)	0.336	-0.4 (-1.4-0.59)	0.401
log(IL-5)	0.16 (-0.9-1.22)	0.758	-1.73 (-5.56-2.1)	0.361	0.7 (-0.19-1.58)	0.113
log(IFN- γ)	0.29 (-0.67-1.25)	0.538	-2.29 (-5.16-0.57)	0.112	-0.07 (-0.75-0.6)	0.828
log(IL-8)	-0.34 (-2.03-1.35)	0.68	-1.85 (-7.87-4.18)	0.534	-0.17 (-1.44-1.09)	0.774
log(IFN- γ)/log(IL-5)	0.02 (-0.05-0.09)	0.483	0.24 (0.06-0.42)	0.012	0.03 (-0.02-0.07)	0.191

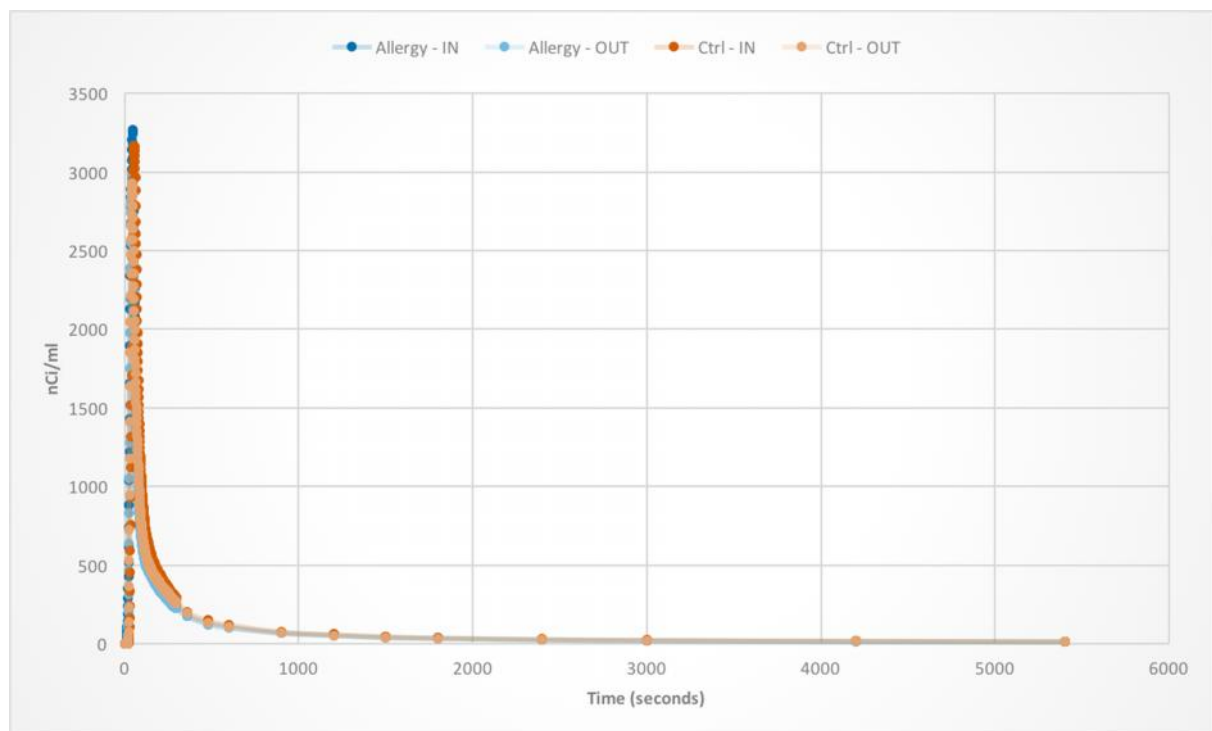


Figure S6. Metabolite-corrected plasma input function for both groups, in and out of pollen season.