Molecular Imaging of Neuroinflammation in Alzheimer's disease.

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Published: 16 September 2015

by Springer

in Clinical and Translational Imaging

Clinical and Translational Imaging. 2015;3:437-447.

Doi: 10.1007/S40336-015-0137-8

Clinical and Translational Imaging: Reviews in Nuclear Medicine and Molecular Imaging

Neuroinflammation and Alzheimer´s disease

--Manuscript Draft--

Manuscript Number:	CATI-D-15-00026R1
Full Title:	Neuroinflammation and Alzheimer´s disease
Article Type:	Review
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Funding Information:	Vetenskapsrådet (SE) (05817) — MD, PhD Agneta Nordberg Stiftelsen för Strategisk Forskning (SE) — MD, PhD Agneta Nordberg Seventh Framework Programme (BE) (HEALTH-F2-2011-278850) — Dr. Andrea Varrone, MD, PhD Agneta Nordberg
Abstract:	Neuroinflammatory changes are observed in the brain of patients with Alzheimer´s disease (AD). Studies have shown the presence of activated microglia and astrocytes surrounding the amyloid plaques, along with the presence of cytokines and other mediators of inflammation. The role of inflammation in AD is not yet completely understood. More specifically, some inflammatory processes, such as the activation of microglia, may have detrimental or beneficial effects on the underlining neuropathology, by promoting inflammation and tissue damage or rather phagocytic activity and tissue repair. Imaging of neuroinflammation with positron emission tomography (PET) is the only technology that enables the visualization of microglia and astrocyte activation in the living human brain. PET studies with first- or second generation radioligands binding to the 18-kD translocator protein (TSPO) ([^{11}C]-R-PK11195, [^{11}C]DAA1106, [^{11}C]PBR28, [^{18}F]FEMPA) have shown some conflicting results, demonstrating on average a ~30% difference in TSPO availability between controls and AD patients, with a few studies showing no statistically significant difference between the two groups. Similar conflicting evidences have been shown when comparing subjects with mild cognitive impairment (MCI) and control subjects. Therefore, whether TSPO is a good marker for detecting in vivo microglia activation in AD is still a matter of debate. Imaging of MAO-B as a marker for astrocyte activation in AD is a valid alternative to TSPO imaging in the context of neuroinflammation. Only limited MAO-B imaging studies with [^{11}C]L-Deprenyl-D2 are available so far in AD and MCI, showing increased MAO-B binding in MCI patients compared with controls with a degree higher than that observed in AD. There are two unmet questions that are still under discussion. The first question is which neuroinflammatory process, microglia or astrocyte activation, occurs earlier in the natural course of AD from prodromal to dementia stage? Comparative studies using these two markers in MCI and AD could be important to clarify which marker could be used for earliest detection of neuroinflammatory changes in vivo. The second question is whether imaging of

	microglia or astrocytes per se is a useful marker of neuroinflammation associated with neurodegeneration. The development of new radioligands for other targets that are more directly associated with the pro- or anti-inflammatory activity of microglia could help understanding the relevance of neuroinflammation in the pathological processes leading to neurodegeneration in AD. Molecular imaging with PET can be a useful tool to determine the nature and temporal evolution of inflammation in early stages of AD in relation to other pathological markers as deposition of amyloid plaques and tau as well as clinical presentation of the disease.
Response to Reviewers:	See attachment for reply to Reviewers

Neuroinflammation and Alzheimer´s disease.

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Abstract

Neuroinflammatory changes are observed in the brain of patients with Alzheimer´s disease (AD). Studies have shown the presence of activated microglia and astrocytes surrounding the amyloid plaques, along with the presence of cytokines and other mediators of inflammation. The role of inflammation in AD is not yet completely understood. More specifically, some inflammatory processes, such as the activation of microglia, may have detrimental or beneficial effects on the underlining neuropathology, by promoting inflammation and tissue damage or rather phagocytic activity and tissue repair. Imaging of neuroinflammation with positron emission tomography (PET) is the only technology that enables the visualization of microglia and astrocyte activation in the living human brain. PET studies with first- or second generation radioligands binding to the 18-kD translocator protein (TSPO) ([^{11}C]-*R*-PK11195, [^{11}C]DAA1106, [^{11}C]PBR28, [^{18}F]FEMPA) have shown some conflicting results, demonstrating on average a ~30% difference in TSPO availability between controls and AD patients, with a few studies showing no statistically significant difference between the two groups. Similar conflicting evidences have been shown when comparing subjects with mild cognitive impairment (MCI) and control subjects. Therefore, whether TSPO is a good marker for detecting in vivo microglia activation in AD is still a matter of debate. Imaging of MAO-B as a marker for astrocyte activation in AD is a valid alternative to TSPO imaging in the context of neuroinflammation. Only limited MAO-B imaging studies with [^{11}C]L-Deprenyl-D2 are available so far in AD and MCI, showing increased MAO-B binding in MCI patients

compared with controls with a degree higher than that observed in AD. There are two unmet questions that are still under discussion. The first question is which neuroinflammatory process, microglia or astrocyte activation, occurs earlier in the natural course of AD from prodromal to dementia stage? Comparative studies using these two markers in MCI and AD could be important to clarify which marker could be used for earliest detection of neuroinflammatory changes in vivo. The second question is whether imaging of microglia or astrocytes per se is a useful marker of neuroinflammation associated with neurodegeneration. The development of new radioligands for other targets that are more directly associated with the pro- or anti-inflammatory activity of microglia could help understanding the relevance of neuroinflammation in the pathological processes leading to neurodegeneration in AD. Molecular imaging with PET can be a useful tool to determine the nature and temporal evolution of inflammation in early stages of AD in relation to other pathological markers as deposition of amyloid plaques and tau as well as clinical presentation of the disease.

Key Words: TSPO; Alzheimer; microglia; astrocytes

Neuroinflammation in Alzheimer´s disease

The involvement of inflammation in Alzheimer´s disease (AD) is suggested by different experimental findings. Epidemiological studies have shown an inverse association between the onset of AD and the treatment with anti-inflammatory drugs [1]. The use of anti-inflammatory drugs might be important in the pre-dementia stage of AD, since clinical trials have failed to demonstrate efficacy of anti-inflammatory treatments in patients with mild-to-moderate dementia [2, 3]. A range of inflammatory processes, such as activated microglia, cytokines, activation of complement cascade have been found in the AD brain [4, 5]. The role of inflammation in the natural progression of AD is still a matter of debate. Microglia and astrocytes are the glial cells involved in the modulation of the inflammatory response and repair in the brain [6, 7]. Post-mortem data indicates that microglia and astrocytes are associated with amyloid deposition in AD. In post-mortem AD brain tissue, microglia and astrocytes are found in the proximity of amyloid plaques [6, 7] (Figure 1) and pre-clinical studies in animal models of AD have shown that microglia and astrocytes are recruited around the amyloid plaques quite rapidly [8]. Astrocytes are cells that are involved in the reparative processes following inflammation and tissue damage. Although it is well acknowledged that both microglia and astrocytes are important cells involved in neuroinflammation, their relative contribution to the overall neuroinflammatory process is not fully understood.

Microglia is activated in response to cell damage and might promote a pro-inflammatory reaction that contributes to tissue damage and sustained inflammation, or can actively promote phagocytosis and tissue repair. A conventional way to indicate these two different functions of microglia is the M1/M2 polarization [9]. The M1 phenotype is conventionally considered pro-inflammatory, whereas the M2 phenotype is classically seen as anti-inflammatory. The balance between the two functional types of microglia activation might determine the end result of the inflammatory process and neurodegeneration [10]. In the

APP/PS1 mouse models of AD, it has been shown that in the hippocampus microglia undergoes an age-related phenotypic switch. At 6 months of age, when the Aβ plaque pathology develops, the M2 or alternative state with phagocytic capacity is predominant, whereas at 18 months of age there is a transition to the classical M1 state, associated with the formation of Aβ oligomers and with pyramidal degereneration [11]. The microglia displaying M2 phenotype was located mainly around the Aβ plaques and was present also at 18 months, at the time of maximal activation of the M1 phenotype [11]. In AD patients it is more difficult to examine this transition and studies have found the presence of both activation phenotypes of microglia [10]. Therefore, the development of specific radioligands for the two activated states of microglia could be helpful to study in vivo the relative contribution and longitudinal progression of the M1/M2 phenotypes in relation to AD pathology.

PET Imaging of neuroinflammation in AD

The most established target for noninvasive molecular imaging of neuroinflammation in AD is the 18-kD translocator protein (TSPO) [12]. TSPO is expressed on the inner mitochondrial membrane of several cells including monocytes, macrophages, microglia and astrocytes [13]. The activation of microglia following tissue damage results in an increased density of TSPO in the mitochondrial membranes [14]. The increased TSPO expression is not limited to only microglia, but also to astrocytes. However, in vitro studies with [^{3}H]-*R*-PK11195 have shown that the binding of this TSPO radioligand in AD brain is more directly associated with immunohistochemical markers of microglia rather then astrocytes [15]. Therefore, TSPO imaging in AD is classically viewed as a tool to image microglia activation rather than astrocyte activation.

A marker that is more specifically associated with astrocyte activation is the measurement of monoamino-oxidase-B (MAO-B) activity. MAO-B in the brain is present in astrocytes and serotonin containing neurons [16, 17]. An autoradiographic study using double staining with [^{3}H]L-Deprenyl and GFAP-immunohistochemistry showed that the binding of [^{3}H]L-Deprenyl was in agreement with the GFAP-staining in the white matter of controls and patients with amyotrophic lateral sclerosis (ALS) [18]. The selectivity of [^{3}H]L-Deprenyl for the binding in reactive astrocytes in ALS patients was confirmed in subsequent studies [19-21], suggesting that the MAO-B can be a suitable marker for imaging reactive astrocytosis. In AD brain, [^{3}H]L-Deprenyl has been shown histochemically to co-localize with GFAP immunoreactivity in cell clusters of astrocytes around senile plaques, in amydgala, hippocampus and insular cortex [22]. Autoradiographic studies using the tritiated MAO-B inhibitors [^{3}H]lazabemide [23], [^{3}H]L-Deprenyl [24] or [^{11}C]L-Deprenyl [25] have also shown increase MAO-B binding in the cortex of AD patients and correlation of MAO-B signal with GFAP immunoreactivity [23]. Based on these evidences, PET imaging of MAO-B

with [^{11}C]L-Deprenyl or the deuterated analog [^{11}C]L-Deprenyl-D2 is considered a marker of astrocyte activation in neurodegenerative disorders, including AD. Since L-Deprenyl is a suicide inactivator of the MAO-B enzyme [26] and the uptake and distribution of [^{11}C]L-Deprenyl in the brain has been shown to be influenced by the tracer delivery, particularly in regions with high MAO-B content [27], the deuterated analog [^{11}C]L-Deprenyl-D2 has been developed [28]. The introduction of deuterium in the molecule decreases the rate of cleavage of the carbon-hydrogen bond alpha to the amino group in the propargyl function of L-deprenyl, thereby decreasing the irreversible trapping of ^{11}C in the brain [28]. The end result is that [^{11}C]L-Deprenyl-D2 displays a less irreversible kinetic behavior in the brain and its uptake and distribution is more associated to MAO-B activity and less dependent on blood-flow. For these reasons [^{11}C]L-Deprenyl-D2 is at present the radioligand most widely used to image MAO-B activity in the brain with PET. Previous imaging studies with [^{11}C]L-Deprenyl-D2 have been performed in different neurological disorders such as such as epilepsy, Creutzfeldt–Jakob disease and amyotrophic lateral sclerosis (ALS) [29-32]. These studies demonstrated increased binding of [^{11}C]L-Deprenyl-D2 in the brain areas involved in the pathology of the different disorders, such as the epileptic lobe, the frontal, parietal and occipital cortices in Creutzfeldt–Jakob disease, and the white matter and the pons in ALS. One aspect to be considered when using [^{11}C]L-Deprenyl-D2 for MAO-B imaging is that smokers have reduced brain MAO-B activity as compared with nonsmokers [33] and that there is a difference in the arterial input function of [^{11}C]L-Deprenyl-D2 between smokers and nonsmokers [34]. These effects of smoking on the delivery and brain uptake of [^{11}C]L-Deprenyl-D2 may influence the quantification of MAO-B activity in the brain, therefore it is recommended to match control and patient groups for smoking or better include only nonsmokers in research studies, to avoid possible bias related to the effect of smoking and to potential differences in the pharmacokinetic of the radioligand in the body.

TSPO imaging in AD

TSPO has been the target most widely examined in AD and at present there is a plethora and redundancy of PET radioligands for that target. From the early development and application of [^{11}C]-*R*-PK11195 [35], a large series of second-generation TSPO radioligands have been developed and some of them, such as [^{11}C]DAA1106 [36], [^{18}F]FEDAA1106 [37], [^{11}C]PBR28 [38], [^{18}F]FEMPA [39], [^{18}F]DPA-714 [40], and [^{18}F]FEPPA [41], have been used to compare TSPO binding in AD vs. control subjects. We focused our review only on TSPO imaging in AD and MCI. Previous reviews have discussed the role of imaging of neuroinflammation in other forms of dementia, such as Parkinson´s disease dementia, frontotemporal dementia and dementia with Lewy bodies [42, 43]. A search of the literature using as kew words "translocator protein", "TSPO", "peripheral benzodiazepine receptor", "PBR", "Alzheimer´s disease" and the different radioligands listed above gave 23 results for in vivo clinical studies. In three papers, different quantification approaches for [^{11}C]-*R*-PK11195 were presented and although the dataset included also PET data from AD patients or MCI subjects, the studies were primarily intended to evaluate the performance of different reference tissue models [44-46]. One paper examined the correlation between binding of [^{11}C]PBR28 to TSPO in subjects with PARP1 gene variation, but did not provide separate data for AD patients and controls [47]. Another paper used data from the ADNI cohort and examined the effect of TSPO polymorphism on amyloid load and clinical progression of AD but did not included TSPO imaging data in AD patients. The remaining 18 studies were designed to specifically compare TSPO binding in AD patients, MCI subjects and elderly control subjects and were considered for this review. The summary of the studies conducted with [^{11}C](*R*)-PK11195 and second-generation TSPO radioligands in AD and MCI are summarized in Table 1. We have also included one SPECT study using the ^{123}I-labelled version of PK11195 [48] and one study with the TSPO radioligand [^{11}C]vinpocetine [49].

Twelve studies have reported different degrees of difference in TSPO binding between AD patients and controls [35, 36, 38, 39, 41, 48, 50-52], whereas the remaining six studies have not shown any statistically significant difference [37, 40, 49, 53-55]. The brain regions in which TSPO binding was found to be significantly higher in AD patients than in controls included the prefrontal cortex [36, 38, 41, 48, 50-52, 56], the temporal cortex [35, 36, 38, 39, 41, 50, 51, 56, 57], the parietal cortex [35, 36, 38, 41, 50, 51, 56, 57], the precuneus [38, 52], the anterior cingulate [36, 50-52, 56], the posterior cingulate [35, 38, 39, 50, 52], the occipital cortex [38, 41, 50, 56], the amygdala [35, 56], the hippocampus [38, 56], the parahippocampal cortex [52, 57], the enthorinal cortex [38, 57], the fusiform gyrus [35], the striatum [35, 36, 39, 50, 51], and the thalamus [39]. If only the studies showing positive results are considered, and excluding one study that used a quantification approach that might have artificially produced several-fold differences in BP_{ND} between AD patients and control subjects [52], AD patients showed higher TSPO binding as compared with control subjects, approximately by 30% on average. Those studies have used different radioligands and different quantification methods and outcome measures, based on the use of a reference time-activity curve or based on the measurement of the arterial input function. In four of the studies using second generation TSPO radioligands, data were stratified according to the TSPO polymorphism or binding status. Interestingly, although the first study conducted with [^{11}C]-*R*-PK11195 and quantification using a supervised cluster analysis has shown very promising results [35], the data were replicated only in one subsequent study using the same radioligand [50], whereas no statistically significant differences were found in two other [^{11}C]-*R*-PK11195 studies [54, 55]. Negative findings have been reported also in two studies using either [^{18}F]FEDAA1106 [37] or [^{18}F]DPA-714 [40]. The lack of statistically significant differences between AD patients and controls can be partly explained with the fact that for both [^{18}F]FEDAA1106 and [^{18}F]DPA-714 there was no stratification of the patients by the binding status, likely

contributing to the variability of the data and reduced effect size. In the case of the [^{11}C]-*R*-PK11195 studies, no obvious reason could explain the lack of statistically significant differences between AD patients and controls. One possible explanation could be that the measurement of neuroinflammation in vivo using TSPO imaging is not sensitive enough to detect differences between patients and controls that can be clearly seen in post-mortem studies. Therefore, although [^{11}C]-*R*-PK11195 is a radioligand with clear limitations for its sub-optimal imaging properties, the development of second generation TSPO radioligands has not really provided imaging tools that outperform [^{11}C]-*R*-PK11195.

In support of the possible explanation that in vivo imaging might not be sensitive enough to detect differences between AD patients and controls, two post-mortem studies conducted in the late ´80s and early ´90s using [^{3}H]Ro5-4864 (4'-chlorodiazepam) [58] and [^{3}H]PK11195 [59] did show differences in the binding of the two radioligands between AD patients and controls. In the first study using [^{3}H]Ro5-4864 the authors reported a 2-fold higher density of peripheral benzodiazepine binding sites in the Broca´s area, postcentral and precentral gyrus of AD patients compared with controls. The differences in midtemporal gyrus and frontal pole were not statistically significant, although the binding of [^{3}H]Ro5-4864 in AD patients was 50% to 80% higher than the binding in controls. The second study used Scatchard plots of [^{3}H]PK11195 binding in frontal and temporal cortex of controls and AD patients. In frontal cortex there was a moderate increase by 40% in B_{max} values which showed a trend towards statistical significance (p=0.07). On the other hand in temporal cortex, the B_{max} values were 120% higher in AD patients compared with controls.

These findings were replicated in subsequent post-mortem studies. A 2-fold higher B_{max} values in hippocampus of AD patients vs. controls was reported using [^{3}H]PK11195 [60]. A 4-fold higher Bmax values in frontal cortex of AD patients compared with controls were found using either [^{3}H]-*R*-PK11195 or [^{3}H]DAA1106 [15]. Finally, in a recent post-mortem

study a 2-fold higher [^{125}I]desfluoro-DAA1106 specific binding was found in the temporal and parietal cortex of AD patients as compared with controls [61].

There is a major discrepancy between post-mortem and in vivo data concerning TSPO in AD. While post-mortem data show 100%-200% higher TSPO binding in AD patients compared with controls, the in vivo data show only approximately 30% difference. This discrepancy cannot be explained only by the difference in resolution between ARG and PET or by the difference in sensitivity between in vitro and in vivo analyses. The discrepancy cannot be explained also by the fact that the outcome measure in vitro is B_{max} and in vivo is B_{max}/K_D*f_{ND} (BP_{ND}) or V_T (which contains specific and nonspecific binding). In vitro studies have not shown differences in K_D between AD patients and controls [15, 59] and differences in f_{ND} should not be expected. One explanation could be that there are other sources of differences between in vitro and in vivo conditions. For instance, in hippocampus specimens from AD brain it has been shown that 54-kD trimers represent the most abundant form of TSPO [60]. It is not known whether the TSPO radioligands bind to TSPO polymers with the same affinity as they bind to monomers. If they bind with different affinity to monomers or polymers some of the discrepancy might be attributed to the different status of polymerization between in vitro and in vivo conditions and between control and AD brains.

Considering all the possible caveats discussed so far, the main conclusions from the majority of the in vivo imaging studies that showed positive results are that only a moderate increase of TSPO binding (30%) in AD patients compared with controls can be detected, that a large overlap is observed between patients and controls and that when using second generation TSPO radioligands it is necessary to stratify for TSPO binding status (Figure 2). If the data of the different studies showing positive results are carefully examined, it is clear that beside the large overlap, only a fraction of AD patients show TSPO binding levels that are clearly higher than controls, see for instance [50] for [^{11}C]-*R*-PK11195, [36] for [^{11}C]DAA1106, [39] for

[^{18}F]FEMPA, [41] for [^{18}F]FEPPA. Therefore, it is possible that not all AD patients have increased TSPO expression or microglia activation as measured with TSPO-PET.

Methodological aspects of TSPO imaging in AD

A major aspect that must be considered when reviewing the different studies examining TSPO imaging in AD and MCI, is that no consensus has been reached in terms of quantification of TSPO binding. Because of the difficulty of measuring adequately the arterial input function of [^{11}C]-*R*-PK11195, the method of choice for the quantification of the binding of [^{11}C]-*R*-PK11195 to TSPO has been the supervised cluster analysis [35, 46, 50, 55, 56]. This method uses the combination of different kinetic clusters that represent different kinetic behaviors of normal GM tissue, pathologic tissue and vascular component. The outcome measure derived using this method is the binding potential (BP_{ND}). The estimation of BP_{ND} is based on the assumption that the time-activity curve used as reference tissue is devoid of specific binding. This assumption is not true in the case of TSPO, since the density of TSPO is similar to the density of other G protein coupled neuroreceptors and cannot be considered negligible [14]. In the case of [^{11}C]-*R*-PK11195 it has been conventionally accepted that the outcome measure of choice is BP_{ND}, but in reality the outcome measure obtained with [^{11}C]-*R*-PK11195 is a surrogate of BP_{ND}, reflecting the ratio between [^{11}C]-*R*-PK11195 binding to "pathological" and "physiological" tissue. The acceptance of this violation to the basic assumptions of PET quantification has partly been an advantage, since BP_{ND} values close to zero can be expected in control subjects, whereas BP_{ND} values as low as 0.2-0.5 in AD patients already provide a large difference at group level between AD patients and controls [35].

The introduction of second generation TSPO radioligands has contributed to examine the quantification in more details. Since second generation TSPO radioligands have higher affinity for TSPO compared with [^{11}C]-*R*-PK11195, the level of specific binding associated with physiological TSPO expression cannot be neglected and PET quantification must rely on accurate measurement of arterial input function and kinetic analysis with compartmental modeling. In such case the outcome measure of choice is the total distribution volume, V_T.

The drawback of V_T is not only that arterial cannulation and radiometabolite analysis are required, but also that V_T contains also a proportion of nondisplaceable binding. In the case of [^{11}C]PBR28, for instance, it has been shown that the proportion of nondisplaceable binding is approximately 40% of the total binding in all brain regions [62]. Therefore, the use of V_T as outcome measure for differentiating AD patients from controls might be affected by the variability introduced by the measurement of the arterial input function and because of the proportion of specific vs total binding that can be different for different radioligands, depending on the affinity for TSPO and the amount of nonspecific binding. Two recent studies with [^{18}F]FEMPA [39] and [^{18}F]FEPPA [41] have used V_T as outcome measures and have shown statistically significant higher TSPO binding in AD vs controls when stratifying for TSPO binding status. Therefore, V_T can be a useful outcome measure provided that methodological or biological sources of variability are considered.

Contrary to the above-mentioned studies, a recent study with [^{11}C]PBR28 has shown that using V_T as outcome measure does not permit to differentiate statistically AD patients from control subjects, whereas using V_T normalized by the plasma free fraction of [^{11}C]PBR28, f_P, permits to detect statistically significantly differences [57]. The use of f_P is somewhat controversial, because the measurement of f_P has itself larger test-retest variability than V_T and measurements conducted at different centres show indeed different values. In the same study, using SUV ratio with the cerebellum as reference region there was a more statistically significant difference between AD and controls (higher SUVR values in AD than controls), with lower variability of the data. Therefore, using an outcome measure which is less accurate than V_T but less affected by noise can in principle paradoxically increase the possibility to show increased neuroinflammation and TSPO binding in AD. The major question is of course if a cerebellum is a suitable reference region in AD. Amyloid plaques can develop in the cerebellum, although more likely in the later stages of the disease. In addition, some studies

have shown differences in TSPO binding in the cerebellum between AD patients and controls [36, 39, 50]. Therefore, the suitability of cerebellum as pseudo-reference region should be checked for each TSPO radioligand using full quantification with arterial input function.

Besides the different quantification methods that might have contributed to the variability of the findings reported so far, another aspect to be considered with regards to quantification is also the potential interference of radiolabelled metabolites entering the brain. To our knowledge, this aspect has not been systematically addressed in the studies reported so far. If radiometabolites enter the brain, in theory differences in metabolism between patient groups could determine differences in the outcome measures. At least in two studies, the parent fraction of [^{18}F]FEDAA1106 [37] and [^{18}F]FEMPA [39] did not differ significantly between AD patients and controls, although the findings of the two studies were different (Table 1). If this is the case for the other radioligands too, then the differences in metabolism of the TSPO tracers should not contribute largely to the differences in the findings observed so far.

Another methodological aspect that should be considered is related to the analysis of the data. For instance, using SPM analysis applied to parametric BP_{ND} images clusters of increased binding of [^{11}C]-*R*-PK11195 to TSPO in AD brain have been observed [50, 55], even when the ROI-results were negative [55], suggesting perhaps that depending upon the ROI size microglia activation might be underestimated using ROI analysis, because of "dilution" of the increased signal with the normal signal.

Neuroinflammation in prodromal AD

Neuroinflammation is a condition that is clearly related to amyloid deposition in the brain and that most likely follow a certain time course in parallel with amyloid deposition. To our knowledge there are no longitudinal studies examining the changes of TSPO binding over time. However, cross sectional studies have examined subjects with mild cognitive impairment (MCI), a condition representing a prodromal stage of AD. These studies have used [^{11}C]-*R*-PK11195 and second generation TSPO radioligands and have reported either no statistically significant differences between MCI subjects and controls or AD patients [38, 54, 55] or a mild-to-moderate increase of TSPO binding between 17% and 41% in MCI subjects compared with either AD patients or controls [51, 63] [56]. The regions in which TSPO binding was significantly higher in MCI subjects than in controls included the prefrontal cortex [51, 56, 63], the temporal cortex [51, 56], the parietal cortex [51, 56], the anterior cingulate [51, 56], the posterior cingulate [56], the amygdala [56], the hippocampus [56], and the striatum [51]. The amyloid load does not seem to always correlate with the level of TSPO binding in AD [50, 63] and the differences in TSPO binding between MCI and controls or between MCI and AD do not seem to be largely influenced by the presence of absence of amyloid in the brain [38, 54, 63].

Some studies have though shown significant correlation between amyloid load and TSPO binding in the posterior cingulate cortex [52] or in several brain regions typically involved in AD pathology such as frontal cortex, temporo-parietal cortex, cingulated cortex and parahippocampal gyrus [56]. This last study used an advanced method of voxel-based analysis based on the correlation of binding potential between voxels of [^{11}C]PIB and [^{11}C]PK11195 parametric images. In the same study a correlation between amyloid load and TSPO binding was also found in MCI subjects in similar brain regions as in AD patients [56].

At present, it is difficult to conclude whether neuroinflammation can be detected in vivo with PET already at early or prodromal stages of AD and whether the presence of neuroinflammation can be a finding predictive of longitudinal progression of the disease or of clinical deterioration in MCI subjects. The only study that attempted to examine the relationship between TSPO binding and conversion to dementia reported that MCI subjects with binding potential values of [^{11}C]DAA1106 higher than the control mean +0.5 standard deviation developed dementia within 5 years [51]. Further studies are needed to confirm these findings and to establish whether the presence of neuroinflammation can be predictive of conversion to dementia.

Imaging of neuroinflammation can be extremely valuable as imaging marker to assess direct or indirect anti-inflammatory effects of new drugs with potential disease modifying properties. Therefore, imaging neuroinflammation with PET in MCI or AD can be a valuable tool for proof-of-mechanism studies and clinical trials with anti-inflammatory drugs.

Neuroinflammation and cognitive function

It is acknowledged that in AD there is no straightforward correlation between amyloid load and cognitive function and that most likely tau pathology is more correlated with cognitive impairment. The relationship between neuroinflammation, measured with TSPO imaging, and cognitive function has been investigated in several studies. Studies with [^{11}C]-*R*-PK11195 [50], [^{11}C]PBR28 [38] and [^{18}F]FEPPA have reported a statistically significant negative correlation between MMSE or neuropsychological tests of memory function and TSPO binding in brain regions typically involved in AD, such as posterior cingulate, frontal, temporal and parietal cortex. Other studies did not report any statistically significant correlation between measures of cognitive function and TSPO binding examined with [^{11}C]-*R*-PK11195 or [^{11}C]DAA1106 [51, 52, 55]. One study has reported statistically significant negative correlation between [^{11}C]-*R*-PK11195 binding and MMSE in amyloid-negative but not amyloid-positive AD patients [63]. Despite some controversies in the results obtained by different groups, the overall findings suggest some link between neuroinflammation and cognitive impairment in AD.

Microglia or astrocytes?

As discussed previously, TSPO is conventionally seen as a marker of activated microglia, although the protein is expressed also in astrocytes, whereas MAO-B is considered a marker of activated astrocytes. At present, the only well-established radioligand available for imaging MAO-B activity in humans with PET is [^{11}C]L-Deprenyl-D2. Contrary to the literature on TSPO, there are only a few studies conducted in AD patients and MCI subjects using [^{11}C]L-Deprenyl-D2. These studies show an increase of cortical and hippocampal binding of [^{11}C]L-Deprenyl [25] or [^{3}H]L-Deprenyl [64] in vitro in post-mortem AD brain tissue. The [^{3}H]L-Deprenyl binding showed a distinct different pattern from the [^{3}H]Pittsburgh compound B (PIB) binding fibrillar amyloid plaques. Quantitative autoradiography demonstrated a clear laminar pattern of [^{3}H]L-Deprenyl binding in the frontal cortex whereas high [^{3}H]PIB binding was observed in all layers [64]. The first in vivo study conducted in an AD population examined the inhibition of MAO-B activity measured with [^{11}C]L-Deprenyl-D2 using a selective MAO-B inhibitor [65]. A subsequent study with [^{11}C]L-Deprenyl-D2 has shown high binding in AD patients [66], while interestingly enough in one study PIB positive MCI subjects showed higher binding of [^{11}C]L-Deprenyl-D2 in brain as compared with both AD patients (Figure 3) and healthy controls [67]. In addition, in PIB positive MCI subjects, the binding of [^{11}C]L-Deprenyl-D2 in the para-hippocampus has been shown to be inversely related to the grey matter density in the same region [68], suggesting a link between astrocyte activation and neurodegeneration in prodromal AD. High [^{11}C]L-Deprenyl-D2 binding has been shown also in pre-symptomatic subjects with familiar AD [69] and in transgenic mice carrying the APPswe mutation, in which increased MAO-B activity measured with [^{11}C]L-Deprenyl-D2 microPET was present already at 6 months, whereas amyloid deposition measured with [^{11}C]AZD2184 was significantly increased only at 18-24 months [70]. These

studies in prodromal AD and in animal models of AD suggest that astrocyte activation is an early pathological finding in AD.

PET imaging of TSPO and MAO-B have been combined with amyloid imaging to examine the relationship between amyloid deposition and neuroinflammation. Although amyloid deposition and activated microglia or astrocytes are present in similar brain regions, suggesting the co-localization of both phenomena, the amyloid load does not necessarily correlate with TSPO binding or MAO-B activity in the brain. The relationship between amyloid deposition and neuroinflammation is not completely straightforward but accumulating data suggest that the two processes are linked to each other, which is also indicated by the high presence of astrocytes found close to the amyloid plaque formations [6].

Conclusions

Several evidences from pathological as well as in vivo PET studies in human subjects indicate the involvement of microglia and astrocytes in neuroinflammatory processes associated with AD. Despite many contributions to the field of molecular imaging of neuroinflammation, some important questions remain to be address, to better understand the relative contribution of microglia and astrocytes in AD pathology. How much of the TSPO binding in vivo is associated to microglia or astrocytes? Is the type of microglia that expresses TSPO mainly pro-inflammatory or anti-inflammatory? The first question could benefit of combined PET studies in prodromal and clinical AD stages with TSPO and MAO-B radioligands, to understand which process, microglia or astrocyte activation, occurs first in relation to amyloid deposition. The second question could benefit of the development of specific radioligands targeting M1 or M2 types of microglia. Radioligand development in the area of neuroinflammation beyond TSPO is very important to try to understand better the process of neuroinflammation in neurodegenerative disorders. After many years of work in trying to find the most suitable TSPO PET radioligand, the major challenge for the coming years will be to develop radioligands for novel and more specific targets of neuroinflammation to be used as early diagnostic markers as well as evaluation of new drug targets for treatment of AD and related dementia disorders.

Figure Legend

Figure 1. Amyloid plaques and astrocyte and microglia distribution in the frontal cortex of Alzheimer brain. Sections from the frontal cortex Alzheimer brain were double stained with amyloid antibody 4G8 (Aß17-24), 6E10 (Aß1-17) in combination with anti-GFAP (astrocytes) and Iba1 (microglia) antibodies. 40 times magnification. Photo courtesy of Dr Larysa Voytenko, Karolinska Institutet.

Figure 2. Transaxial images of [^{18}F]FEMPA obtained in high affinity binding subjects, showing higher binding of the radioligand in the brain of AD patients compared with control subjects. The unit of radioactivity was expressed as standardized uptake value (SUV). The PET images were obtained by averaging the frames between 60 and 90 minutes. Image courtesy of Andrea Varrone, Karolinska Institutet, Juha Rinne, Turku PET Centre, Ana Catafau, Piramal Imaging.

Figure 3. High astrogliosis in the brain of patient with cognitive impairment associated with high β-amyloid load (PIB+) indicative of prodromal AD (left panel) in comparison with clinically demented patient with Alzheimer's disease (AD; right panel). Representative parametric images of [^{11}C]-L-deprenyl-D2 binding (that reports monoamine-oxidase activity in astrocytes) were obtained by position emission tomography. The positron emission tomography scans show sagittal sections of the brain at the level of basal ganglia. Color scale: red = very high, yellow = moderate high, green = high, blue = low [^{11}C]-L-deprenyl-D2 binding. Image courtesy of Agneta Nordberg, Karolinska Institutet.

Acknowledgments

The work has been supported by funds from the Swedish Research Council (project 05817), Karolinska Institutet Strategic Neuroscience program, the Stockholm County Council-Karolinska Institutet regional agreement on medical training and clinical research (ALF grant), Swedish Brain Power, the Swedish Brain Foundation, the Alzheimer Foundation in Sweden, Karolinska Institutet's Foundation for Aging Research, Swedish Foundation for Strategic Research (SFF), and by the EU project INMiND, FP7/2007-2013-no HEALTH-F2-2011-278850 (http://www.uni-muenster.de/InMind). Part of the work has been also supported by Bayer Healthcare, Berlin, Germany.

Compliance with Ethics Guidelines

Conflict of interest. Andrea Varrone and Agneta Nordberg declare no conflicts of interest. The work performed using [^{18}F]FEMPA has been supported by Bayer Healthcare, Berlin, Germany.

Human and animal studies. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study. In cases of animal studies, all institutional and national guidelines for the care and use of laboratory animals were followed.

Contribution statement

Andrea Varrone: Literature search and Review, Content planning, Manuscript writing and Editing

Agneta Nordberg: Literature search and Review, Content planning, Manuscript writing and Editing

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Figure 1
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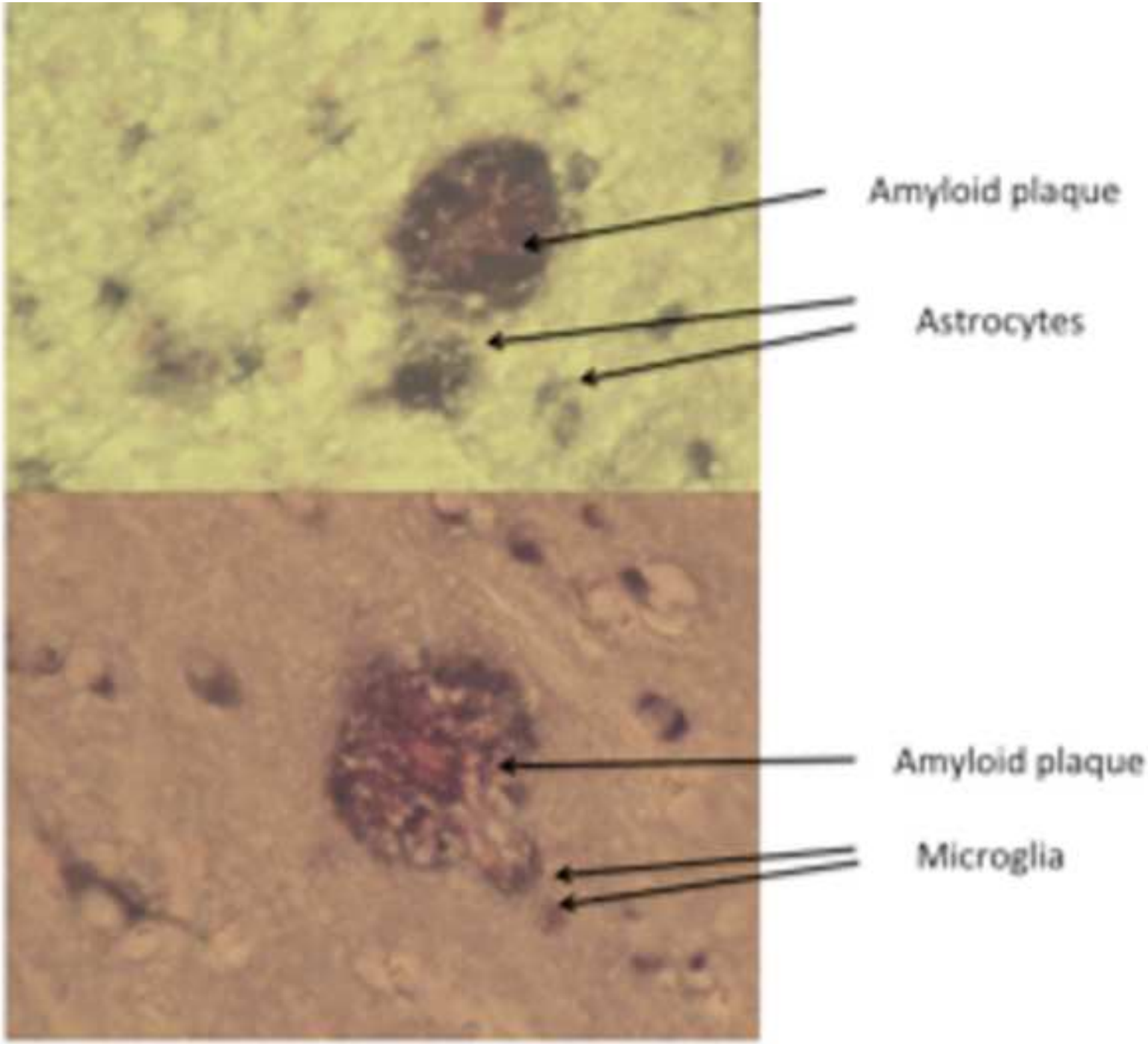

Figure 2
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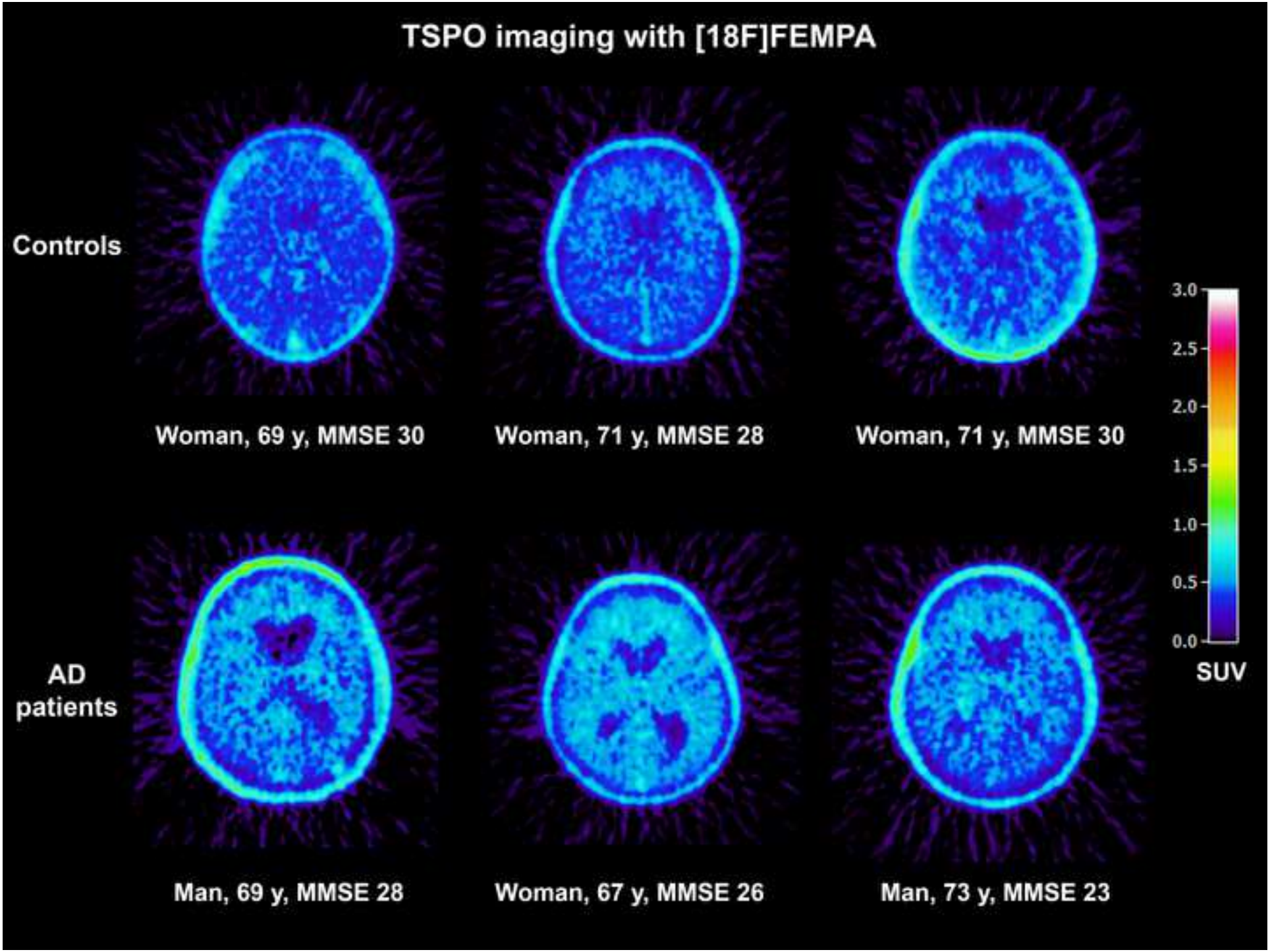
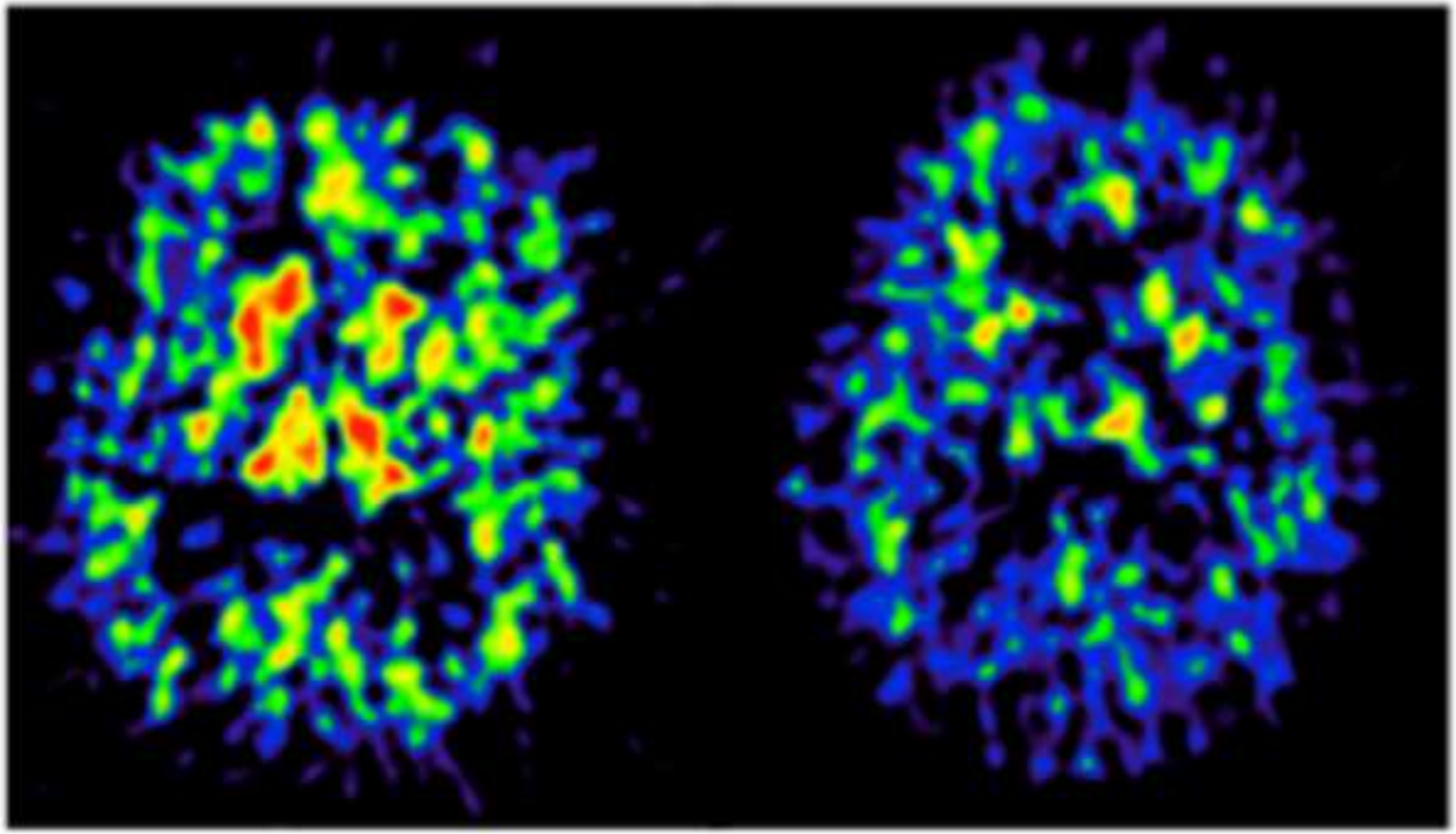


Figure 3
Click here to download Figure: Figure 3.tiff



MCI (PIB+)

AD

Table 1

Table 1. Summary of in vivo TSPO imaging studies in Alzheimer´s disease and MCI.

Author	Patients	Radioligand	PIB	PVEc	Outcome measure – method	Increase of TSPO binding (%) vs. controls
Groom, 1995	8 AD	[^{11}C]PK11195[a]	No	No	Region-to-cerebellum ratio	n.s.
Cagnin, 2001	8 AD, 1MCI	[^{11}C]-*R*-PK11195	No	No	BP_{ND} – supervised cluster analysis	35-188%
Versijpt, 2003	10 AD	[^{123}I]PK11195	No	No	Region-to-cerebellum ratio	32%
Edison, 2008	13 AD	[^{11}C]-*R*-PK11195	Yes	No	BP_{ND} – supervised cluster analysis	23%
Yasuno, 2008	10 AD	[^{11}C]DAA1106	No	No	BP_{ND} (k_3/k_4) – 2TCM	17%
Wiley, 2009	6 AD, 6 MCI	[^{11}C]-*R*-PK11195	Yes	Yes	BP_{ND} – supervised cluster analysis (cerebellum)	n.s.
Okello, 2009	22 AD, 14 MCI	[^{11}C]-*R*-PK11195	Yes	No	BP_{ND} – supervised cluster analysis	9-23% AD, 17-25% MCI
Gulyás, 2011	6 AD	[^{11}C]vinpocetine	No	No	SUV, SUVR and BP_{ND} (cerebellum)	n.s.
Yokokura, 2011[b]	11 AD	[^{11}C]-*R*-PK11195	Yes	No	BP_{ND} – SRTM with normal brain TAC as reference	118-1100%

Yasuno, 2012	10 AD, 7 MCI	[^{11}C]DAA1106	No	No	BP_{ND} (k_3/k_4) – 2TCM	18% AD, 26% MCI
Schuitemaker, 2013	20 AD, 13 MCI	[^{11}C]-*R*-PK11195	No	Yes	BP_{ND} – supervised cluster analysis	n.s.
Varrone, 2013	9 AD	[^{18}F]FEDAA1106	No	No	V_T - Logan GA	n.s.
Kreisl, 2013[c]	19 AD, 10 MCI	[^{11}C]PBR28	Yes	Yes	V_T / f_P – 2TCM	38% AD
Varrone, 2015[c]	10 AD	[^{18}F]FEMPA	No	No	V_T - Logan GA	19.5% (HABs)
Golla, 2015	10 AD	[^{18}F]DPA-714	No	No	V_T – 2TCM	n.s.
Lyoo, 2015[c]	25 AD[c], 11 MCI[c]	[^{11}C]PBR28	Yes	No	V_T – 2TCM V_T / f_P – 2TCM DVR (cerebellum) SUVR (cerebellum)	n.s.(V_T) 22-25% AD (V_T / f_P) 8-14% (DVR) 6-10% (SUVR)
Suridjan, 2015[c]	21 AD	[^{18}F]FEPPA	No	Yes	V_T – 2TCM	44-56%
Fan, 2015	10 AD, 10 MCI, 11 PDD	[^{11}C]-*R*-PK11195	Yes	No	BP_{ND} – supervised cluster analysis	40.5% AD, 41% MCI

[a]In this study it is not specified if the racemic mixture of PK11195 or the *R* enantiomer was used.

[b]This study used the average time-activity curve (TAC) from the control subjects as indirect input function for the estimation of BP_{ND} in both the AD and control groups. This approach might have produced values of BP_{ND} close to 0 in the control group, thereby leading to BP_{ND} in the AD group that was several-fold higher than the controls.

[b]These studies used stratification or adjustment for TSPO binding status.

[c]Some of these patients were already included in a previous study from the same group (Kreisl, 2013).

PVEc=partial volume effect correction.

PDD=Parkinson´s disease dementia.

Reply to Reviewers

We thank the Reviewers for the fruitful comments that improved the quality of the revised review manuscript. Below please find the point-by point reply to Reviewrs´ comments.

Reviewer 1

Comment: Score =1. there is no description of the search strategy employed. My quick Medline search for (TSPO or PK11195 or PBR28 or FEDAA1106 or FEMPA or FEPPA or DAA1106) and (Alzheimer disease) yields ~50 published articles. Only 14 are included in this review. Many papers in my search are outside the desired scope (studies in AD genetic mice, case reports, etc). However, there is no description provided for search strategy nor for inclusion / exclusion criteria used.

Answer. Thank to the Reviewer for this comment. We have now clarified the source criteria for the articles and described the criteria for including or excluding articles in this review. We have also found a few more articles that were overlooked in the initial search (one old study, one SPECT study and one study in press). This section is now included in page 7 or the revised Review.

Quality appraisal of evaluated studies should be undertaken. Have the relative strengths and weaknesses of the literature on the question been clarified? Please score and comment

Reviewer #1

Comment: Score = 1. This is not addressed in the MS

Reviewer #2:

Comment:

Score 4; the review does not go into detail concerning the quality of the studies evaluated, but for the present purpose it should suffice.

Answer. We have included several more sections in the Review trying to address these aspects. See page 9-10, 13, 14, 15. We hope this is sufficient to satisfy the criticism of the Reviewers.

After including and excluding studies based on the quality appraisal, data evaluation and results of the studies should be undertaken. Were bias correctly identified from the literature evaluation? Please score and comment.

Reviewer #1

Comment: Score = 1. Not addressed in the MS.

Reviewer #2:

Score 4; see response to previous question.

Answer. In the sections described in the previous comment, we have tried to identify possible sources of bias. See for instance page 9, 10, and 13.

Are conclusions based on the best available scientific evidence? Have literature conflicts been sorted out? Is general interpretation of the results accurate also in terms of implications for future research? Please score and comment.

Reviewer #1:

Comment: Score = 3. Data are not completely described, and several important (potential) sources of discrepancy and error are not discussed.

Answer. Again we think we have tried to identify these sources of discrepancy in the included sections. See in particular page 9 and 10 for the discrepancy between in vitro and in vivo data. We have also now described the results in AD and MCI in more details. See for instance page 8 and page 14.

Reviewer#1:

Comment: Score = 4. Should consider mentioning the PET imaging approach as unique and important also in determining the temporal nature of inflammation in AD vs clinical features and other biomarkers.

Answer. We have included a final sentence in the abstract to mention this.

Are Figures and Tables complete and adequate in relation to the content? Are titles and captions explanatory of Figures and Tables (so readers can take-home message)? In case of need of reproduction permissions, are the original sources properly addressed in Figure or Table captions? Please score and comment.

Reviewer #1:

Comment: Score = 4. Figure 1 is not clearly necessary. Color scale in Figure 2 does not emphasize distinctions between AD and controls very well - perhaps this is the point?

Answer. We would like to keep Figure 1 that, for a less experienced reader, can provide a visual representation of the link between amyloid deposition and inflammatory glial cells in AD. The Reviewer is correct for Figure 2. It is fare to show that imaging TSPO does not provide the same contrast as seen for amyloid imaging.

General comment to the Author. In addition to the above evaluation grid and scoring, please comment on other points of the review article, if needed, and about its significance, accuracy and clarity. If relevant, also comment on implications for further research or discussion in the field.

Reviewer #1: This review covers a wide range of technical approaches to imaging of neuroinflammation in Alzheimer disease. it has the potential to bring together an apparently disparate range of publications. however, it would be much stronger if the structure of the literature search were systematic and described. Given the lack of consistent results reported, it would seem also very important to emphasize potential sources of the disparity and of error.

Answer. In the sections highlighted above we have tried to cover all these aspects underscored by the Reviewer.

For example, are any of the ligands employed corrected for CNS penetration of radiometabolites? Are the metabolic pathways and products of the tracers known? This issue has significant impact on the specificity of modeling approaches used/described, and could highlight differences between laboratories employing different ligands.

Answer. We have included a paragraph describing this potential source of error. See page 13.

Another important issue to consider is the pattern (expected vs found) in the apparent distributions of TSPO ligands in AD and MCI. The patterns of amyloid deposition and of neurofibrillary degeneration in typical AD are known (at autopsy by Braak - by neuroimaging in amyloid PET and FDG). Do regional distributions of TSPO findings make any sense regarding this framework?

Answer. This is a very good comment by the reviewer. We have discussed more in detail the correlation between amyloid and TSPO binding in page 14 and 15. To the best of our knowledge, though, there is no clear report of a pathological staging of neuroinflammation as in the case of amyloid and we feel that the in vivo imaging data are still not enough adequate to propose such in vivo staging.

Reviewer #2:

1. The second paragraph on page 3 is very general, indicating pro- and anti-inflammatory processes. In a thematic issue on neuroinflammation it is likely that there will be duplication with other contributions. Therefore, if possible, this paragraph should also be focused more on AD.

Answer. We have included a paragraph describing the findings in an animal model of AD and in the brain of AD patients to discuss more specifically the M1/M2 phenotypes in AD.

2. The statement on the top of page 6 that uptake and distribution of deprenyl in the brain is delivery dependent is misleading. Indeed, for an irreversible tracer, uptake is related to Ki, which is K1k3/(k2+k3), clearly showing the relationship with K1. However, MAO-B activity is related to k3 and it has been shown that MAO-B activity can be measured by k3 through careful kinetic modelling (see Lammertsma et al, JCBFM 1991;11: 545-556).

Answer. We agree with the Rewiever´s comment. We have slightly modified the text stating that the tracer uptake can be influenced by the delivery. The point here we want to make is why Deuterium was included in the molecule as originally reported by Fowler et al.

3. On a more general note, Fowler et al have demonstrated a relationship between MAO-B activity and smoking, which would impose a complication in the selection of patients and volusnteers. Should this not be mentioned?

Answer. Thanks to the Reviewer for this comment. We have included a paragraph in page 6 to describe it.

4. The manuscript contains one table and this is referred to as Table. For consistency, would it not be better to refer to Table 1?

Answer. This has been done.

5. Pages 7 and 8 contain a concise presentation of TSPO imaging results in AD, some studies showing significant difference with healthy controls, whilst others are unable to detect a difference. Important in this discussion is the method of analysis. For example, at an ROI level, Schuitemaker et al did not find a difference between AD and healthy controls. Nevertheless, an SPM analysis applied to parametric BPND images showed small clusters of increased binding in AD. Therefore, ROI results may depend on ROI size (dilution with normal signal).

Answer. We have included a paragraph describing this important aspect in the methodological section on page 13.

6. On page 9 the sentence “Because of the high nonspecific binding …” (line 13) seems incorrect. I agree with most of the sentence, but this is not because of the high nonspecific binding. Maybe it is just a matter of formulating the sentence.

Answer. We agree with the Reviewer that the formulation of the sentence was not accurate and we have changed it accordingly.

7. The last sentence of the first paragraph of page 10 is not clear. If the level of nonspecific binding is high, a reference tissue approach should still be able to measure specific binding (although precision will decrease with increasing nonspecific binding). In contrast, VT will be dominated by nonspecific binding and will not be as accurate (although maybe more precise) given the large bias.

Answer. We have modified the order of the sentenced to describe this aspect more clearly.

8. Using the same argument, I wonder whether SUVR may not be a better reflection of BPND than VT if the nonspecific contribution is high and provided there is a kind of equilibrium.

Answer. In principle we agree with the Reviewer, but the use of SUVR still has to be validated for all TSPO radioligands, making sure that no difference in the cerebellum is found. See pages 12-13.

9. On page 12 it may be useful to include studies in which TSPO binding in MCI subjects was correlated with (later) conversion to AD.

Answer. We have included a more detailed description of the findings of the only study addressing this point. See page 15.

10. I think that “crucial” should be removed from the first sentence of the conclusions (page 16). There definitely is involvement, but it is not clear yet how important it is.

Answer. The word “crucial” was removed.