Characterizing postoperative cognitive dysfunction in the elderly

Hovens, Iris Bertha

CHAPTER THREE

—

Postoperative cognitive dysfunction: involvement of neuroinflammation and neuronal functioning

Iris B. Hovens[1], Regien G. Schoemaker[1], Eddy A. van der Zee[1], Anthony R. Absalom[2], Erik Heineman[3] and Barbara L. van Leeuwen[3]

[1]department of Molecular Neurobiology, University of Groningen, Groningen, the Netherlands; [2] department of Anesthesiology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; [3] department of Surgery and Surgical Oncology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

Abstract

Postoperative cognitive dysfunction (POCD) has been hypothesized to be mediated by surgery-induced inflammatory processes, which may influence neuronal functioning either directly or through modulation of intraneuronal pathways, such as the brain derived neurotrophic factor (BDNF) mediated pathway.

To study the time course of post-surgical (neuro)inflammation, changes in the BDNF-pathway and POCD, we subjected 3 months old male Wistar rats to abdominal surgery and implanted a jugular vein catheter for timed blood sampling. Cognition, affective behavior and markers for (neuro)inflammation, BDNF and neurogenesis were assessed at 1, 2 and 3 weeks following surgery.

Rats displayed changes in exploratory activity shortly after surgery, associated with postoperatively elevated IL-6 plasma levels. Spatial learning and memory were temporarily impaired in the first two weeks following surgery, whereas non-spatial cognitive functions seemed unaffected. Analysis of brain tissue revealed increased neuroinflammation (IL-1B and microgliosis) 7 days following surgery, decreased BDNF levels on postoperative day 14 and 21, and decreased neurogenesis until at least 21 days following surgery.

These findings indicate that in young adult rats only spatial learning and memory is affected by surgery, suggesting hippocampal dependent cognition is especially vulnerable to surgery-induced impairment. The observed differences in time course following surgery and relation to plasma IL-6 suggest cognitive dysfunction and mood changes comprise distinct features of postoperative behavioral impairment. The postoperative changes in neuroinflammation, BDNF and neurogenesis may represent aspects of the underlying mechanism for POCD. Future research should be aimed to elucidate how these players interact.

Introduction

Following illness or trauma, patients may experience cognitive impairment. One event that has been associated with cognitive decline is surgery (Newman et al., 2007; Rasmussen, 2006). Postoperative cognitive dysfunction (POCD) involves a wide range of cognitive functions including working memory, long term memory, information processing, attention, and cognitive flexibility (Hovens et al., 2012), adversely affecting quality of life, social dependence, and mortality (Steinmetz et al., 2009). In most cases, cognitive function returns to normal within a month after surgery, but in some patients the cognitive decline persists (Rasmussen, 2006). Although POCD can occur at all ages, the main risk factor for POCD is advanced age (Krenk et al., 2010; Monk et al., 2008). Other risk factors include the severity and duration of the surgical procedure (Krenk et al., 2010).

The pathogenesis of POCD remains largely unknown. Evidence is accumulating for a key role of inflammation in the disease process (Krenk et al., 2010). Local inflammation due to the surgical trauma is paralleled by an increase in systemic inflammatory mediators (Beloosesky et al., 2007; Cibelli et al., 2010; Ramlawi et al., 2006; Shapira-Lichter et al., 2008; Yaffe et al., 2003). Several of these mediators have been shown to influence inflammatory processes in the brain, leading to the activation of microglia, the immune cells of the brain, and the concurrent endogenous production of pro-inflammatory cytokines (Cibelli et al., 2010; Dilger and Johnson, 2008; Ji et al., 2013b; Tang et al., 2011; Wan et al., 2007). Since neuroinflammation has been associated with impaired cognitive functioning, this mechanism is hypothesized to underlie POCD development (Cibelli et al., 2010; Hovens et al., 2012; Wan et al., 2007). Aging has been associated with an exacerbated inflammatory response and more pro-inflammatory profile (Barrientos et al., 2006; Cortese et al., 2011; Dilger and Johnson, 2008), which may account for the increased incidence of POCD in the elderly (Barrientos et al., 2012; Beloosesky et al., 2007; Hudetz et al., 2007; Ramlawi et al., 2006; Wan et al., 2007; Yaffe et al., 2003). In addition to a direct effect of pro-inflammatory cytokines, such as IL-1B and IL-6, on neuronal functions essential for learning and memory, inflammatory factors may also indirectly influence neuronal

functioning (Yirmiya and Goshen, 2011). In particular brain-derived neurotrophic factor (BDNF) and its intraneuronal pathway have been implicated as mediators between neuroinflammation and neuronal dysfunction (e.g. decreased neurogenesis, synaptic plasticity and LTP), leading to cognitive impairment (Barrientos et al., 2004; Cortese et al., 2011; Yirmiya and Goshen, 2011).

Although POCD occurs most frequently in the elderly, it is also known to occur in younger adult patients (Monk et al, 2008). We have previously developed a rat model of POCD after abdominal surgery with mesenteric ischemia-reperfusion (Hovens et al., 2013). Healthy young adult rats coped well with the procedure, with no peri-operative deaths, minimal weight loss, and evidence of a robust inflammatory response to the procedure.

The aim of the current study was to investigate the time course of POCD and the involvement of (neuro)inflammation and changes in the BDNF pathway. We therefore used our existing model to assess aspects of learning and memory, exploratory behavior and markers of (neuro)inflammation, BDNF and neurogenesis at 1, 2 and 3 weeks following surgery in young adult rats.

Methods and materials

Design

Young adult rats were subjected to abdominal surgery and received a jugular vein catheter under anesthesia (n=36). Learning, memory and exploratory behavior were assessed during postoperative week one (S1, n=12), two (S2, n=12), or three (S3, n=12), followed by sacrifice on day 7, 14 or 21 after surgery, respectively (Figure 3.1). Naïve control animals (C, n=12) underwent testing and sacrifice together with the three surgery groups (4 animals at each time point). Animals that only received anesthesia (A, n=12) underwent testing and sacrifice together with S1.

As implantation of a jugular vein catheter is a surgical intervention in itself, in a

control experiment the behavioral consequences of this procedure were studied during the second week following the intervention. Rats that only received a jugular vein catheter (n=10) were compared with rats that remained naïve (n=10).

All behavioral tests were conducted in a room adjacent to the housing room, under dim light conditions, in the first half of the dark phase.
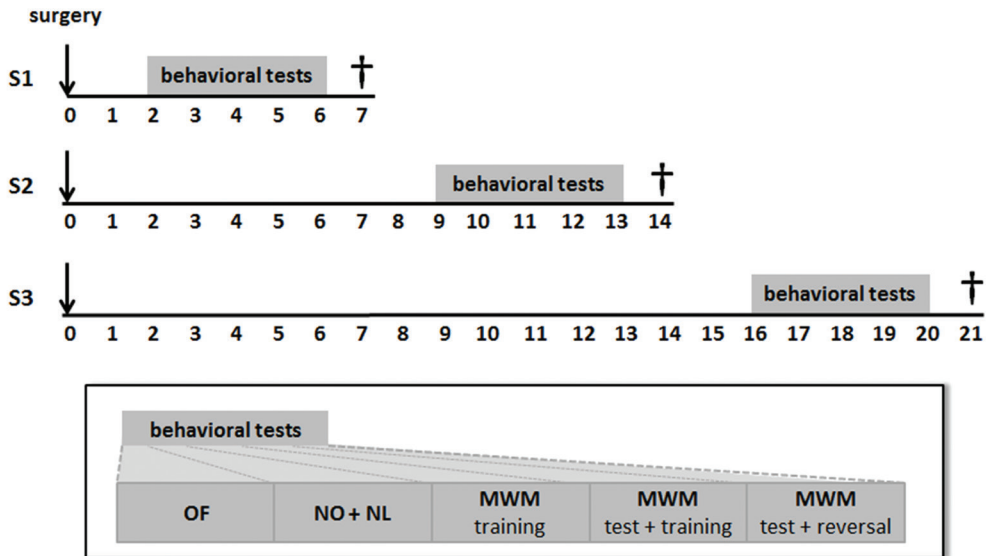

Figure.3.1: *Experimental design. For the experimental groups that underwent behavioral testing during week one (S1), two (S2) or three (S3) following surgery a time axis is shown displaying the number of days following surgery (↓). S1, S2 and S3 underwent a series of behavioral tests during a period of 5 days, starting on day 2, 9 and 16 respectively. The inset shows the order in which the behavioral tests were performed. In this inset each block represents one day. The day after the last behavioral test the animals were sacrificed (†)*

Experimental animals

Three months old male Wistar rats (HsdCpb:WU, Harlan, Venray, NL) were individually housed in cages of 24*24*36cm in a room with a temperature of 20±2 °C and humidity of 50±10%. Rats had ad libitum access to laboratory chow and tap water. The animals were kept on a 12:12 light:dark cycle (lights on at 9.00 a.m.). All

experiments were approved by the local animal experiment and welfare committee (DierExperimentenCommissie, Groningen, the Netherlands).

Surgery

Abdominal surgery was performed on rats (n=36) as described before (Hovens et al., 2013). In short, under sevoflurane anesthesia (3% sevoflurane in O_2 at 0.7 L/min) and buprenorphine analgesia (0.003 mg/kg s.c.) the gastrointestinal tract was exteriorized and the upper mesenteric artery clamped for 30 min. Clamping of the upper mesenteric artery leads to a restriction of blood flow in the mesenteric vascular bed, although the presence of collateral arteries allows some perfusion (Petrat et al., 2010).

We consider this abdominal surgery a model to mimic major abdominal surgery in humans (Grootjans et al., 2010a, 2010b). The animals that received abdominal surgery were equipped with an indwelling jugular vein catheter during the abdominal surgery procedure, to allow timed blood sampling with minimal handling (Steffens, 1969). A group of rats that received only anesthesia and analgesia (n=12) was kept under sevoflurane anesthesia for 1 h. Analgesia and blood sampling were performed in accordance with the surgery group.

Behavioral tests

Open Field

An open field test was performed to assess exploratory activity and anxiety (Schoemaker and Smits, 1994). The test was performed on post-operative day 2, 9 and 16, for S1, S2 and S3, respectively. A square (100*100*40 cm) open field was divided into a center area (60*60 cm), 4 corner areas (20*20 cm), and 4 side areas (20*60 cm). Behavior was recorded for 5 min and analyzed with a computerized image analyzing system (Ethovision, Noldus Information technology, Wageningen, the Netherlands) on percentage of time spent in the center and corners and total distance moved.

Novel object and novel location recognition

A novel object and novel location recognition test was performed to assess visual and spatial short term memory (Dere et al., 2007). The test was performed on post-operative day 3, 10 and 17, for S1, S2 and S3, respectively. The day before testing, rats were habituated by twice being placed in the test box (50*50*40 cm) for 5 min. After a 3 min habituation, the test consisted of 3 phases of 3 min separated by a 45 s pause in which the rat remained in the test box. In the exploration phase, the rat was presented two identical objects (stacked Lego cubes or plastic bottles). In the novel object recognition phase (NO), the rat was presented with one familiar object and one novel object. In the last phase, the novel location recognition phase (NL), the familiar object of the NO was returned to its original location, whereas the novel object of the NO was placed at a novel location. All objects were cleaned with 70% ethanol before they were placed in the test box to remove smell cues. The percentage of time the animal spent exploring each object in each phase was determined using Eline software. The ratio of time the animal spent exploring the novel or relocated object compared to the total object exploration time was taken as a measure of object or location recognition. Trials in which rats spent less than 5 s exploring the objects were removed from further analysis.

Morris water maze

Morris water maze (MWM) testing was performed to assess spatial learning, spatial memory and cognitive flexibility. A round pool (140 cm ID) was filled with water of 26 ± 1 °C and surrounded by external visual cues. The maze was divided in 4 quadrants. In one quadrant (target quadrant) an invisible platform was placed 1 cm below the water surface.

The water maze protocol started on post-operative day 4, 11 and 18, for S1, S2 and S3, respectively, and consisted of two training phases, two probe trials and a reversal training over a period of 3 days. The first day of the protocol the rats were trained to find the hidden platform. This first training phase started at 10 o'clock and consisted of 3 training sessions with a 1 h training interval. Each training session consisted of three

consecutive trials. The rat was sequentially placed in each quadrant not containing the platform and allowed to search for the platform. The order of the quadrants was randomly chosen. Each trial stopped 10 s after the rat found the platform. If the rat did not find the platform within 60 s it was gently guided towards the platform and left there for 10 s. After completion of the three trials the rat was towel dried and returned to the home cage. The average escape latency to the platform per training session was taken as measure for spatial learning.

The second day of the protocol at 10 o'clock a probe trial was performed to assess spatial memory. The platform was removed from the maze. Each rat was randomly placed in one of the quadrants and allowed to explore the maze for 60 seconds. Behavior was analyzed with a computerized image analyzing system (Ethovision, Noldus Information technology, Wageningen, the Netherlands) for time spent in each quadrant of the maze and distance moved. Time spent in the target quadrant was taken as measure for spatial memory. One hour after this probe trial, animals underwent two more training sessions to assure that all the rats learned the platform location, and had a similar base for reversal training. Again the average escape latency to the platform per training session was taken as measure for spatial learning. The average escape latencies of both the first and second training session were used to form a learning curve.

The third day of the protocol at 10 o'clock a second probe trial was performed to assess spatial memory. One hour after the second test animals were subjected to reversal training (D'Hooge and DeDeyn, 2001), in which the platform was moved to the opposite quadrant. The reversal training consisted of 4 consecutive trials. Average escape latency of the reversal training was taken as measure of cognitive flexibility.

Immunohistochemistry

Animals were sacrificed by transcardial perfusion with saline containing 0.1% EDTA, under pentobarbital anesthesia (6%, 2 ml/kg). Half of each brain was immersion fixed in 4% paraformaldehyde for 4 days followed by cryoprotection with 30% sucrose in

PBS. Brains were cut into 30 µm thick sections and transferred to PBS in 15 ml multi-purpose containers (Greiner Bio-One, Alphen aan de Rijn, the Netherlands). Free floating sections were pretreated with 3% H_2O_2 for 20 min.

To visualize microglia, sections were incubated for 3 days with 1: 2500 rabbit-anti IBA-1 (Wako, Neuss, Germany) in 2% BSA, 0.1% TX at 4oC, followed by a 1 h incubation with 1:500 goat-anti rabbit secondary antibody (Jackson, Wet Grove, USA) at room temperature.

To visualize BDNF, sections were blocked for 1 h with 5% normal goat serum, incubated with 1:1000 rabbit-anti BDNF (Alomone labs, Jerusalem, Israel) in 1% BSA, 1% NGS for 3 h at 37°C, overnight at room temperature and 3 nights at 4°C, followed by incubation with 1:500 goat-anti rabbit secondary antibody (Jackson, Wet Grove, USA) overnight at 4°C.

To visualize newly formed neurons, sections were blocked for one hour with 5% normal rabbit serum, incubated with 1:1000 goat-anti DCX (Santa Cruz, Dallas, USA) in 1% BSA, 1% NRS, for 3 h at 37°C, overnight at room temperature and 3 nights at 4°C, followed by incubation with 1:500 rabbit-anti goat secondary antibody (Jackson, Wet Grove, USA) overnight at 4°C. All sections were incubated for 2 h with avidin-biotin peroxidase complex (Vectastain ABCkit, Vector, Burlingame, USA) at room temperature. Labeling was visualized using a 0.075 mg/ml DAB solution activated with 0.1% H_2O_2. All dilutions were made in 0.01M PBS. All sections were thoroughly rinsed with 0.01M PBS between staining steps. Sections were transferred to glass slides and dehydrated through gradients of ethanol and xylol solutions.

Labeling was analyzed blinded to the treatment, in 3 sections per area for each animal. Microglia activation was determined in the dentate gyrus inner blade (DGib), CA1 region and CA3 region of the hippocampus, prefrontal cortex (PFC, Zilles's Cg1) and dorsolateral striatum (STR). Using image analysis software (Image-Pro Plus 6.0), the number of microglia, the average total cell size and average cell body size

were determined in an 0.059 square mm area (400x magnification). Since microglia activation is characterized by an increased cell body size and shortening of the dendritic processes (Kreutzberg, 1996), the cell body to total cell size ratio was used as a measure of microglia activation.

The number of DCX positive cells was counted in the DG using a light microscope at 50x magnification and corrected for the size of the DG (Van der Borght et al., 2007). The number of DCX positive cells per mm was considered a measure for the number of new-formed neurons in the DG and thus neurogenesis. The amount of BDNF staining in the DGib, CA1, CA3, STR and PFC (50x magnification) was determined by analyzing optical density using quantitative imaging software (Leica QWin, Leica Microsystems).

Elisa

Blood samples were taken in all rats equipped with a jugular vein catheter. Blood was sampled 2, 6, 12, and 24 h and 2, 7 and 14 days after surgery at the end of the light phase. Blood was centrifuged for 10 min at 2600 G, plasma was collected and stored at -80 °C until further analysis. Plasma IL-6 concentrations were determined using the Rat IL-6 Elisa-kit (Invitrogen, Frederick, USA) according to manufacturer's instruction.

After sacrifice, half of each brain was dissected into different brain areas. The hippocampus (HIP), PFC and striatum (STR) were homogenized in a 50 mM Tris-HCL buffer (150 mM NaCl, 0.002% Tween-20 and 1 protease inhibitor tablet/ 10 ml) followed by 2 x 5 s sonification. Homogenates were centrifuged for 15 min at 12000 RPM, and supernatant was collected. Using a Bradford assay in 96-well microplates (CELLSTAR, Greiner Bio-One, Alphen aan den Rijn, the Netherlands), the supernatant was diluted to 5 mg/ml protein. IL-6 and IL-1B concentrations were determined using the Rat IL-6 Elisa-kit and Rat IL-1B Elisa-kit (Invitrogen, Frederick, USA) according to manufacturer's instruction.

Statistical analysis

Data are displayed as group averages ± SEM. Statistical analysis was performed using SPSS Statistics 20.0 (IBM). Spatial learning in the MWM was assessed using repeated measures ANOVA, with training sessions as within subject factor and experimental group as between subject factor. All other outcomes were compared using an ANOVA and 2 sided Dunnett's post hoc analysis with experimental group as between subject factor and C as reference group. Pearson correlation coefficients were determined to examine whether there was a relationship between plasma IL-6 concentrations at 24 h following surgery and behavioral test outcomes. Outcomes that deviated by more than 2 SD from group average were considered outliers and excluded from analysis. Effects were regarded statistically significant when p ≤ 0.05.

Results

Weight

Body weight at the start of the experiment was 372 ± 2 g (C: 372 ± 4, A: 373 ± 3, S1: 372 ± 3, S2: 372 ± 4, S3: 342 ± 7 g; F4,55=0.04, p=0.997). Maximum relative weight loss (C: 0.1 ± 0.1%, A: 0.8 ± 0.2, p=0.839, S1: 6.8 ± 0.5%, p=0.000, S2: 4.5 ± 0.9%, p=0.000, S3: 5.4 ± 1.4%, p=0.000) differed significantly between experimental groups ($F_{4,55}$=35.90, p= 0.000).

Exploratory behavior

The distance moved in the open field (Figure 3.2A) seemed to be decreased for S1 although the difference between groups did not reach significance ($F_{4,55}$=1.65, p=0.176). Preference for the corners of the open field (Figure 3.2B), the zone perceived to be most safe, differed between groups ($F_{4,55}$=2.55, p=0.049) and was significantly increased in S1, whereas time spent in the center of the open field was significantly decreased in S1 (C: 10 ± 3%, A: 10 ± 5%, S1: 5 ± 4%, S2: 9 ± 3%, S3: 8 ± 5%; $F_{4,55}$=2.77, p=0.037).
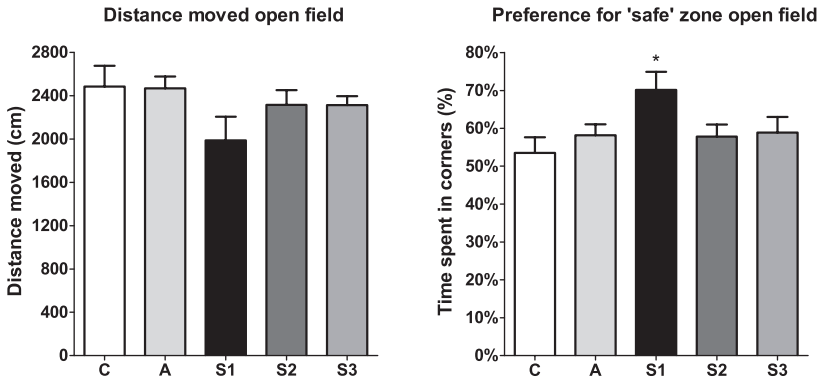
Figure 3.2: *Open field behavior following surgery. A) Distance moved in the open field (cm). B) Time spent in the corners of the open field, considered to be a measure for safe zone preference. C=control, A=anesthesia only, S1, S2 and S3 = animals that underwent behavioral testing during week 1, 2 and 3 after surgery. *p<0.05 compared to control.*

Learning and memory

There was a significant effect of training session on escape latency in the maze ($F_{4,212}$=50.76, p=0.000), indicating all rats were able to learn where the platform was located. The learning curve (Figure 3.3A) differed significantly between groups ($F_{4,212}$=1.71, p=0.047) with S1 showing impaired learning compared to C (p=0.033). Rats of group S1 and S2 showed a decreased dwell time in the target quadrant in the first MWM probe trail, (Figure 3.3B, $F_{4,52}$=4.20, p=0.005). Dwell time in the other quadrants did not differ significantly. Distance moved during this probe trial did not differ between groups (Figure 3.3D, $F_{4,52}$=0.49, p=0.74). Test performance in the second MWM probe trial, did not differ between groups (C: 40 ± 3%, A: 41 ± 4%, S1: 36 ± 2%, S2: 40 ± 3%, S3: 34 ± 4%; $F_{4,47}$=0.54, p=0.71), indicating all animals had learned the platform location before reversal training started. There was no group difference in escape latency during the reversal trials (Figure 3.3E, $F_{4,55}$=1.15, p=0.34). In the exploration, NO and NL phase, 1, 7 and 11 out of 60 trials respectively had to be excluded from analysis because the animals spent less than 5 s on object exploration. Although on average object exploration decreased in consecutive phases (Exploration phase: 21±1%, NO: 13±1%, NL: 10±1%, $F_{4,55}$=104.32, p=0.000), there was no difference between groups ($F_{4,55}$=0.87, p=0.49). Object preference in the exploration phase ($F_{4,54}$=0.66, p=0.62) and

object recognition (Figure 3.3F, $F_{4,49}$=0.84, p=0.51) did not differ significantly between groups. Location recognition was decreased in the first 2 weeks after surgery (Figure 3C, $F_{4,45}$=4.62, p=0.003).
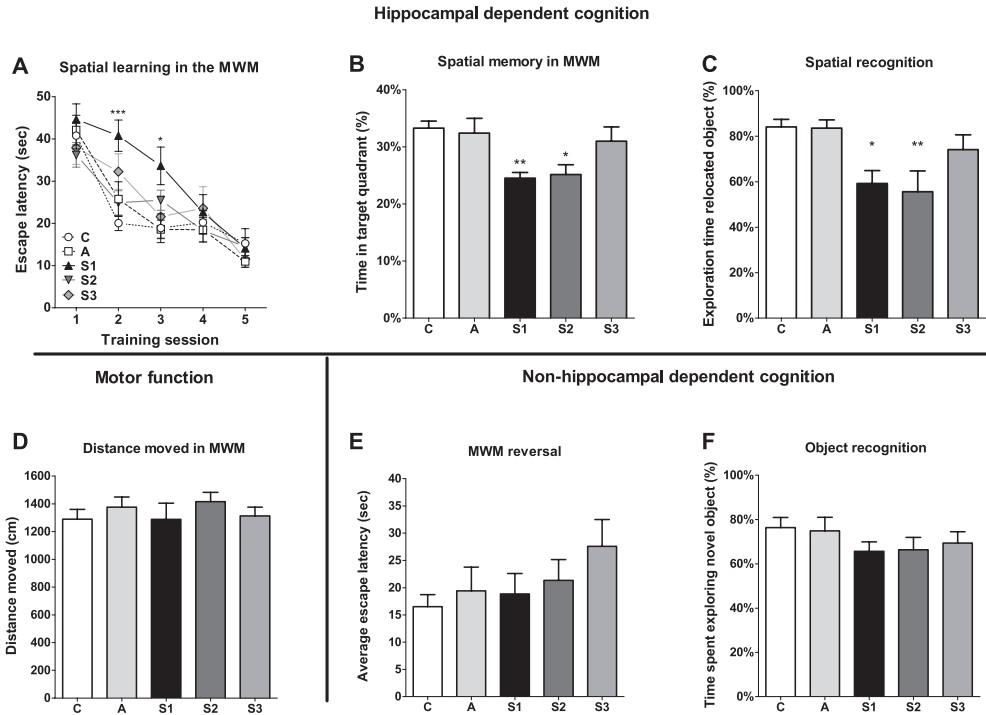
Hippocampal dependent cognition

Motor function

Non-hippocampal dependent cognition

Figure 3.3. *Learning and memory performance following surgery. A) Spatial learning in the MWM. Average escape latency (seconds) is shown for the 5 training sessions in the maze. B) Spatial memory in the MWM. The percentage of time in the target quadrant (Target Q) and time in the quadrant opposite to the target quadrant (Opposing Q) is shown. Dotted line = reference line for expected time in the target quadrant (25%) for a random swimming pattern, C) Spatial recognition in the novel location test. Exploration time of the relocated object as percentage of total object exploration is shown. Dotted line = reference line for expected exploration time (50%) if there is no object preference. D) Distance moved (cm) in the MWM during the first test as a measure for motor function. E) Cognitive flexibility in the MWM. Average escape latency (sec) during the four MWM reversal trials. F) Object recognition in the novel object test. C=control, A=Anesthesia only, S1, S2 and S3 = animals that underwent behavioral testing during week 1, 2 and 3 after surgery. *p<0.05, **p<0.01, ***p<0.001 compared to C.*

Animals that only received a jugular vein catheter did not differ significantly from naïve control animals on any of the behavioral tests, except for an impaired performance in the novel location test (t (11) = 2.455, p=0.032).

Plasma IL-6

Plasma IL-6 levels (Figure 3.4) were increased significantly the first 12 h after surgery ($F_{7,57}$=21.71, p=0.000). Although plasma IL-6 concentrations at 24 h following surgery did not deviate significantly from baseline, the concentrations still varied considerably in the measurable range. As this 24 h time point was the closest time point to the behavioral tests that still showed variation, these values were correlated with behavioral test outcomes. Exploratory behavior, but not learning and memory, was significantly correlated with plasma IL-6 levels 24 h following surgery (distance moved in the open field: R^2 = 0.73, p=0.013, time spent in the safe zone of the open field: R^2 = 0.64, p=0.031).

Figure 3.4: *Plasma IL-6 concentrations (pg/ml) following surgery. ***p<0.001 and *p<0.05 compared to baseline (day 14 following surgery).*
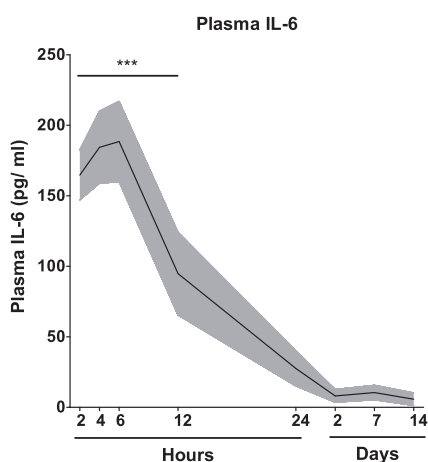
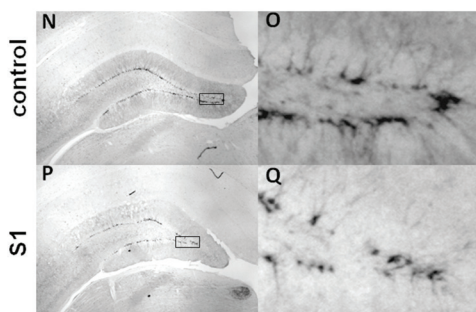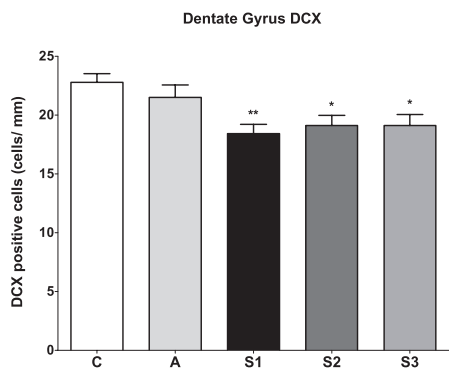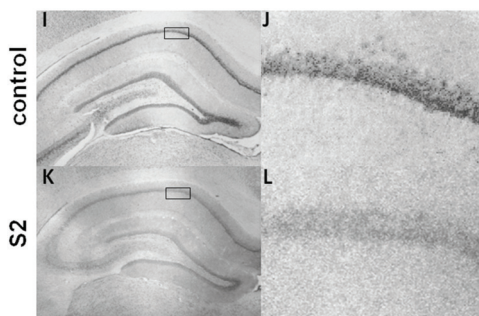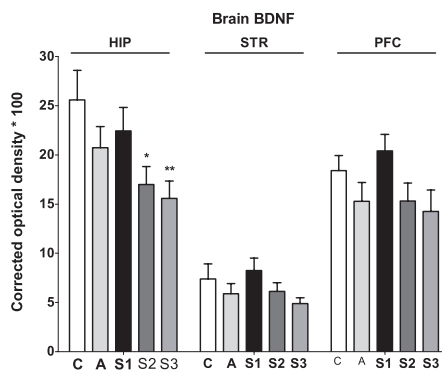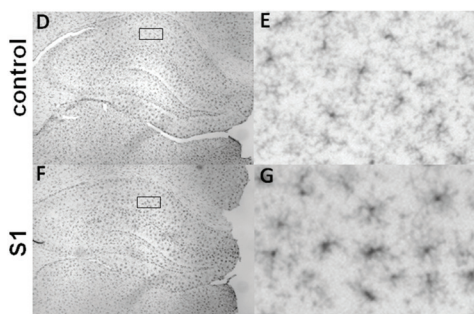### Brain cytokine levels, microglia activation, BDNF and DCX

IL-1B concentrations (Figure 3.5A) differed significantly between the experimental groups in the hippocampus ($F_{4,47}$=8.30, p=0.000) and PFC ($F_{4,52}$=5.94, p=0.001), but not in the striatum ($F_{4,45}$= 1.17, p=0.339). IL-6 levels (Figure 3.5B) differed significantly between experimental groups only in the hippocampus (HIP: $F_{4,47}$=3.03, p=0.028; STR: $F_{4,43}$=1.84, p=0.14; PFC: $F_{4,50}$=0.68, p=0.608), although no group differed significantly from control.

Figure 3.5D-G, 2.5I-L and 3.5N-H are representative images of IBA-1, BDNF and DCX staining of hippocampal sections respectively. The number of microglial cells did

not differ in any of the analyzed brain areas, nor did the average total cell size of the microglia (data not shown). The average microglial cell body size as percentage of total microglia size was used as a measure of microglial activation. Figure 3.5C shows the microglial activation in hippocampus (average of the DGib, CA1, and CA3 region) the striatum and the PFC. There was a significant difference in microglial activation between groups in the hippocampus (average: $F_{4,57}$=11.69, p=0.000; DGib; $F_{4,50}$=10.84, p=0.000; CA1: $F_{4,56}$=8.36, p=0.000; CA3: $F_{4,57}$=2.53, p=0.051) and the PFC ($F_{4,53}$=2.76, p=0.037). Microglial activation in the striatum did not differ significantly between groups ($F_{4,53}$=0.94, p=0.448).

Figure 3.5H shows the optical density of the BDNF staining in the hippocampus (average of the DGib, CA1, and CA3 region), the striatum and the PFC. There was a significant difference between groups in the hippocampus (average: $F_{4,51}$=3.44, p=0.015; DGib; $F_{4,50}$=3.28, p=0.018; CA1: $F_{4,56}$=3.56, p=0.013; CA3: $F_{4,57}$=3.84, p=0.008), but not in the striatum ($F_{4,56}$=1.50, p=0.215) or PFC ($F_{4,52}$=1.95, p=0.117). The number of DCX positive cells (Figure 3.5M) differed significantly between groups ($F_{4,55}$= 4.19, p=0.005).s

Figure 3.5 (see next page): Changes in the rat brain following surgery. A) IL-6 and B) IL-1B concentrations (pg/ mg protein) in the hippocampus, striatum and prefrontal cortex. C) Microglial activation in the hippocampus, striatum, and prefrontal cortex. Microglial activation is determined by measuring the microglial cell body as percentage of total cell size, with a higher cell body% indicating increased microglia activation. D-G) Overview (10x magnification) and detail photographs of IBA-1 staining of the hippocampus of a C (D and E) and S1 (F and G) animal. H) Optical density measurements (*100) of BDNF staining in the hippocampus, striatum and prefrontal cortex. I-L) Overview (10x magnification) and detail photographs of BDNF staining of the hippocampus of a C (D and E) and S2 (F and G) animal. M) Number of DCX positive cells in the dentate gyrus (cells/ mm). N-Q) Overview (10x magnification) and detail photographs of DCX staining of the hippocampus of a C (D and E) and S1 (F and G) animal. HIP= hippocampus, STR = striatum, PFC= prefrontal cortex, C=control, A=anesthesia only, S1, S2 and S3 = animals that underwent behavioral testing during week 1, 2 and 3 after surgery. #p<0.01, *p<0.05, **p<0.01, ***p<0.001 compared to C.

Brain IL-1B

HIP   STR   PFC

[IL-1B] (pg/mg)

C A S1 S2 S3   C A S1 S2 S3   C A S1 S2 S3

Brain IL-6

HIP   STR   PFC

[IL-6] (pg/mg)

C A S1 S2 S3   C A S1 S2 S3   C A S1 S2 S3

Microglia activation

HIP   STR   PFC

Cellbody size (% total cell size)

C A S1 S2 S3   C A S1 S2 S3   C A S1 S2 S3

control

S1

Brain BDNF

HIP   STR   PFC

Corrected optical density * 100

C A S1 S2 S3   C A S1 S2 S3   C A S1 S2 S3

control

S2

Dentate Gyrus DCX

DCX positive cells (cells/ mm)

C   A   S1   S2   S3

control

S1

Discussion

The aim of the current study was to investigate postoperative changes in learning, memory and exploratory behavior in relation to neuroinflammation and parameters of neuronal function.

Two days following surgery, rats (S1) showed changes in exploratory behavior in the open field, including decreased distance moved and an increased preference for the corners, which are behaviors associated with reduced interest for the environment and increased anxiety (Capuron and Miller, 2011; Hovens et al., 2013; Schoemaker and Smits, 1994). Since this was the only time point when changes in exploratory behaviors were observed, it may reflect the acute behavioral response to illness or trauma. This so-called sickness-response occurs in the first days following an inflammatory stimulus, and is related to the systemic inflammatory response (Dantzer, 2001; Dantzer et al., 2008). Accordingly, the changes in exploratory behavior in our experiment were correlated to plasma IL-6 concentrations at 24 h following surgery.

Learning and memory functions were impaired for two weeks following surgery, but restored at 3 weeks. The duration of POCD varies in animal studies, depending on age, species and surgical techniques used, but mostly lasts between 1 and 7 days (Barrientos et al., 2012; Cibelli et al., 2010; Degos et al., 2013; Fidalgo et al., 2011a; M. Li et al., 2013; Rosczyk et al., 2008; Terrando et al., 2011, 2010a; Wan et al., 2010; Wan et al., 2007). This hampers differentiation between POCD and more acute postsurgical cognitive deficits, such as delirium (Hovens et al., 2012). We, and others (Fidalgo et al., 2011b; Kamer et al., 2012), have however shown POCD lasting for more than 1 week in rodents, and thus likely beyond the delirium phase (Hovens et al., 2012). Accordingly, clinical literature shows that POCD is quite common in the first weeks following surgery, but persists only in approximately 10% of elderly patients (Moller et al., 1998; Newman et al., 2007; Rasmussen, 2006). Although the risk and duration of POCD differs between aged and young individuals, it seems likely that the same mechanism underlies the postoperative cognitive changes that these patients experience.

We observed postsurgical impairment in performance in the MWM and novel location test. These spatial tasks are generally considered to depend on hippocampal function (D'Hooge and De Deyn, 2001; Dere et al., 2007; Miyoshi et al., 2012). Cognitive flexibility in the MWM reversal task and novel object recognition, both thought to be independent of hippocampal function (D'Hooge and De Deyn, 2001; Dere et al., 2007), were not affected by surgery. Hence, our findings suggest that hippocampal dependent learning and memory is specifically vulnerable to surgery-induced impairment in young adult rats. Similarly, previous studies in young rodents showed that hippocampal dependent contextual fear memory, but not hippocampal independent auditory cued fear memory was impaired following surgery (Barrientos et al., 2012; Cibelli et al., 2010; Vizcaychipi et al., 2011). Kamer et al.(2012) showed impaired object recognition in young mice following splenectomy. The 24 h delay between the acquisition and recognition phase in their novel object test may explain these contrasting findings, since longer delays in the novel object test have been associated with increased involvement of the hippocampus (Dere et al., 2007).

Our study showed increased IL-1B concentrations and microglia activation in the hippocampus (DGib, CA1 and CA3) and PFC 1 week following surgery. This indicates that surgery led to neuroinflammation in these brain regions, which lasted well beyond the systemic inflammatory response, but subsided after 1 week. Our findings are in accordance with clinical and pre-clinical studies indicating a co- occurrence of (neuro)inflammation and cognitive impairment following surgery (Beloosesky et al., 2007; Chu et al., 2013; Cibelli et al., 2010; Fidalgo et al., 2011b; Hudetz et al., 2011a, 2011b; Ji et al., 2013b; Ramlawi et al., 2006; Shapira-Lichter et al., 2008; Yaffe et al., 2003). Evidence for a causal relation was recently provided by Cibelli et al. (2010) and Barrientos et al. (2012) who demonstrated that POCD could be attenuated by blocking central IL-1B signaling.

In other pre-clinical studies that reported increased hippocampal levels of pro-inflammatory cytokines, most notably IL-1B, and microgliosis following surgery (Barrientos et al., 2012; Chu et al., 2013; Cibelli et al., 2010; Fidalgo et al., 2011b;

Terrando et al., 2011, 2010b; Wan et al., 2007) neuroinflammation resolved within a few days following the surgical intervention. Furthermore, the only other study measuring neuroinflammation in brain regions other than the hippocampus (Fidalgo et al., 2011b), showed no postoperative increase in IL-1B levels in the PFC.

The abdominal surgery model we used leads to injury of the villi of the small intestines and increased intestinal permeability (Grootjans et al., 2012, 2010b; Petrat et al., 2010). This relatively severe surgical procedure, in combination with an indwelling jugular vein catheter, may have caused the more severe and long-lasting neuroinflammatory changes.

Interestingly, IL-1B concentrations and microglia activation in the hippocampus seemed to decrease below control levels in the second and third week following surgery. Although, to our knowledge, we are the first to show this pattern in relation to POCD, it may be in accordance with the compensatory anti-inflammatory response that is described in patients following severe inflammatory insults, such as surgery (Mokart et al., 2002; Ward et al., 2008).

Whereas neuroinflammation seemed to resolve after the first postoperative week, cognitive impairment remained present for two weeks following surgery. Moreover, even though neuroinflammation was present in the PFC, object recognition, a supposedly PFC dependent task (Dere et al., 2007) was not impaired in our rats. Although literature shows that neuroinflammation may play a key role in POCD (Cibelli et al., 2010; Barrientos et al., 2012), our findings may indicate that dysfunction in particular cognitive domains only occurs when specific underlying pathways are significantly affected.

Inflammatory factors may indirectly influence neuronal functions essential for learning and memory by modulating intraneuronal pathways (Yirmiya and Goshen, 2011). The BDNF-pathway has been implicated in particular as mediator between neuroinflammation and cognitive impairment (Barrientos et al., 2004; Cortese et al., 2011;

Yirmiya and Goshen, 2011). BDNF activates several intra-neuronal signaling cascades (Cortese et al., 2011; Moonat et al., 2010) that, regulate neural plasticity including LTP (Lu et al., 2008), synaptogenesis (Tong et al., 2012) and neurogenesis (Li et al., 2008; Pérez- Gómez and Tasker, 2013). BDNF was shown to be strongly associated with learning and memory formation (Barnes and Thomas, 2008; Barrientos et al., 2004; Yirmiya and Goshen, 2011). For example, Lee et al. (2004) showed that inhibiting BDNF expression impaired contextual fear conditioning in rats. Concentrations of BDNF were shown to decrease in the presence of neuroinflammation (Gibney et al., 2013; Guan and Fang, 2006; Tanaka et al., 2006), which could be prevented by administration of a IL-1 receptor antagonist, indicating that IL-1B plays a crucial role in regulation of BDNF levels.

We hypothesize that postoperative neuroinflammation leads to decreased BDNF signaling, decreased neuronal plasticity (among others reduced neurogenesis), and as a consequence decreased cognitive performance. In accordance with this hypothesis, we were able to show that hippocampal BDNF levels and neurogenesis, as indicated by decreased DCX staining (Bloch et al., 2011), were decreased following surgery. BDNF levels in the striatum and PFC however remained intact, as well as the cognitive functions mediated by these brain regions. This may suggest that the BDNF pathway in the hippocampus is specifically vulnerable to neuroinflammation, and cognitive impairment only occurs if the BDNF pathway is significantly affected. Indeed, the literature indicates that the hippocampus dependent learning and memory is especially vulnerable for inflammatory insults (Yirmiya and Goshen, 2011). This finding may explain why, in spite of the increase in neuroinflammation in the PFC, only hippocampal dependent functions were impaired in our rats.

Whereas markers of neuroinflammation were increased in the first week after surgery, BDNF levels were decreased in postoperative week 2 and 3. This finding may hypothetically be due a delay between neuroinflammation and the occurrence of decreased BDNF levels, which could occur if increased IL-1B levels slowly decreases BDNF expression over time. However, the literature gives no evidence for this theory

and indicates that BDNF levels decrease as soon as neuroinflammation occurs (Cortese et al., 2011; Fidalgo et al., 2011b; Gibney et al., 2013). We therefore do not have a fitting explanation for the delay found in our experiment.

Neurogenesis was reduced in the first three weeks following surgery. In a previous experiment we showed that the number of DCX positive neurons did not differ from control levels in rats that had undergone surgery 6 weeks before, suggesting that the postoperative decrease in neurogenesis is temporary (Hovens et al., 2013). Reduced neurogenesis was observed before the decrease in BDNF levels occurred. This may suggest that in our experiment neurogenesis decreased independent of the BDNF pathway. However, as described above, IL-1B can interfere downstream in the BDNF signaling pathway (Barrientos et al., 2004; Tong et al., 2012), and thus possibly affect BDNF regulated neuronal plasticity when BDNF levels are still intact.

Even though rats showed decreased hippocampal BDNF levels and neurogenesis in the third week after surgery, spatial learning and memory were not impaired in these animals. Hypothetically, when BDNF or neurogenesis is reduced for a longer time period, compensatory mechanisms may preserve spatial performance. Carretón et al. (2012) suggested that upregulation of the TrkB receptor preserved spatial learning and memory performance in heterogeneous BDNF knockout mice with reduced BDNF levels.

Conclusions

We demonstrated temporary postoperative changes in behavior, neuroinflammation and the BDNF pathway in young adult rats. The differences in time course and relation to plasma IL-6 suggest sickness behavior and learning and memory impairment comprise distinct features of postoperative behavioral dysfunction. Hippocampal dependent learning and memory seems to be specifically vulnerable to impairment as a consequence of surgery. Although the findings presented here shed some light on possible roles for neuroinflammation, BDNF and neurogenesis in POCD, it remains

to be elucidated how these players interact. Future research should look in more detail at the dynamics of the BDNF pathway following surgery.

Acknowledgments