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

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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-\(\alpha\), IL-5, IL-6, IL-8 and IFN-\(\gamma\) 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-\(\alpha\) 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.

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 hypothetic 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...
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 are produced in response to allergen challenges. Th2-driven inflammation was thought to be reflected in a decreased IFN-γ/IL-5 ratio. We also hypothesized that the degree of [11C]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 [11C]PBR28 grey matter binding in the brain, as indicating immune-to-brain signaling.

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 [11C]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 [11C]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 [11C]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 rhinocconjunctivitis 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 consent of Stockholm (no: 2011/1846-31/1).
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 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 course of the study, we also added collection of saliva samples at screening, for analysis of polymorphisms of CRP.

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.

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 antihistamines 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. [11C]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, Mariefjord, 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 (Colliste 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 (Colliste 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. $[^{11}\text{C}]$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 $[^{11}\text{C}]$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 (Colliste 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 $-80^\circ$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-1β, IL-2, IL-4, IL-6, IL-8, TNF-α), single spot V-PLEX Human IL-5 Kit, K15105D (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-1β, 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 INF-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 sleep data was used to determine a measure of subjective sleep quality (Åkerstedt et al., 1994) and a single frontal EEG electrode (MyZeo, Zoo 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 (Activwatch, Philips Respironics), a wristwatch 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 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. 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 subject 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 medicated with oral antihistamines. Some university

3.2. \([^{11}C]\)PBR28 binding

All results are described as effect estimates with 95% confidence intervals in hard brackets. As expected, HAB genotype was associated with increased \([^{11}C]\)PBR28 binding in the total grey matter in the brain (1.35 [0.84, 1.85], \(p < 0.0001\)), compared to MAB. There was no group-pollen season interaction (0.49 [–0.48, 1.46], \(p = 0.30\), Figs. 2 and 3A) for \([^{11}C]\)PBR28 binding in the brain. Further, there was no main effect of group (allergic subjects vs healthy subjects) (0.29 [–0.21, 0.80], \(p = 0.24\), Fig. 3) or pollen season (in vs out of season) (0.01 [–0.47, 0.50], \(p = 0.96\), Fig. 3A) on \([^{11}C]\)PBR28 binding. Male sex was significantly associated with lower \([^{11}C]\)PBR28 binding (–0.69 [–1.16, –0.23], \(p = 0.005\)), but adjusting the model for sex did not appreciably change results. For all factors, allergic patients during pollen season.

3.3. Plasma cytokines

For IFN-\(\gamma\) and IL-8, 60/60 of the observations were above the detection limit. For TNF-\(\alpha\) 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-\(\alpha\) and log-IL-5. When decomposed, allergic subjects had higher levels of TNF-\(\alpha\) during pollen season compared to outside of pollen season (0.37 [0.00, 0.73], \(p = 0.048\), Fig. 3B), whereas healthy subjects did not (–0.06 [–0.22, 0.10], \(p = 0.40\), Fig. 3B). Outside pollen season, there was no difference between allergic subjects and healthy subjects (–0.04 [–0.47, 0.38], \(p = 0.84\), Fig. 3B). Outside pollen season, allergic subjects had higher levels of log-IL-5 compared to healthy subjects (0.20 [0.02, 0.38], \(p = 0.03\), Fig. 3D) and were even higher during pollen season (0.14 [0.00, 0.27], \(p = 0.048\), Fig. 3D). In healthy subjects, no difference was observed between seasons (–0.05 [–0.14, 0.04], \(p = 0.29\), Fig. 3D). For neither log-IL-6, log-INF-\(\gamma\) 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-INF-\(\gamma\)/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 1

| Variables          | Allergy | Healthy subjects |
|--------------------|---------|------------------|
| Age (median, IQR)  | 34 (30.2–44.2) | 34 (27.0–46.0) |
| BMI                | 25.3 (±4.4)   | 22.4 (±3.0)      |
| Sample             | 18       | 13               |
| Education          |          |                  |
| 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%)        |
| Genotype           |          |                  |
| 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%)        |
| Demographics       |          |                  |
| Age (median, IQR)  | 34 (30.2–44.2) | 34 (27.0–46.0) |
| Sex (females)      | 8 (44.4%) | 5 (38.5%)        |
| University graduate | 9 (50.0%) | 3 (30.0%)        |
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 (−0.30 [−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 [11C]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 [11C]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).
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

### 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.

| Cytokine | Intercept Estimate + CI | Group (allergic vs healthy subjects) Estimate + CI | Season (in vs out) Estimate + CI | Group*season Estimate + CI |
|----------|------------------------|-----------------------------------------------|-------------------------------|----------------------------|
| **Cytokine** | | | | |
| TNF-α | 2.38 (2.19–2.58) | 0.18 (0.21 to 0.57) | 0.15 (0.06 to 0.36) | 0.43 (0.01–0.85) |
| log(IL-6) | −0.35 (−0.40–0.15) | 0.05 (−0.04 to 0.19) | 0.04 (−0.05 to 0.14) | 0.78 (−0.25 to 0.11) |
| log(IL-5) | −0.44 (−0.42–0.25) | 0.34 (0.13–0.48) | 0.05 (−0.04 to 0.13) | 0.26 (0.02–0.35) |
| log(IFN-γ) | 0.75 (0.67–0.83) | 0.13 (−0.03 to 0.29) | 0.04 (−0.11 to 0.19) | 0.12 (−0.18 to 0.42) |
| log(IL-8) | 0.96 (0.91–1.01) | 0.01 (0.16 to 0.09) | 0.01 (−0.07 to 0.05) | 0.06 (−0.06 to 0.19) |
| log(IFN-γ)/log(IL-5) | −0.88 (−1.98 to 0.23) | 0.63 (−1.57 to 2.84) | 0 (−2.22 to 2.21) | 0.59 (−3.79 to 5.06) |

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**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-alpha 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-gamma) concentration. No difference between patients and healthy subjects was observed. F. Log(IL-8) concentration. No difference between patients and healthy subjects was observed.
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 Sarluis 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 [11C] 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
(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

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 | p | Estimate + CI | p | Estimate + CI | p |
| MFI-20                 |            |    |            |    |            |    |
| General fatigue        | 9.89 (8.83–10.95) | <.001 | 1.5 (0.12–2.88) | .035 | 3 (0.23–5.77) | .035 |
| Physical fatigue       | 8.43 (7.3–9.56)  | .007 | 1.52 (0.24–2.8) | .022 | 3.34 (0.79–5.9) | .012 |
| Mental fatigue         | 8.31 (7.4–9.23)  | .016 | 0.86 (0.43 to 2.15) | .185 | 2.48 (0.1 to 5.07) | .059 |
| Reduced motivation     | 6.71 (5.88–7.55)  | .059 | 1.23 (–0.09 to 2.55) | .066 | 3.08 (0.45–5.72) | .024 |
| Reduced activity       | 8.31 (7.38–9.23)  | .016 | 1.47 (0.24–2.71) | .021 | 3.87 (1.4–6.34) | .003 |

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.
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 (Trikajot 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 (Shambrook 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 [11C]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.

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