



Brain Region-Specific Microglial Phenotypes and Responses in Parkinson's Disease

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Summary

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1-2% of the population over 65 years. While PD has traditionally been regarded as a more or less isolated disorder of the nigrostriatal dopaminergic system, recent studies indicate that this is an oversimplification; its complex symptomatology comprises not only classic motor deficits but also non-motor symptoms, including autonomic dysfunction, sensory and cognitive impairments, neuropsychiatric and sleep disturbances. These non-motor symptoms greatly impact the quality of the patients' lives and are associated with extra-nigral changes, largely comprising accumulation of alpha-synuclein (α -synuclein) pathology as well as microglial activation throughout the brain.

Whereas the involvement of microglia and α -synuclein has been studied in relation to the dopaminergic cell loss in the substantia nigra and/or the motor symptoms, little attention has been paid to such changes in extra-nigral regions that are involved in non-motor symptoms. In addition, microglia form a diverse group of cells with possible brain region-specific phenotypes that can exert either beneficial or detrimental effects, depending on their local phenotype and context.

Hence, the **overall aim** of this thesis was to gain insight into differences in microglial phenotype between brain regions that are differently affected in PD, and to study whether such differences relate to the neuropathological outcome. Several aspects were studied: a) region-specific microglial characteristics and properties like morphology, distinct receptor expression and proliferation. This was studied in relation to the proteopathology and/or neuronal loss in brain regions differentially affected by PD, i.e. the substantia nigra, olfactory bulb and hippocampus (**chapters 2, 3 and 4**), b) intrinsic differences in the genomic profile of microglia when isolated directly from different rat brain regions under basal conditions (**chapter 5**), and c) spatio-temporal changes in microglial morphology in response to local α -synuclein overexpression, studied in an adeno-associated viral (rAAV) rat model (**chapter 6**).

In **chapter 1.2** we review the literature on the motor and non-motor symptoms of PD, its main neuropathological hallmarks, and propose a role for neuroinflammation, primarily microglia, as important modulators of PD etiology. We propose that regional differences in microglial responses are present in brain regions that are differently affected in PD. Such

differences may relate to the patterns of α -synuclein pathology and neuronal loss that differ between different brain regions. As such, such differences may contribute to the (development of) non-motor symptoms in PD.

Whereas previous studies primarily identified amoeboid types of microglia in the substantia nigra and hippocampus of PD and Alzheimer's disease (AD) patients respectively, little was known about such changes in the olfactory bulb, a region affected early in both PD and AD and related to smell dysfunction. In **chapter 2**, we therefore focused 1) on the activation status of microglial cells in the human olfactory bulb of well-characterized AD and PD patients compared to age-matched control subjects, and on their relationship with α -synuclein pathology or neuronal degeneration.

While a significant increase was present in amoeboid microglia within the anterior olfactory nucleus of AD and PD patients, notably in close proximity to β -amyloid, hyperphosphorylated tau or α -synuclein deposits, no uptake of pathological proteins by, or inside, microglia could be detected. This implied, different from *in vitro* work, that microglia in the human anterior olfactory nucleus do not exert overt phagocytic activity towards disease-specific protein deposits at end-stage disease. Moreover, no correlation was found between amoeboid microglial cell density in the anterior olfactory nucleus and the level of local α -synuclein pathology, suggesting that proportional changes are not apparent, and that thus the presence of α -synuclein pathology per se may already be sufficient to evoke microgliosis. In this respect, the response to α -synuclein pathology appears to differ between the anterior olfactory nucleus and the substantia nigra. Furthermore, despite clear changes in the neuronal network, no overt neuronal loss was present in the anterior olfactory nucleus. We thus propose that rather than neuronal loss, it is the microglial activation and/or neuronal network changes in the anterior olfactory nucleus that contributes to the related functional deficits in PD, like hyposmia.

We next compared microglial phenotypes in the hippocampus and substantia nigra of PD patients in **chapter 3**. The hippocampus is affected from PD Braak stage 4 onwards and is implicated in dementia and depression, i.e. important non-motor symptoms in PD. We investigated tissue of established PD patients (stage 4-6), age- and gender-matched control subjects (stage 0), and included incidental Lewy body disease (iLBD) cases (stage 1-3) that did not have clinical PD symptoms, but displayed α -synuclein deposition at autopsy. As such, these iLBD cases are considered a prodromal state of PD. In 75% of the cases, hippocampus

and substantia nigra tissue was obtained from the same patient, reducing 'between patient' variability. Also the Alzheimer Braak stages and scores were matched between the 3 groups, ruling out possible effects of AD pathology.

Similar to the substantia nigra and anterior olfactory nucleus, the hippocampus showed a clear α -synuclein pathology-related increase in amoeboid microglia in PD. Since recent experimental studies suggested an important role for toll-like receptor 2 (TLR2) in the α -synuclein-triggered microglial activation, we studied whether TLR2 expression is related to the pathology in iLBD and PD patients. Particularly in iLBD cases, primed/reactive microglial cells coincided with a profound increase in microglial TLR2 expression, indicative of an early activational response to PD pathology. Moreover, in PD, TLR2 expression remained upregulated in the substantia nigra but not in the hippocampus, consistent with a region-specific pattern of microglial activation. As such, we show for the first time in human brain, that TLR2 is an important player in the early neuroinflammatory response during PD progression.

In summary, **chapter 2 and 3** demonstrate that microglial activation extends beyond the substantia nigra, involving several other regions as well, with the pattern of microglial responses differing between regions. Outside the substantia nigra, microglial activation largely coincides with α -synuclein deposition, but is not associated with neuronal loss. Although we cannot rule out differences in (genetic) neuronal susceptibility, this suggests that microglial responses may modify the neuropathology in PD.

In **chapter 4**, we studied cell proliferation in the hippocampus in relation to microglia. The hippocampus contains stem cells that, in rodents, are implicated in function and can proliferate in response to e.g. neurodegeneration. We here questioned whether proliferation is altered in the PD hippocampus. We used minichromosome maintenance protein 2 (MCM2) as proliferation marker, that was double-labeled with Iba-1 to assess the contribution of microglia. MCM2-positive cells were significantly increased in the hippocampus of presymptomatic iLBD cases, but not in established PD patients. Interestingly, the majority of the proliferating cells in the PD hippocampus were microglia, indicating that, similar to our TLR-2 results, microglia respond early during PD development.

Overall, **chapters 3 and 4** support the idea that secreted proteins like α -synuclein oligomers can affect microglia and may be 'sensed' through TLRs, which could lead to microglial

proliferation and/or 'priming'. These chapters further indicate that the latter occurs early in PD, with clear differences between the substantia nigra and hippocampus in later stages of PD.

Next to the region-specific differences in microglia in PD, we studied in **chapter 5** whether the gene expression profiles of rat microglia are already brain region-specific under baseline conditions. Knowledge of this microglia 'gene signature', and whether that is region-specific, may be important for our understanding of their responses during disease conditions. Using an optimized isolation protocol and cell sorting (FACS), we studied gene expression in microglia isolated from discrete gray matter regions of the rat brain. Under control conditions, already subtle differences were found in microglia gene expression patterns between the substantia nigra, hippocampus, olfactory bulb, striatum and amygdala. Whereas no differences were found in general microglial markers like CD11b (itgam), AIF-1 (Iba1) and pathogen recognition receptor TLR2 between these regions, CD68 (microglial activation) and Interleukin-1 β (IL-1 β ; pro-inflammatory cytokine) were found to be increased in the olfactory bulb. Tumor necrosis factor (TNF; pro-inflammatory cytokine) and C-C chemokines receptor 2 (CCR2; monocyte chemotaxis) were increased in the substantia nigra, and P2X purinoreceptor 7 (P2X7R; receptor for ATP) was increased in the hippocampus. These intrinsic, brain region-specific differences in genomic profile under baseline conditions indicate that microglia are no uniform population and such differences may contribute to region-specific responses of microglia to e.g. aberrant protein accumulation.

Various studies support the idea that α -synuclein triggers microglial activation in several brain regions affected by PD. They also suggest this occurs before and independent of neuronal loss. In order to study this experimentally, we used in **chapter 6** an animal model in which α -synuclein was locally overexpressed using a viral vector in the rat substantia nigra and hippocampus *in vivo* that allowed studying the response in microglial activation and neuronal loss 3 and 8 weeks later.

Eight weeks after α -synuclein transduction, Tyrosine Hydroxylase cell loss was found in the substantia nigra and striatum while at 3 weeks, increases in microglia were already observed in the substantia nigra, indicating that α -synuclein overexpression triggers a microglial response prior to the degeneration of dopaminergic neurons. This increase in microglia remained present at 8 weeks in the substantia nigra. Increases were further observed in CD68-positive amoeboid microglia only after 8 weeks of α -synuclein

overexpression in the substantia nigra. This is consistent with earlier research suggesting that microglia fulfill an antigen-presenting role during the first stages of α -synuclein-mediated neurodegeneration and may become more sensitive, or 'primed', which could eventually promote neurotoxicity in the substantia nigra. In contrast, neither neuronal loss was observed in the hippocampus, nor did we observe any activation of microglia or increases in their density in this brain region following local α -synuclein overexpression. Although certain technical limitations apply, as discussed in **chapter 6**, these studies are consistent with the concept that microglial responses, occurring in a brain region and time dependent manner, can contribute to neurodegeneration.