Neurobiology of Aging 52 (2017) 98–105

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Rod-shaped microglia morphology is associated with aging in 2 human autopsy series

Adam D. Bachstetter [a,b,*], Eseosa T. Ighodaro [a,c], Yasmin Hassoun [a], Danah Aldeiri [a], Janna H. Neltner [c,d], Ela Patel [c], Erin L. Abner [c,e], Peter T. Nelson [c,d]

[a] Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY, USA
[b] Department of Neuroscience, University of Kentucky, Lexington, KY, USA
[c] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA
[d] Division of Neuropathology, Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington, KY, USA
[e] Department of Epidemiology, University of Kentucky, Lexington, KY, USA

ARTICLE INFO

Article history:
Received 20 November 2016
Received in revised form 22 December 2016
Accepted 28 December 2016
Available online 5 January 2017

Keywords:
Aging
Microglia activation
Neurodegeneration
Neuroinflammation
Neuropathology
Hippocampus
Alzheimer's disease
Traumatic brain injury

ABSTRACT

A subtype of microglia is defined by the morphological appearance of the cells as rod shaped. Little is known about this intriguing cell type, as there are only a few case reports describing rod-shaped microglia in the neuropathological literature. Rod-shaped microglia were shown recently to account for a substantial proportion of the microglia cells in the hippocampus of both demented and cognitively intact aged individuals. We hypothesized that aging could be a defining feature in the occurrence of rod-shaped microglia. To test this hypothesis, 2 independent series of autopsy cases (total n = 168 cases), which covered the adult lifespan from 20 to 100+ years old, were included in the study. The presence or absence of rod-shaped microglia was scored on IBA1 immunohistochemically stained slides for the hippocampus and cortex. We found that age was one of the strongest determinants for the presence of rod-shaped microglia in the hippocampus and the cortex. We found no association with the presence of rod-shaped microglia and a self-reported history of a TBI. Alzheimer's disease–related pathology was found to influence the presence of rod-shaped microglia, but only in the parietal cortex and not in the hippocampus or temporal cortex. Future studies are warranted to determine the functional relevance of rod-shaped microglia in supporting the health of neurons in the aged brain, and the signaling processes that regulate the formation of rod-shaped microglia.

1. Introduction

Microglia are the resident tissue macrophage of the central nervous system. In the healthy central nervous system, microglia form a network of nearly uniformly distributed cells throughout the tissue, with highly thin-ramified cell processes. Changes in microglia morphology away from the ramified or 'surveying' type of cell are well described in the literature, but largely are centered around the hypertrophic or "activated" morphology. Despite recent studies defining a number of additional microglia morphologies (Bachstetter et al., 2015; Roth et al., 2014; Streit, 2006; Ziebell et al., 2012), little is known about the relevance of these morphological changes to human brain health and disease.

First described by Franz Nissl over 100 years ago (reviewed by [Graeber, 2010]), rod-shaped microglia are a particularly intriguing morphologically defined subtype. The modern literature describing rod-shaped microglia is sparse and is dominated by case reports, with the exception of a recent study that determined the relative amount of rod-shaped microglia in the hippocampus of different age-related neurodegenerative diseases (Bachstetter et al., 2015). Rod-shaped microglia were found in approximately 60% of the cases, including a subset of nondemented control cases, as well as in cases with different neurodegenerative disease (Bachstetter et al., 2015). The high prevalence of rod-shaped microglia in individuals of 65 years or older suggested that aging or an age-related degenerative process might be an important predictor for the presence of rod-shaped microglia.

In this study, we sought to determine if aging, Alzheimer's disease, or traumatic brain injury (TBI) could be a defining feature in the occurrence of rod-shaped microglia in the human brain. To this end, we used 2 independent series of cases. The first series included 61 cases that covered the adult lifespan from 20 to

* Corresponding author at: Department of Neuroscience, Spinal Cord and Brain Injury Research Center, University of Kentucky, B459 BBSRB, 741 S. Limestone St., Lexington, KY 40536, USA. Tel.: +1-859-218-4315; fax: +1-859-257-5737.
E-mail address: adam.bachstetter@uky.edu (A.D. Bachstetter).

0197-4580/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2016.12.028

96 years of age, which were free of advanced neurodegenerative pathology. The second set of 107 cases was from an aged population–based series, with an age range of 77–100+ years, which included nondemented controls and cases with Alzheimer's disease. We found that older chronological age was a strong predictor for the presence of rod-shaped microglia, even when controlling for Alzheimer's disease pathology. Our data suggest that there may be an age-related change to neurons or microglia, which we are yet to define, that predisposes the aged brain to the presence of rod-shaped microglia.

2. Materials and methods

2.1. UK series: University of Kentucky human subjects and tissue processing

A set of 61 autopsy cases were collected from the University of Kentucky (UK) bio tissue repository (Table 1). The cases were selected to cover the adult lifespan from 20 to 96 years of age. Cases were selected by the investigators (J. H. N. and P. T. N.) to be free of advanced neurodegenerative pathology. Exclusion criteria included pathologically confirmed neurodegenerative disease: specifically, but not limited to, advance disease pathology associated with Alzheimer's disease, dementia with Lewy bodies, hippocampal sclerosis of aging, and vascular dementia. To identify rod-shaped microglia, brains were stained with the ionized calcium binding adapter molecule 1 (IBA1) antibody, which is used as a pan marker of macrophages/microglia in the brain. Paraffin-embedded tissue was processed, 8μm-thick sections were cut, and immunohistochemical staining was done using the primary antibody: IBA1 (rabbit polyclonal, 1:1000 immunohistochemical, Wako Pure Chemical Industries, Richmond, VA, USA). A biotinylated secondary antibody (Vector Laboratories) was amplified using avidin-biotin substrate (ABC solution, Vector Laboratories catalog no. PK-6100), followed by color development in Nova Red (Vector Laboratories). The Aperio ScanScope XT digital slide scanner was used to image the entire stained slide at 40× magnification to create a single high-resolution digital image. A tissue section from the hippocampus and the frontal cortex was analyzed by 2 observers blind to the experimental conditions (Y. H. and D. A.). The observers exhaustively inspected the gray matter of the entire slides for the presence of rod-shaped microglia at a minimum of 8× magnification. Presence of rod-shaped microglia was defined as at least 1 clearly defined rod-shaped microglia: Fig. 1 shows examples of cells defined as rod-shaped microglia.

Table 1
Case series characteristics

Characteristics	UK series	AI-ACT series
Number of cases	61	107
Age		
20–29	6 (9.8%)	0 (0%)
30–39	12 (19.7%)	0 (0%)
40–49	7 (11.5%)	0 (0%)
50–59	6 (9.8%)	0 (0%)
60–69	4 (6.6%)	0 (0%)
70–79	5 (8.2%)	12 (11.2%)
80–89	16 (26.2%)	45 (42.1%)
90–99	5 (8.2%)	43 (40.2%)
100+	0 (0%)	7 (7.5%)
Sex		
Male	27 (43.3%)	63 (58.9%)
Female	31 (50.8%)	44 (41.1%)
Dementia status		
No dementia	61 (100%)	57 (53.3%)
Dementia	0 (0%)	50 (46.7%)
Braak NFT stage		
0/I/II	61 (100%)	28 (26.2%)
III/IV	0 (0%)	44 (41.1%)
V/VI	0 (0%)	32 (29.9%)
CERAD rating		
None	61 (100%)	24 (22.4%)
Sparse	0 (0%)	33 (30.8%)
Moderate	0 (0%)	25 (23.4%)
Frequent	0 (0%)	25 (23.4%)

Number of cases and percent of total cases for the series are shown in the table. University of Kentucky bio tissue repository series of cases (UK series). The Aging, Dementia and Traumatic Brain Injury Study series (2016 Allen Institute for Brain Science. Aging, Dementia and TBI. Available from: http://aging.brain-map.org/overview/explore) (AI-ACT series). Braak neurofibrillary tangle (NFT) Stage. Consortium to Establish a Registry for Alzheimer's Disease (CERAD).
Key: ACT, Adult Changes in Thought; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; NFT, neurofibrillary tangle.

2.2. AI-ACT series: aging, dementia, and TBI study of the Allen Institute (AI) for Brain Science

The Aging, Dementia and Traumatic Brain Injury Study is a series of 107 autopsy cases with and without a history of TBI drawn from the Adult Changes in Thought (ACT) study (AI-ACT; Table 1). Data used for the current project are publically available through the AI website (2016 Allen Institute for Brain Science. Aging, Dementia and TBI. Available from: http://aging.brain-map.org/overview/explore). High-magnification digital histopathological images of the entire physical slides are available to download for 3 brain regions—hippocampus, temporal cortex, and parietal cortex—for a number of neuropathological stains. Additional information is available in the AI-ACT database, including results from extensive biochemical assays, and clinical information about the cases. To determine how often rod-shaped microglia were found in this sample population, the digital histopathological images of IBA1 for the hippocampus, temporal cortex, and parietal cortex were downloaded (all regions were not available for all cases) and were independently scored by 3 observers blind to the experimental conditions (Y. H., D. A., and A. D. B.) for the presence or absence of rod-shaped microglia in the gray matter. Presence of rod-shaped microglia was defined as at least 1 clearly defined rod-shaped microglia; Fig. 1 shows examples of cells defined as rod-shaped microglia.

2.3. Statistics

JMP Pro Software version 12.0 or SAS 9.4 (SAS Institute, Inc.; Cary, NC, USA) was used for statistical analysis. Statistical significance was set at 0.05. Contingency tables of categorical variables (presence of rod-shaped microglia, sex, age group, history of TBI) were compared using the Pearson χ^2 test or Fisher's exact test. The Cochran-Armitage Test was used to assess for a linear trend in the association between age and frequency of rod-shaped microglia. Logistic regression was used to assess the variable of interest (age) and potential confounders (sex, dementia status, Braak neurofibrillary tangle (NFT) stage, and Consortium to Establish a Registry for Alzheimer's Disease [CERAD] neuritic plaque rating) and to estimate odds ratios (ORs) for the presence of rod-shaped microglia. Age groups were generated by a median split into 20–69 and 70 years or above for the UK series, and 70–89 and 90 years or above for the AI-ACT series. Availability of confounder variables differed in the UK and AI-ACT cohorts. For UK, only sex (coded male = 1, female = 2) was available for all the cases. For the AI-ACT series, additional variables were available: sex was coded as male = 1, female = 2, Braak NFT stage was coded as low (stage 0/I/II), moderate (III/IV), or high (V/VI); CERAD neuritic plaque rating was coded as none, sparse, moderate, or frequent; dementia status was coded as no dementia versus dementia (DSM-IV criteria). Multivariable regression models to adjust clinicopathological associations for potential confounding factors have been

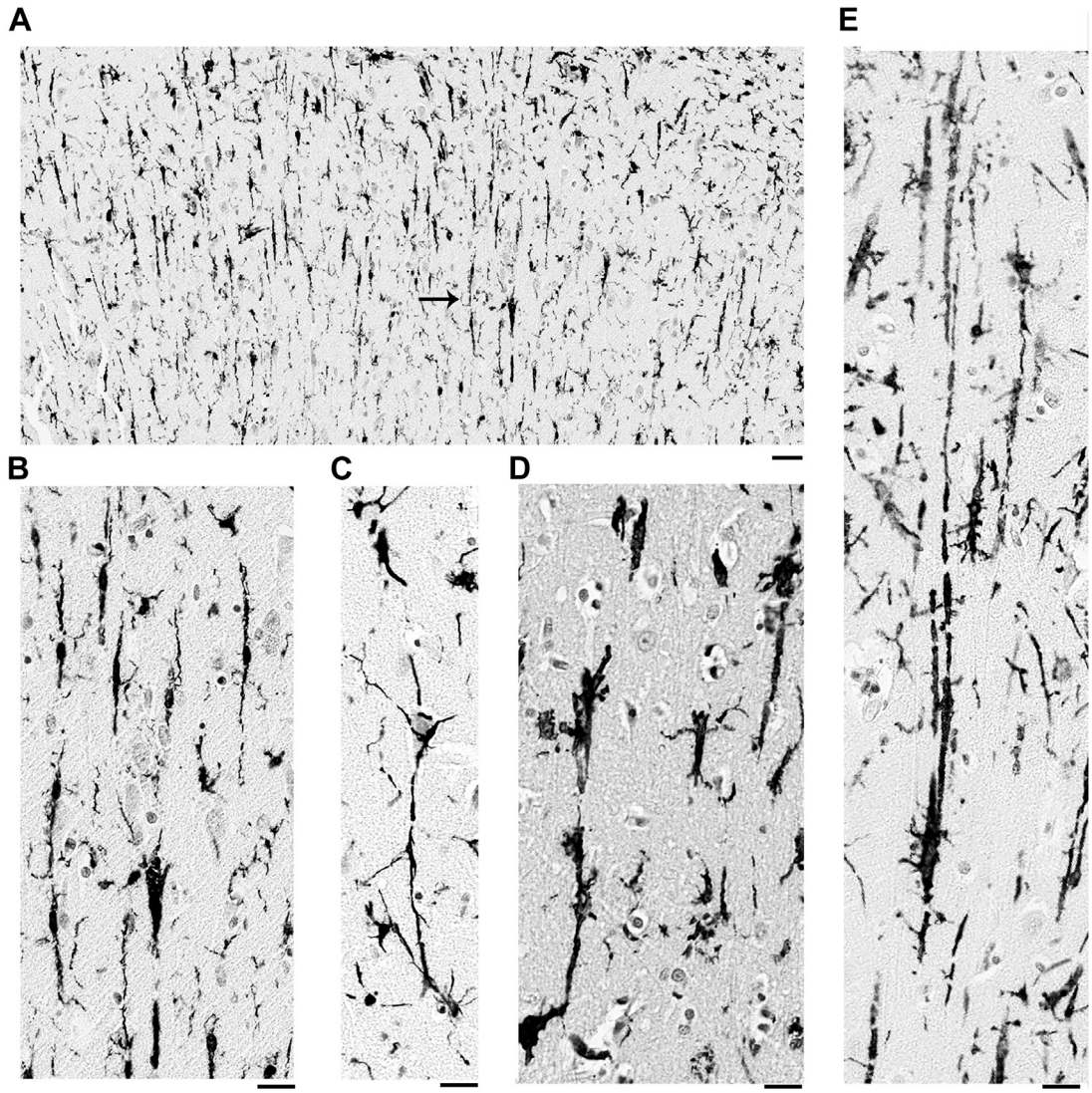

Fig. 1. Examples of rod-shaped microglia from the UK series (A) Numerous rod-shaped microglia are found throughout the CA1 region of the hippocampus of an 86-year-old female. The arrow indicates the area shown at a higher magnification in (B). (C) Long thin rod-shaped microglia, which appear to be surrounding a pyramidal neuron, in the subiculum of a 39-year-old male, who had hypoxia/ischemia pathology, and a pulmonary-associated cause of death. (D) Example of rod-shaped microglia seen in the frontal cortex of a 28-year-old female, who died of atherosclerotic disease. (E) A number of long trains of rod-shaped microglia were found in the CA1 region of the hippocampus of a 25-year-old female, who had hypoxia/ischemia pathology, and a pulmonary-associated cause of death. Scale bar = 50 μm.

used multiple times by our group (Abner et al., 2014, 2016; Ighodaro et al., 2017; Nelson et al., 2010). Comparisons between the AI-ACT series versus UK series were done using a logistic regression to determine ORs for the presence of rod-shaped microglia in the hippocampus of older aged individuals (70–89 year old).

In preclinical animal models, TBI has been shown to be a strong initiator for the presence of rod-shaped microglia; therefore, we sought to determine if a history of TBI exposure in the AI-ACT series could be associated with the presence of rod-shaped microglia. As a part of the ACT study design, participants were asked at initial enrollment to the study and at the biannual re-evaluation about exposure to a brain injury that caused a loss of consciousness (Dams-O'Connor et al., 2013). Cases reporting a history of TBI with a loss of consciousness were compared with those without a history of TBI for the presence of rod-shaped microglia. To determine if more severe TBI could be associated with the presence of rod-shaped microglia, cases with a self-reported loss of consciousness of more than 10 minutes were compared with the cases with no TBI exposure.

3. Results

3.1. Age is associated with increased presence of rod-shaped microglia in the University of Kentucky (UK) aging series

The primary research question was to elucidate the clinical-pathological correlations associated with the occurrence of rod-shaped microglia in postmortem hippocampal and cortical tissue. We first sought to determine if aging was accompanied by an increase incidence of IBA1$^+$ rod-shaped microglia. Examples of rod-shaped microglia identified in the tissue of the aging UK series are shown in Fig. 1. In the hippocampus, a significant trend for an increased occurrence of any rod-shaped microglia with increasing age was found (Fig. 2A). Separating the cases into younger adults (20–69 year old; n = 35) to the older adults (70+ years old; n = 26), at the time of death (using a median split of the cases), we found a significantly greater proportion of any rod-shaped microglia in hippocampus of older adults (46.2%) compared with younger adults (11.4%; Fig. 2B). The age-related increase in the presence of

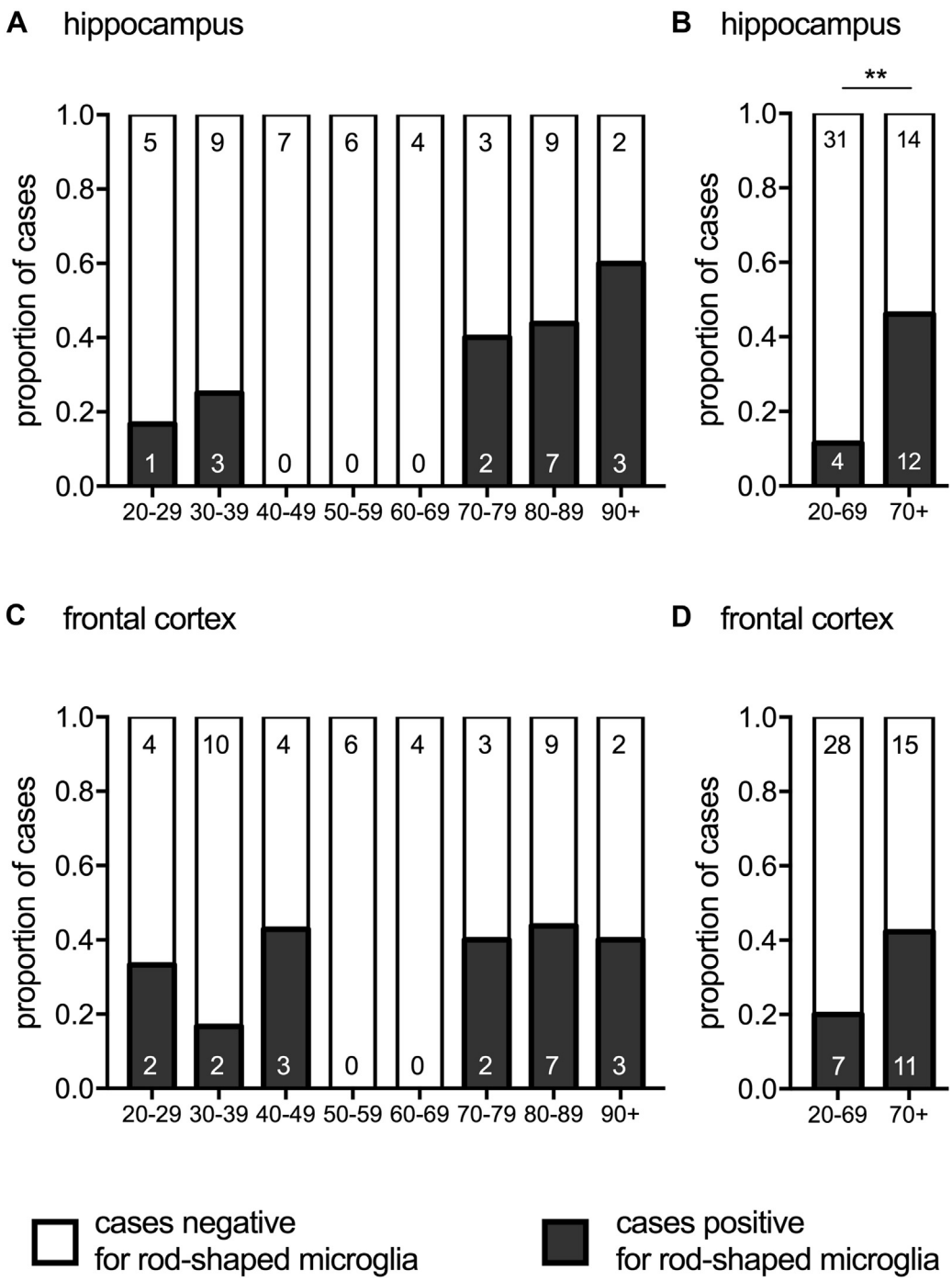

Fig. 2. Association of aging on the presence of rod-shaped microglia. (A) In the hippocampus, a significant trend was found for an increasing occurrence of rod-shaped microglia with increasing age ($p = 0.017$, Cochran-Armitage Trend Test). (B) A significant increase in the occurrence of rod-shaped microglia in the hippocampus was found in those individuals of 70-years or older (70+) at the time of death, compared with those individuals 20–69 year old at the time of death ($p = 0.0033$, Fisher exact test) (C) A similar pattern was found for the frontal cortex when plotted as age by decade ($p = 0.222$, Cochran-Armitage Trend Test), or (D) split into young adults (20–69 year old) and older adults (70+; $p = 0.089$, Fisher Exact test). The number of cases in each group is shown in the bars. $^{**}p < 0.01$.

rod-shaped microglia remained significant when corrected for sex ($p = 0.005$). The OR for presence of rod-shaped microglia in the older adults versus the younger adults was 6.64 (95% confidence interval [CI] 1.94–27.25), and when corrected for sex, the OR was 5.87 (95% CI 1.66–24.8); the reduction in magnitude was likely due to the fact that females in the UK series were both more likely to be older and more likely to have rod-shaped microglia in the hippocampus. In the frontal cortex, a similar age-related increase in the proportion of individuals with any rod-shaped microglia was found (Fig. 2C and D); however, this increase was not statistically significant.

To further explore the age-related differences in the occurrence of rod-shaped microglia, the cases were subdivided by which brain region(s) rod-shaped microglia were found, as follows: (1) in the hippocampus only; (2) in the cortex only; or (3) in both the cortex and the hippocampus (Fig. 3). In those individuals less than 70 years of age, rod-shaped microglia were found in the frontal cortex, or the hippocampus, but not in both the hippocampus and frontal cortex

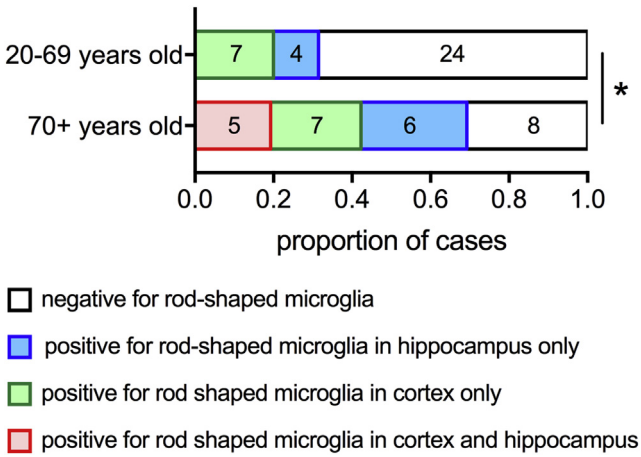

Fig. 3. Regional heterogeneity in the occurrence of rod-shaped microglia in younger adults versus aged adults. Cases were subdivided by which brain region(s) rod-shaped microglia were found, as follows: (1) in both the cortex and the hippocampus; (2) in the hippocampus only; and (3) in the cortex only. In younger adults (20–69 year old), rod-shaped microglia were found in the hippocampus, or in the frontal cortex, which is in contrast to the older adults (70+ years old; $p = 0.047$, χ^2 test). The number of cases in each group is shown in the bars. $^*p < 0.05$.

(Fig. 3). This was in contrast to the older adults, where 19.2% of the cases were found to have rod-shaped microglia in the frontal cortex and hippocampus.

3.2. Age, and not TBI or Alzheimer's disease–related pathology, was associated with the presence of rod-shaped microglia in the AI-ACT series

To further explore the occurrence of rod-shaped microglia in older individuals, we used the public data repository released by the Allen Institute (AI) for Brain Science (2016 Allen Institute for Brain Science. Aging, Dementia and TBI. Available from: http://aging.brain-map.org/overview/explore). Overall, we found that the percentage of cases positive for rod-shaped microglia in the hippocampus in the older aged individuals (70–89 year old) was higher in the AI-ACT series compared with the UK series (UK = 42.9% 9/21; AI-ACT = 65.1% 28/430). The OR for AI-ACT (70–89 year old) versus UK series (70–89 year old) to have rod-shaped microglia in the hippocampus was 2.49 (95% CI, 0.86–7.44). The data suggested that increased age and/or Alzheimer's disease pathology found in the AI-ACT series in comparison to the UK series (Table 1) may be associated with the increased presence of rod-shaped microglia in the AI-ACT series.

Since the AI-ACT series contained the oldest-old individuals, we also sought to determine whether oldest-old age (90+ years) was associated with increased presence of rod-shaped microglia relative to younger age (70–89 years). In the hippocampus, the unadjusted OR for 90+ versus 70–89 was 1.30 (95% CI 0.54–1.86), suggesting no significant effect of oldest-old age on the presence of rod-shaped microglia in this region. In the parietal cortex, the OR was 0.86 (95% CI 0.34–2.16), and in the temporal cortex, the OR was 3.27 (95% CI 1.43–7.73), which may suggest that age effects are region-specific (Table 2). To evaluate if age was independently associated with presence of rod-shaped microglia after controlling for Alzheimer's disease pathology in the AI-ACT series, a logistic regression analysis was done to determine if sex, Braak NFT Stage, CERAD neuritic plaque score, and dementia status were the potential confounders of the age-microglia relationship. The age effect remained significant in the temporal cortex (Fig. 4A, Table 2) after adjustment, but there were also strong effects for dementia and sparse and moderate plaques in the parietal cortex, although CIs were wide (Table 2). There were no significant associations between rod-shaped microglia and AD pathology in the hippocampus or temporal cortex. In all the 3 regions, men were less likely than women to have rod-shaped microglia, but this effect was only significant in the parietal cortex (Fig. 4B, Table 2).

Finally, comparing a self-reported history of TBI with a loss of consciousness to the presence of rod-shaped microglia yielded no significant association (Fig. 5A). No association was found between the severity of TBI, as determined by an extended period of loss of consciousness and the presence of rod-shaped microglia (Fig. 5B). As some of the cases with a history of TBI had the exposure more than 6 decades before their death, we sought to compare only those cases with a TBI more proximal to the time of death. However, no significant association was found between the presence of rod-shaped microglia, and the individuals who had a first TBI exposure after the age of 60 years (Fig. 5C).

4. Discussion

The present study investigated the clinical-pathological correlations associated with the occurrence of rod-shaped microglia in

Table 2
Odds ratios (95% CI) for presence of rod-shaped microglia in the AI-ACT series

Comparison	Hippocampus		Parietal cortex		Temporal cortex	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Age						
90+ vs. 70–89	1.30 (0.54–1.86)	1.06 (0.40–2.80)	0.86 (0.34–2.16)	1.04 (0.32–3.29)	3.27** (1.43–7.73)	4.05** (1.63–10.79)
Sex						
Male vs. Female	0.42 (0.16–1.05)	0.47 (0.17–1.21)	0.26** (0.09–0.66)	0.20** (0.06–0.57)	0.63 (0.27–1.42)	0.72 (0.29–1.75)
Dementia status						
Dementia vs. no dementia	1.15 (0.48–2.83)	0.76 (0.25–2.23)	1.97 (0.78–5.13)	4.03* (1.18–15.7)	1.76 (0.78–3.96)	2.55 (0.96–7.20)
Braak neurofibrillary tangle stage						
III/IV vs. 0/I/II	1.47 (0.52–4.20)	1.47 (0.47–4.67)	0.56 (0.18–1.77)	0.37 (0.89–1.40)	1.21 (0.45–3.25)	0.96 (0.31–2.88)
V/VI vs. 0/I/II	2.81 (0.88–9.73)	3.54 (0.72–19.52)	0.68 (0.21–2.24)	0.41 (0.58–2.57)	1.42 (0.49–4.17)	0.91 (0.19–4.24)
CERAD neuritic plaque rating						
Sparse vs. none	0.76 (0.21–2.67)	0.60 (0.15–2.28)	2.50 (0.62–12.82)	6.00* (1.11–42.54)	1.38 (0.44–4.39)	2.11 (0.59–7.99)
Moderate vs. none	0.80 (0.21–2.90)	0.51 (0.12–2.07)	3.21 (0.78–16.78)	5.81* (1.02–43.33)	1.65 (0.51–5.44)	1.57 (0.41–6.23)
Frequent vs. none	1.21 (0.31–4.71)	0.52 (0.09–2.84)	1.00 (0.19–5.71)	1.10 (0.11–11.04)	1.30 (0.40–4.27)	0.75 (0.15–3.58)

Odds ratio and 95% confidence intervals for logistic regression analysis are shown for the presence of rod-shaped microglia for each given independent variable (unadjusted), or with all the independent variables included in the regression model (adjusted). $^*p < 0.05$, $^{**}p < 0.005$.
Key: ACT, Adult Changes in Thought; CERAD, Consortium to Establish a Registry for Alzheimer's Disease.

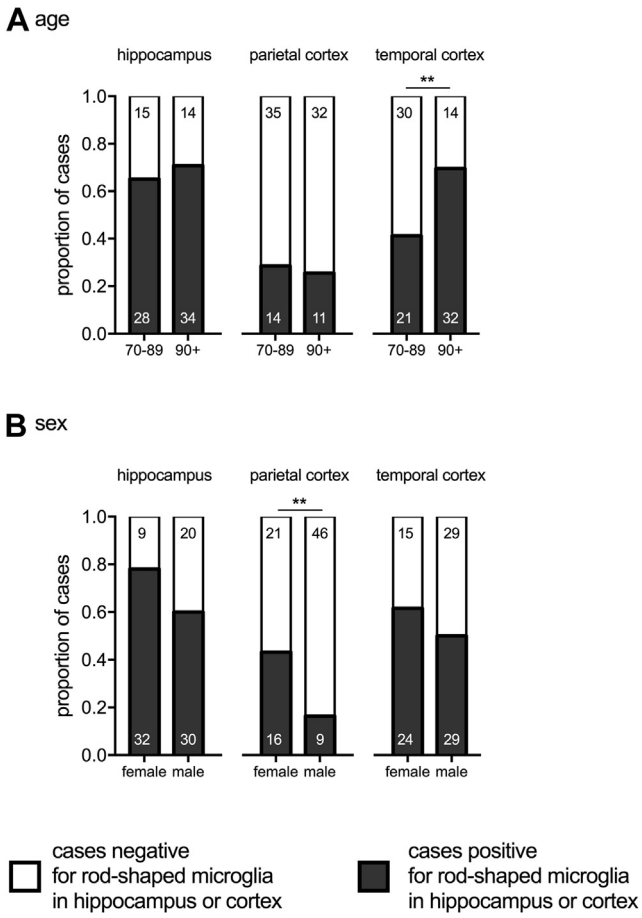

Fig. 4. Association of age and sex on the presence of rod-shaped microglia. The presence of rod-shaped microglia was determined in cases from the AI-ACT series. (A) Age at the time of death was found to significantly contribute to an increase percentage of cases positive for the presence of rod-shaped microglia. (B) Sex was also found to effect the odds ratio that rod-shaped microglia would be found. See Table 2 for summary or results. The number of cases in each group is shown in the bars. $^{**}p < 0.01$.

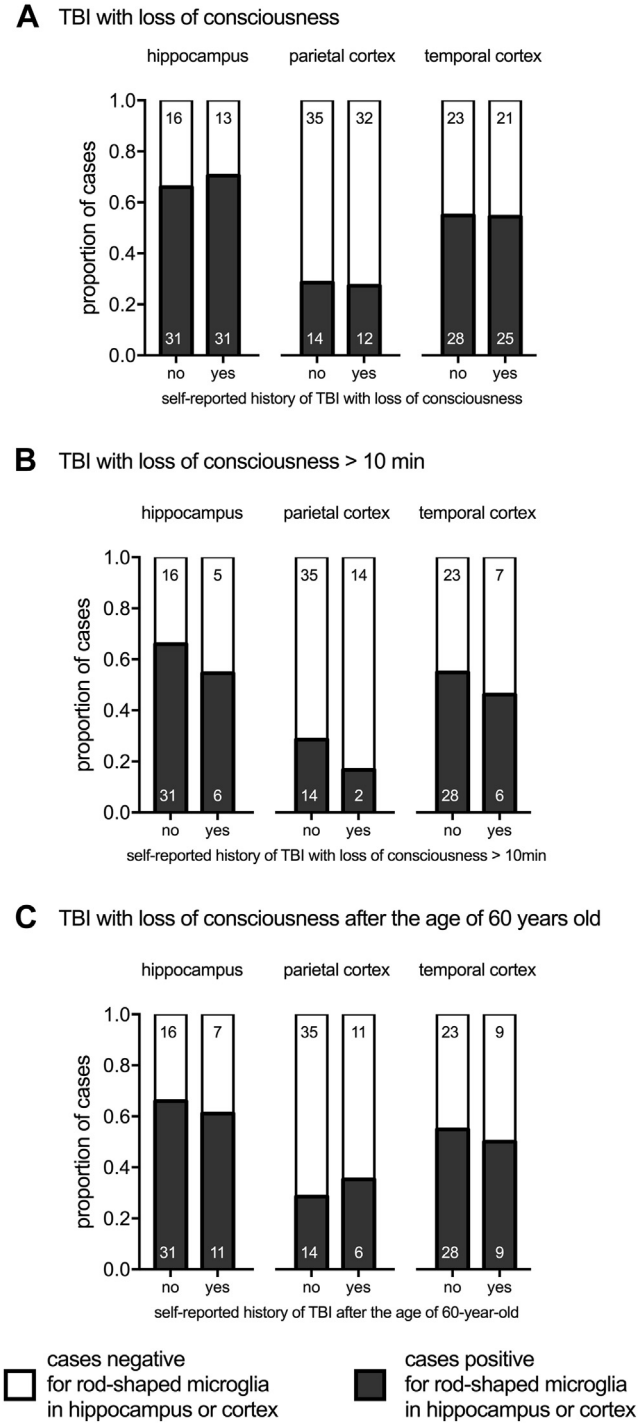

Fig. 5. Association between a history of traumatic brain injury (TBI) and the presence of rod-shaped microglia in AI-ACT series. (A) A self-reported history of at least one TBI with a loss of consciousness of at least a few seconds was not found associated with the presence of rod-shaped microglia. No association with the presence of rod-shaped microglia was found with either: (B) a self-reported history of at least 1 TBI with a loss of consciousness of more than 10 minutes; or (C) a first TBI exposure after the age of 60 years. The number of cases in each group is shown in the bars.

postmortem hippocampal and cortical tissue. Using 2 independent autopsy series, we found that the age was one of the strongest determinants of the presence of rod-shaped microglia in the hippocampus and the cortex. We found no association with the presence of rod-shaped microglia and a self-reported history of a TBI. Alzheimer's disease–related pathology was found to influence the presence of rod-shaped microglia, but only in the parietal cortex and not in the hippocampus or temporal cortex. This suggests that increased activation of microglia (number or hypertrophic morphology) as seen in Alzheimer's disease does not explain the increase number of rod-shaped microglia in the brain. This is in agreement with our previous study, where we found a very weak correlation between the total number of microglia in the CA1 region of the hippocampus and the number of rod-shaped microglia ($R^2 = 0.008$; $p = 0.28$ Spearman r; Bachstetter et al., 2015). Thus, our results highlight that rod-shaped microglia may be more common than once believed, particularly in individuals over the age of 70 years, and that the increased prevalence of rod-shaped microglia cannot be explained by simply larger numbers of microglia or more activated microglia.

4.1. Potential functions of rod-shaped microglia

Experimentally, the most faithful way to induce rod-shaped microglia is by an interruption in axonal transport either caused by a TBI, optic nerve transection, or axotomy (Ackman et al., 2006; Bachstetter et al., 2015; de Hoz et al., 2013; Sanders et al., 2014; Streit et al., 1999; Yuan et al., 2015; Ziebell et al., 2012). Deficits in axonal transport are thought to be an early molecular mechanism leading to neuronal loss in age-related neurodegenerative diseases

(Morfini et al., 2009). In the present study, it is not possible to determine if the rod-shaped microglia are associated specifically with neurons with deficits in axonal transport. The occurrence of rod-shaped microglia in more than one brain region in older individuals, as we found in this study, could indicate an age-related brain-wide change inhibiting axonal transport. The lack of a strong association of rod-shaped microglia with any one neurodegenerative disease, as we found in this study and in our previous work (Bachstetter et al., 2015), could be explained by the shared degenerative mechanism of axonal transport deficits seen in aging and age-related neurodegenerative diseases (Morfini et al., 2009).

The function of rod-shaped microglia is largely unknown. There has been some speculation that rod-shaped microglia might provide a protective function to neurons, potentially by surrounding less damaged neurons to promote survival of the neurons (Streit et al., 1999; Trapp et al., 2007; Ziebell et al., 2012). Mechanistically, the neuroprotection could be imparted through the process of synaptic stripping, to alter the excitability of the weakened neuron (Trapp et al., 2007). Rod-shaped microglia may also provide trophic support through some yet to be identified mechanism that would appear to be contact dependent (Streit et al., 1999). An intriguing possibility would be that rod-shaped microglia are involved in a process of mitochondria transfer, as was recently shown for astrocytes following stroke (Hayakawa et al., 2016), however more work is needed to test this possibility.

4.2. Potential chemotaxis signals for the formation of rod-shaped microglia

The presence of rod-shaped microglia associated with specific neurons, whereas adjacent neurons are not associated with rod-shaped microglia, would suggest an active neuron-microglia signal regulating the specificity of rod-shaped microglia to individual neurons. Neurons are able to interact directly with microglia through a number of neuron-microglia–specific signaling pathways. A well-documented example of such a signaling pathway is the neuron ligand, CX3CL1 and the microglia receptor, CX3CR1. The CX3CR1-CX3CL1 pathway is vital for the suppression of inflammatory responses from microglia. Importantly, this pathway has been shown to be dysfunctional with age (Bachstetter et al., 2011; Corona et al., 2010; Lyons et al., 2009). The age-related loss of CX3CL1 may reduce the contact inhibition signals, thereby priming the aged brain for the formation of rod-shaped microglia. Yet, rod-shaped microglia are not a defining feature in CX3CR1 KO mice (Cardona et al., 2006; Rogers et al., 2011), so it is unlikely that this pathway is a sole driver for the formation of rod-shaped microglia. Thus, there must also be a chemotaxis signal that is released from the neuron to cause the recruitment of the microglia to the neuron. As microglia have been shown to constantly make brief contact with neurons (Tremblay et al., 2010), the chemotaxis signal could be contact dependent and not a soluble factor released from the neurons.

The AI-ACT study includes an extensive array of histological and biochemical information, including RNAseq. A superficial exploration of this data did not reveal any proteins or genes at the tissue level that were associated with the presence or absence of rod-shaped microglia. As rod-shaped microglia account for only a small percentage of the total microglia population (Bachstetter et al., 2015), and the total microglia population only accounts for 5%–20% of the total glia population (van Rossum and Hanisch, 2004), it is likely at the tissue level, a rod-shaped microglia molecular signature may not be evident. Future studies using laser capture microdissection would be needed to isolate individual neurons that are associated with rod-shaped microglia and adjacent neurons and microglia which are not in close association. Then a subtractive analysis could be done to determine the molecular signature unique to the neuron associated with the rod-shaped microglia and that of the rod-shaped microglia, compared to the adjacent neurons and microglia.

4.3. Limitations and future directions

A limitation of the study is that the AI-ACT series over-represents males and individuals with a history of TBI, compared with the community-based sample of the larger ACT study. Methods for weighted analyses to extrapolate results to the larger cohort are provided by AI (2016 Allen Institute for Brain Science. Aging, Dementia and TBI. Available from: http://aging.brain-map.org/overview/explore) but were not used for this study. Therefore, findings reported should be seen as specific to the AI-ACT series and may not be representative of the larger cohort or the population in general. A second limitation of our study is that more than half of the cases with a history of TBI, had the first exposure to TBI many decades before death; thus, it is reasonable to assume that rod-shaped microglia may have resolved over time. A third limitation of our study was that it was not possible with the tissue available to provide a design-based stereological quantification of the total number of microglia; thus, we cannot account for the effect of age-related increase in microglia density that may influence the likelihood of detecting rod-shaped microglia in the older aged individuals.

A striking finding was the lack of rod-shaped microglia in the hippocampus of individuals aged 40–69 years. The leading causes of death are known to change across age groups. Therefore, we hypothesized that differences in cause of death in the 40- to 69-year-old group could be associated with the lack of rod-shaped microglia in this group. In the younger individuals (aged 20–69 years), rod-shaped microglia were not found in the cases where the cause of death was either cardiac associated, drug abuse, or sepsis. Rod-shaped microglia were found in cases of pulmonary embolism, liver failure, or cancer. A limitation of this study was that cause of death was known for only a subset of the individuals 60 year old or above, so associations between cause of death and rod-shaped microglia could not be fully determined. Future studies specifically investigating the relationship of cause of death and rod-shaped microglia may be warranted.

5. Conclusion

In summation, our results highlight that aging, but not necessarily Alzheimer's disease or a history of TBI, is a defining feature in the presence of rod-shaped microglia in the human brain. Future studies are warranted to determine the functional relevance of rod-shaped microglia in supporting the health of neurons in the aged brain, and the signaling processes that regulate the formation of rod-shaped microglia.

Disclosure statement

The authors have no actual or potential conflicts of interest.

Acknowledgements

The authors are profoundly grateful to all of the study participants who made this research possible. The corresponding author, Adam Bachstetter, PhD, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Research reported in this publication was supported by National Institutes of Health under award numbers P30 AG028383, R00 AG044445. The content is solely the responsibility of the authors and does not represent the official views of the National Institutes of Health.

References

Abner, E.L., Nelson, P.T., Kryscio, R.J., Schmitt, F.A., Fardo, D.W., Woltjer, R.L., Cairns, N.J., Yu, L., Dodge, H.H., Xiong, C., Masaki, K., Tyas, S.L., Bennett, D.A., Schneider, J.A., Arvanitakis, Z., 2016. Diabetes is associated with cerebrovascular but not Alzheimer's disease neuropathology. Alzheimers Dement 12, 882–889.

Abner, E.L., Nelson, P.T., Schmitt, F.A., Browning, S.R., Fardo, D.W., Wan, L., Jicha, G.A., Cooper, G.E., Smith, C.D., Caban-Holt, A.M., Van Eldik, L.J., Kryscio, R.J., 2014. Self-reported head injury and risk of late-life impairment and AD pathology in an AD center cohort. Dement Geriatr. Cogn. Disord. 37, 294–306.

Ackman, J.B., Siddiqi, F., Walikonis, R.S., LoTurco, J.J., 2006. Fusion of microglia with pyramidal neurons after retroviral infection. J. Neurosci. 26, 11413–11422.

Bachstetter, A.D., Morganti, J.M., Jernberg, J., Schlunk, A., Mitchell, S.H., Brewster, K.W., Hudson, C.E., Cole, M.J., Harrison, J.K., Bickford, P.C., Gemma, C., 2011. Fractalkine and CX3CR1 regulate hippocampal neurogenesis in adult and aged rats. Neurobiol. Aging 32, 2030–2044.

Bachstetter, A.D., Van Eldik, L.J., Schmitt, F.A., Neltner, J.H., Ighodaro, E.T., Webster, S.J., Patel, E., Abner, E.L., Kryscio, R.J., Nelson, P.T., 2015. Disease-related microglia heterogeneity in the hippocampus of Alzheimer's disease, dementia with Lewy bodies, and hippocampal sclerosis of aging. Acta Neuropathol. Commun. 3, 32.

Cardona, A.E., Pioro, E.P., Sasse, M.E., Kostenko, V., Cardona, S.M., Dijkstra, I.M., Huang, D., Kidd, G., Dombrowski, S., Dutta, R., Lee, J.C., Cook, D.N., Jung, S., Lira, S.A., Littman, D.R., Ransohoff, R.M., 2006. Control of microglial neurotoxicity by the fractalkine receptor. Nat. Neurosci. 9, 917–924.

Corona, A.W., Huang, Y., O'Connor, J.C., Dantzer, R., Kelley, K.W., Popovich, P.G., Godbout, J.P., 2010. Fractalkine receptor (CX3CR1) deficiency sensitizes mice to the behavioral changes induced by lipopolysaccharide. J. Neuroinflammation 7, 93.

Dams-O'Connor, K., Gibbons, L.E., Bowen, J.D., McCurry, S.M., Larson, E.B., Crane, P.K., 2013. Risk for late-life re-injury, dementia and death among individuals with traumatic brain injury: a population-based study. J. Neurol. Neurosurg. Psychiatry 84, 177–182.

de Hoz, R., Gallego, B.I., Ramirez, A.I., Rojas, B., Salazar, J.J., Valiente-Soriano, F.J., Aviles-Trigueros, M., Villegas-Perez, M.P., Vidal-Sanz, M., Trivino, A., Ramirez, J.M., 2013. Rod-like microglia are restricted to eyes with laser-induced ocular hypertension but absent from the microglial changes in the contralateral untreated eye. Plos One 8, e83733.

Graeber, M.B., 2010. Changing face of microglia. Science 330, 783–788.

Hayakawa, K., Esposito, E., Wang, X., Terasaki, Y., Liu, Y., Xing, C., Ji, X., Lo, E.H., 2016. Transfer of mitochondria from astrocytes to neurons after stroke. Nature 535, 551–555.

Ighodaro, E.T., Abner, E.L., Fardo, D.W., Lin, A.L., Katsumata, Y., Schmitt, F.A., Kryscio, R.J., Jicha, G.A., Neltner, J.H., Monsell, S.E., Kukull, W.A., Moser, D.K., Appiah, F., Bachstetter, A.D., Van Eldik, L.J., 2017. Alzheimer's Disease Neuroimaging I, Nelson PT. Risk factors and global cognitive status related to brain arteriolosclerosis in elderly individuals. J. Cereb. Blood Flow Metab. 37, 201–216.

Lyons, A., Lynch, A.M., Downer, E.J., Hanley, R., O'Sullivan, J.B., Smith, A., Lynch, M.A., 2009. Fractalkine-induced activation of the phosphatidylinositol-3 kinase pathway attenuates microglial activation in vivo and in vitro. J. Neurochem. 110, 1547–1556.

Morfini, G.A., Burns, M., Binder, L.I., Kanaan, N.M., LaPointe, N., Bosco, D.A., Brown Jr., R.H., Brown, H., Tiwari, A., Hayward, L., Edgar, J., Nave, K.A., Garberrn, J., Atagi, Y., Song, Y., Pigino, G., Brady, S.T., 2009. Axonal transport defects in neurodegenerative diseases. J. Neurosci. 29, 12776–12786.

Nelson, P.T., Schmitt, F.A., Jicha, G.A., Kryscio, R.J., Abner, E.L., Smith, C.D., Van Eldik, L.J., Markesbery, W.R., 2010. Association between male gender and cortical Lewy body pathology in large autopsy series. J. Neurol. 257, 1875–1881.

Rogers, J.T., Morganti, J.M., Bachstetter, A.D., Hudson, C.E., Peters, M.M., Grimmig, B.A., Weeber, E.J., Bickford, P.C., Gemma, C., 2011. CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J. Neurosci. 31, 16241–16250.

Roth, T.L., Nayak, D., Atanasijevic, T., Koretsky, A.P., Latour, L.L., McGavern, D.B., 2014. Transcranial amelioration of inflammation and cell death after brain injury. Nature 505, 223–228.

Sanders, D.W., Kaufman, S.K., DeVos, S.L., Sharma, A.M., Mirbaha, H., Li, A.M., Barker, S.J., Foley, A.C., Thorpe, J.R., Serpell, L.C., Miller, T.M., Grinberg, L.T., Seeley, W.W., Diamond, M.I., 2014. distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron 82, 1271–1288.

Streit, W.J., 2006. Microglial senescence: does the brain's immune system have an expiration date? Trends Neurosci. 29, 506–510.

Streit, W.J., Walter, S.A., Pennell, N.A., 1999. Reactive microgliosis. Prog. Neurobiol. 57, 563–581.

Trapp, B.D., Wujek, J.R., Criste, G.A., Jalabi, W., Yin, X.H., Kidd, G.J., Stohlman, S., Ransohoff, R., 2007. Evidence for synaptic stripping by cortical microglia. Glia 55, 360–368.

Tremblay, M.E., Lowery, R.L., Majewska, A.K., 2010. Microglial interactions with synapses are modulated by visual experience. Plos Biol. 8, e1000527.

van Rossum, D., Hanisch, U.K., 2004. Microglia. Metab. Brain Dis. 19, 393–411.

Yuan, T.F., Liang, Y.X., Peng, B., Lin, B., So, K.F., 2015. Local proliferation is the main source of rod microglia after optic nerve transection. Sci. Rep. 5, 10788.

Ziebell, J.M., Taylor, S.E., Cao, T.X., Harrison, J.L., Lifshitz, J., 2012. Rod microglia: elongation, alignment, and coupling to form trains across the somatosensory cortex after experimental diffuse brain injury. J. Neuroinflammation 9, 247.