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Rapid Communication

Postoperative cognitive dysfunction and microglial activation in associated brain regions in old rats

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ABSTRACT

Research indicates that neuroinflammation plays a major role in postoperative cognitive dysfunction (POCD) in older patients. However, studies have mainly focused on hippocampal neuroinflammation and hippocampal-dependent learning and memory, which does not cover the whole spectrum of POCD. We hypothesized that regional differences in postoperative neuroinflammation in the brain may underlie variation in postoperative cognitive impairment. We aimed to investigate this hypothesis in a rat-model for POCD, by analyzing postoperative impairment in behavioral task performance and microglial activation in related brain areas.

We subjected 25 months old Wistar rats to surgery and assessed spatial learning and memory, object and location recognition, reversal learning and exploratory behavior in the second postoperative week. The number and morphology of microglia were analyzed in the hippocampus, prefrontal cortex, striatum and amygdala on postoperative day 14. Control groups consisted of 3 and 25 months old rats that did not undergo surgery.

We observed age related impairment in learning, memory and behavior, which was aggravated following surgery. Additionally, in old rats surgery was associated with signs of classical microglial activation in brain areas related to the impaired cognitive functions. These outcomes suggest that indeed neuroinflammation may be involved in POCD. Moreover, effects of age and surgery on cognition and microglial morphology seem to be area specific and hence cannot be generalized to the whole brain. This underpins the importance for expanding the research of POCD beyond the hippocampus.

1. Introduction

Surgery can lead to transient cognitive decline in patients of all ages, but persisting POCD is mainly seen in patients aged over 60 years (Monk et al., 2008). Postoperative (neuro)inflammation is considered to play a major role in the development of POCD (Cao et al., 2010; Peng, Xu, & Ouyang, 2013; Rosczyk, Sparkman, & Johnson, 2008). It is hypothesized that an altered reactivity of the immune system may account for the persisting POCD in older persons (Barrientos, Hein, Frank, Watkins, & Maier, 2012). Microglia, the primary immune cells of the central nervous system, may play a pivotal role in this process (Barrientos et al., 2006; 2012; Dilger & Johnson, 2008).

POCD affects multiple cognitive domains such as learning and memory, information processing and executive functions (Hovens et al., 2012; Price, Garvan, & Monk, 2008). However, studies investigating the involvement of neuroinflammation in POCD have focused primarily on hippocampal neuroinflammation and hippocampal-dependent learning and memory. This approach may lead to incomplete assumptions, since regional differences in microglial reactivity and vulnerability to inflammatory signaling exist throughout the brain (Olah, Biber, Vinet, & Boddeke, 2011; Vinet

Abbreviations: BLA, Basolateral Amygdala; CA1, Cornu Ammonis 1; CA3, Cornu Ammonis 3; DGib, Dentate Gyrus inner blade; PFC, the medial Prefrontal Cortex Zilles cg1 region; DMS, dorso-medial striatum; IBA-1, ionized calcium binding adaptor molecule 1; MWM, Morris water maze; NL, novel location recognition; NO, novel object recognition; OC, Old Control group; OS, Old Surgery group; POCD, postoperative cognitive dysfunction; YC, Young Control group.

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et al., 2012; Yirmiya & Goshen, 2011). We hypothesized that regional differences in the postoperative neuroinflammatory response may underlie variation in POCD. As POCD is mainly seen in the elderly, we aimed to study postoperative cognitive performance and microglial activation in the hippocampus, striatum, prefrontal cortex and amygdala in old rats.

2. Methods

Rats (18 months, originating from HsdCpb:WU) were obtained from the Semmelweis University (Budapest, Hungary) and aged until 25 months. The old rats underwent surgery (OS[1], $n = 11$) or remained untreated (OC[1], $n = 12$). Behavior was assessed between day 10–13 after surgery. To control for the effects of aging, experimental outcomes of 3 months young rats (YC[1], $n = 12$, HsdCpb:WU obtained from Harlan Laboratories, Venray) that were obtained from an experiment that ran in parallel to the experiment in the old rats were reanalyzed (Hovens, Schoemaker, et al., 2014). Rats were housed under controlled conditions: temperature of 20 ± 2 °C; humidity of 50 ± 10%; 12:12 reversed light dark cycle; and ad libitum access to food and water. Starting one week before the experiment, rats were housed individually. All experimental procedures were approved by the local animal experiment and welfare committee (Dierexperimentencommissie, Groningen, the Netherlands).

Rats were weighed daily at the beginning of the dark phase. Maximum postoperative weight loss as percentage of body weight on the day of surgery (baseline) was determined.

Surgery was performed as described before (Hovens, Schoemaker, et al., 2014). Under sevoflurane anesthesia (3–4% in O_2 with a flow of 0.8% l min^{-1}) and buprenorphine analgesia (0.003 mg/kg s.c, Temgesic) the gastrointestinal tract was exteriorized the upper mesenteric artery was clamped for 30 min. This procedure was set-up to mimic major abdominal surgery in humans (Grootjans et al., 2010; Petrat, Swoboda, de Groot, & Schmitz, 2010). Additionally, a permanent catheter was implanted in the left jugular vein and attached to the skull of the rat to allow repeated blood sampling with minimal handling (Steffens, 1969). Control rats underwent a single blood sample from the tail vein and did not receive any additional anesthesia or analgesia.

Behavioral testing was performed in the second postoperative week as described previously (Hovens, Schoemaker, et al., 2014) and included the following tests: an open field test on day 9 in which behavior was recoded for 5 min and analyzed using Eline software for the percentage of time spent on exploratory behavior (sniffing, rearing and walking), anxiety related behavior (scanning, pressure breathing and freezing), grooming and resting. Alterations in these behaviors are considered indicative of a depression-like profile, which are thought to be mediated by the hippocampus and amygdala (Capuron & Miller, 2011; Schoemaker & Smits, 1994; Walf & Frye, 2006); A NO[1] and NL[1] test on day 10 to determine short term visual recognition, considered to depend primarily on prefrontal function (Dere, Huston, & De Souza Silva, 2007), and spatial recognition, considered to depend mainly on hippocampal function (Dere et al., 2007). The time spent exploring de novel/relocated object as percentage of the total object exploration, during 3 min, was analyzed using Eline software; A MWM[1] protocol consisting of three training sessions of three trials each on day 11, a 60 s probe trial and two additional training sessions on day 12 and a reversal training of 4 trials on day 13. Average escape latency to the platform, located in the center of the target quadrant, during each training session was used as measure for spatial learning. In the probe trial, swimming distance and time in the target quadrant were determined using Ethovision (Noldus Information technology, Wageningen, the Netherlands) and used as measure for motor performance and spatial memory respectively. For the reversal trials, escape latency to the platform, now located in the opposing quadrant, was used as measure for cognitive flexibility. Both spatial learning and memory are considered to depend mainly on hippocampal function, whereas the striatum has been shown to be involved in cognitive flexibility (D'Hooge and De Deyn, 2001).

Rats were terminated 14 days after surgery by transcardial perfusion with saline (0.1% EDTA) under terminal anesthesia (2 ml/kg 6% sodium pentobarbital i.p.). Half of each brain was processed for immunohistochemical staining and stained against IBA-1[1] as described previously (Hovens, Nyakas, & Schoemaker, 2014). Briefly, immersion-fixed 30 μm sections were pretreated with 0.3% H_2O_2, incubated with 1:2500 rabbit-anti IBA-1 (Wako, Neuss, Germany) in 2% BSA, 0.1% TX for two days at 4 °C, with 1:500 goat-anti rabbit secondary antibody (Jackson, Wet Grove, USA) for two hours, and avidin–biotin peroxidase complex (Vectastain ABCkit, Vector, Burlingame, USA) for one hour. Sections were DAB-labeled (0.075 mg/ml DAB). Pictures were taken at 100* magnification of the molecular layer of the DGib, the radial layer of the CA1[1], the stratum lucidum of the CA3[1], the BLA[1], the medial PFC[1] Zilles cg1 region, and the DMS[1] in three brain sections per rat (Leica Qwin, Leica microsystems, Rijswijk, the Netherlands). Pictures were analyzed using image analysis software (Image-pro plus 6.0, Media Cybernetics, Rockville, MD, USA) as described before (Hovens, Nyakas, et al., 2014). The number of microglia were counted and corrected for the size of the area of interest. Based on area coverage, the total microglial cell size and total microglial cell body size were determined. The total cell body size was divided by the total cell size to obtain the cb/c[1] as measure of microglial activation in all brain area's separately and averaged in the DGib, CA1 and CA3 for microglial activation in the hippocampus.

Results are displayed as mean ± SEM. Outcomes deviating more than two SD from the mean were considered outliers and excluded from further analysis. Statistical analysis was performed using SPSS (IBM SPSS statistics 22). Escape latencies in the MWM and exploration in the NO and NL test were analyzed using Repeated measures ANOVA. All other experimental outcomes were analyzed with a one-way ANOVA, followed by a Tukey Post Hoc analysis or Kruskal–Wallis analysis followed by Mann–Whitney U Post Hoc analysis. $p \leqslant 0.05$ was regarded statistically significant. Cognitive test outcomes and microglial activation in related brain areas was correlated using Pearson's or Spearman's correlations, with a Bonferonni–Holmes correction for the significance level.

3. Results

3.1. Weight

Baseline body weight was 372 ± 4 g and 591 ± 16 g in young and old rats respectively. Maximum weight loss differed significantly between groups ($F_{2,33} = 60.17$, $p = 0.000$). OS lost significantly more weight (7.4 ± 2.7%) than OC (1.7 ± 1.1%, $p = 0.000$) and YC (0.1 ± 0.2%, $p = 0.000$).

3.2. Behavioral test outcomes

In the NO and NL test, there was no place preference during the exploration phase ($F_{2,32} = 0.16$, $p = 0.86$). Object recognition declined due to surgery in old rats (Fig. 1A, $F_{2,29} = 6.08$, $p = 0.007$). Location recognition differed significantly between control groups (Fig. 1B, $F_{2,29} = 12.29$, $p = 0.000$). Although there was no phase*group interaction effect on object exploration in the NO and NL test, the average object exploration in the test phases differed

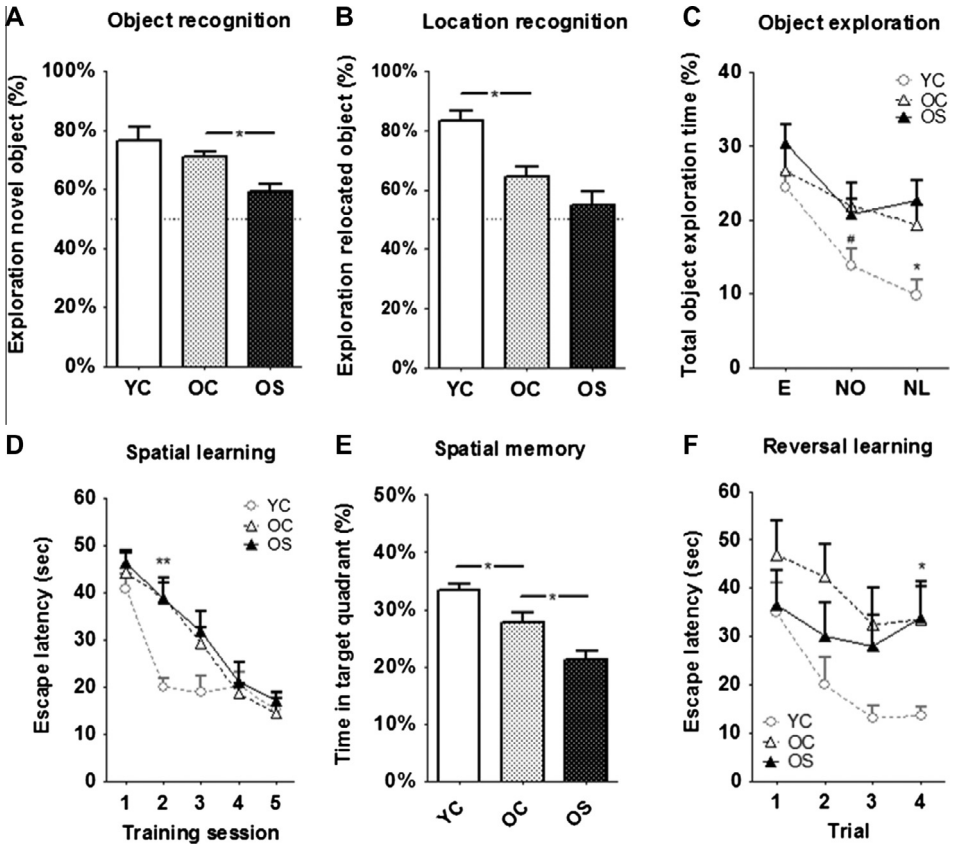

Fig. 1. Cognitive performance. (A) Location recognition as time spent on exploration of the relocated object relative to total object exploration (%). (B) Object recognition as the time spent on exploration of the novel object relative to total object exploration (%). (C) Object exploration during the exploration phase (E), object recognition phase (NO) and location recognition phase (NL) of the novel object and novel location test. (D) Spatial learning in the MWM as average escape latency for each training session. (E) Spatial memory in the MWM as percentage of time spent in the target quadrant during the probe trial. (F) Reversal learning in the MWM as average escape latency (sec) for each reversal trial. YC = Young Control, OC = Old Control, OS = Old Surgery, $^{\#}p < 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$.

significantly (Fig. 1C, $F_{2,32} = 5.80$, $p = 0.007$), with old rats spending more time on object exploration than young rats.

The learning curve of the MWM-training sessions declined significantly over time in all groups (Fig. 1D, $F_{4,116} = 36.07$, $p = 0.000$), but did not differ between groups. However, the average escape latency over all five training sessions differed significantly ($F_{2,29} = 4.797$, $p = 0.016$), with a better performance in young compared to old rats. Performance in the MWM probe trial (Fig. 1E) differed significantly between groups ($F_{2,30} = 15.60$, $p = 0.000$), with an aging effect exaggerated by surgery. There were no group differences in the MWM reversal learning curve (Fig. 1F). However, there was a significant difference in the average escape latency over all reversal trials ($F_{2,29} = 6.857$, $p = 0.004$), with a better performance in young compared to old rats. Swimming distance in the MWM did not differ between groups.

In the open field (Fig. 2) there was a significant difference in resting behavior ($\chi^2(233) = 14.98$, $p = 0.001$) with old rats spending more time resting than young rats and a trend for increased resting behavior following surgery in old rats. Additionally there was an overall trend for a difference in exploratory behavior ($F_{2,32} = 3.04$, $p = 0.063$).

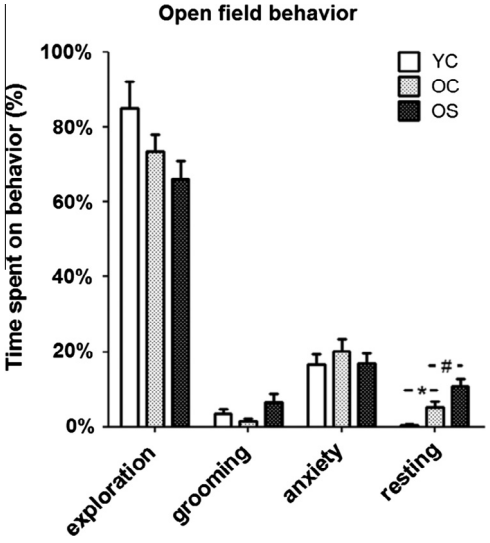

Fig. 2. Behavior in the open field test. Percentage of time spent on exploratory behavior, grooming, anxiety related behavior and resting behavior in the open field. YC = Young Control, OC = Old Control, OS = Old Surgery, $^{\#}p < 0.10$, $^{*}p < 0.05$.

3.3. Microglial activation

The cb/c of microglia was used as a measure of microglial activation (Fig. 3). There were significant group differences in cb/c in the Dgib ($F_{2,29} = 20.36$, $p = 0.000$), CA1 ($F_{2,29} = 23.94$, $p = 0.000$), CA3 ($F_{2,33} = 20.36$, $p = 0.000$), PFC ($\chi^2(231) = 14.77$, $p = 0.001$), and STR ($\chi^2(233) = 16.43$, $p = 0.000$), but not in the BLA. In the DGib and CA3 cb/c was increased in old rats, whereas in the CA1 and PFC cb/c was increased only in old rats following surgery. Strikingly, in contrast to the other brain areas, STR cb/c was reduced in old rats.

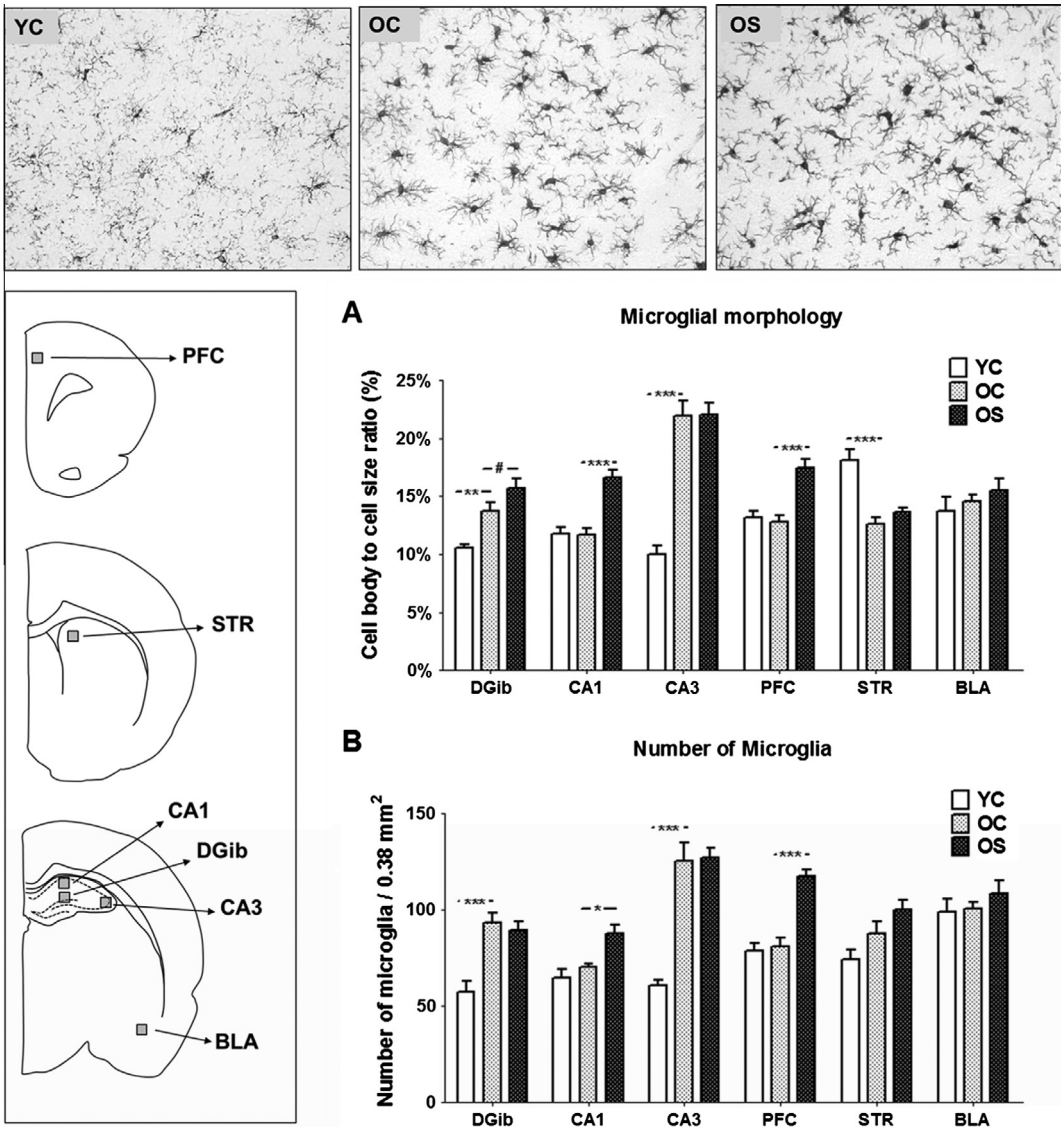

Fig. 3. Microglial activation (A) the cell body to cell size ratio of microglia, and (B) the number of microglia in an area of 0.38 mm^2. Images: example of IBA-1 staining in the CA3 for each experimental group. Box: analyzed areas. DGib = Dentate Gyrus inner blade, PFC = Prefrontal Cortex, STR = Striatum, BLA = Basolateral Amygdala, YC = Young Control, OC = Old Control, OS = Old Surgery, $^{\#}p < 0.01$, $^{**}p < 0.01$, $^{***}p < 0.001$.

The number of microglia appears to follow a similar pattern with significant group differences in the Dgib ($F_{2,31} = 15.49$, $p = 0.000$), CA1 ($F_{2,31} = 10.16$, $p = 0.000$), CA3 ($\chi^2(231) = 21.85$, $p = 0.000$), PFC ($F_{2,31} = 29.33$, $p = 0.000$), but not in the BLA. In the STR there was also a significant group difference ($F_{2,31} = 5.79$, $p = 0.007$) where OS had a significantly higher number of cells than YC.

Overall, hippocampal cb/c was significantly correlated with performance in the MWM probe trial ($r = -.612$, $p = 0.000$) and location recognition ($r = -.446$, $p = 0.017$), but not with spatial learning in the MWM. Cb/c in the PFC was significantly correlated with object recognition ($r_s = -.505$, $p = 0.006$). Cb/c in the STR was significantly correlated with reversal learning ($r_s = -.425$, $p = 0.017$).

4. Discussion

Evidence is accumulating for the role of neuroinflammatory processes in POCD development. The studies linking neuroinflammation to POCD have mainly focused on the hippocampus. Indeed, in a previous study, we showed that young rats display temporary impairments in hippocampal-dependent memory only, as well as hippocampal neuroinflammation (Hovens, Schoemaker, et al., 2014). However, POCD mainly occurs in elderly patients and involves a wide range of cognitive impairments. Therefore, in the present study, we aimed to investigate postoperative cognitive impairment and microglial activation in old rats.

In addition to age-related alterations in cognitive and behavioral task performance, old rats displayed impaired spatial memory, object recognition and resting behavior following surgery. Thus, our results indicate that old rats show a more generalized cognitive and behavioral dysfunction following surgery. This seems to be more in line with the cognitive impairments associated with POCD in older humans than the isolated spatial impairments observed in young rats (Hovens, Schoemaker, et al., 2014; Hovens et al., 2012).

Interestingly, spatial and reversal learning were unaffected by surgery indicating that in old rats postoperative cognitive impairment it is still limited to specific cognitive domains. Reversal

learning is considered to depend primarily on striatal function (D'Hooge and De Deyn, 2001). In spatial navigation tasks young adults are considered to prefer a hippocampal-dependent strategy, relying on external spatial cues. However, old individuals often switch to a more striatal-dependent navigation strategy, memorizing a specific set of actions or body movements (Rodgers, Sindone, & Moffat, 2012). Taking this into consideration, it may be that striatal-dependent tasks are less sensitive to the effects of surgery than tasks that depend primarily on other brain regions (Dere et al., 2007). In contrast to our findings, others reported a postoperative decrease in reversal learning in aged rats (Cao et al., 2010; Li et al., 2013; Rosczyk et al., 2008). Since these changes in reversal learning were observed in the first week following surgery, it may be that postoperative impairment in cognitive flexibility did occur in our rats, but was short-lived.

After surgery old rats did not show further impairment in location recognition. This may be due to a flooring effect in the task, since OC rats already explored little more than 50%, indicating no object discrimination.

In old rats, microglial cell body to cell size ratio and number in the DGib and particularly the CA3 was increased, indicating an effect of age per se on microglial activation in these hippocampal regions. Indeed, previous research indicates that aging is associated with hippocampal microglial activation (Dilger & Johnson, 2008; Frank, Barrientos, Watkins, & Maier, 2010, 2006).

In young rats we previously observed that hippocampal microglial activation was increased in the first postoperative week, and had returned to control levels one week later (Hovens, Schoemaker, et al., 2014). In the current experiment, old rats show signs of increased microglial activation in the CA1 as well as a trend towards increased microglial cell body to cell size ratio in the DGib, two weeks following surgery. This suggests that, in concurrence with the literature, old age is associated with prolonged hippocampal microglial activation, co-occurring with increased postoperative spatial memory impairment (Barrientos et al., 2012; Cao et al., 2010; Rosczyk et al., 2008). Additionally, we observed a significant correlation between hippocampal microglial activation and spatial memory in the MWM and NL test. Similarly, old rats displayed a postoperative increase in microglial activation in the PFC, which was correlated to impaired object recognition. These outcomes suggest that postoperative microglial activation in the hippocampus and PFC may be associated with impaired spatial and visual memory.

Contrary to our expectations, old age was associated with a marked decrease in the cell body to cell size ratio in the striatum. Although microglial activation has classically been associated with an increased cell body size and retraction of processes, it has become apparent that, depending on the stimulus and environment, other morphological changes occur (Olah et al., 2011; Ransohoff & Perry, 2009). These different types of microglial activation may have different consequences for neuronal functioning (Olah et al., 2011; Ransohoff & Perry, 2009). Notably, microglial phenotype and responsiveness to inflammatory stimuli was shown to be brain area dependent (Olah et al., 2011). Hypothetically, characteristics of striatal microglia may underlie the relative resilience of striatal-dependent learning to surgery. However, reduced striatal microglial activation was correlated to reduced reversal learning performance, suggesting that a reduced cell body to cell size ratio of microglia in this brain area in old rats is associated with reduced cognitive flexibility.

Of note to the findings in this study is that the observed alterations in microglial morphology were relatively mild compared to the alterations associated with an acute inflammatory event, such as LPS injection. To investigate the functional consequences of this microglial activation and confirm the preliminary hypotheses formed in this study, future studies should include a more extensive analysis of microglial activation and markers for neuroinflammation and neuronal functioning.

We found that old rats spend an increased time on object exploration in the novel object and novel location test. This differences may represent an alteration in motivational state. However, old age is usually associated with a loss rather than a gain of interest. Indeed, in the open field old age and surgery were related to increased resting behavior and a trend towards decreased exploration. Alternatively, the increased object exploration may reflect an alteration in information processing. This may be in concurrence with the increased microglial activation observed in the DGib and particularly the CA3 of old rats. These hippocampal regions have been associated with pattern separation and pattern completion respectively (Kesner, 2007a,b; Neunuebel & Knierim, 2014). Moreover, these regions were believed to be involved in rapid encoding of novel information (Kesner, 2007a,b). If these processes are impaired in old rats they may need a longer time and more information before a memory pattern is formed.

In the current study, we have not included animals that received only anesthesia for a period comparable to the duration of the surgical procedure. Although, our previous studies (Hovens et al., 2013; Hovens, Schoemaker, et al., 2014) and those of others have not found an effect of anesthesia on POCD development, neurological side effects of anesthesia have been reported (Cao, Li, Lin, & Zuo, 2012; Chen, Gong, Yan, & Zhang, 2013). Therefore, we cannot exclude that the outcomes of our experiment were partly caused by the anesthetic agent.

To conclude, whereas young rats were previously found to display a postoperative impairment in spatial memory only, old rats show a more general postoperative cognitive impairment that seems to be associated with microglial activation in functionally related brain regions. These outcomes suggest that neuroinflammatory signaling may indeed be involved in the pathogenesis of POCD. However, our results also indicate that the effects of age and surgery on cognition and microglial morphology are area specific and cannot be generalized to the brain as a whole. This underpins the importance for expanding the research of POCD beyond spatial memory and the hippocampus.

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