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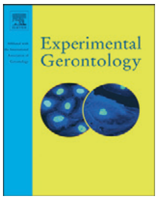

Surgery-induced behavioral changes in aged rats

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ABSTRACT

Elderly patients may experience impairments in cognition or mood following surgery. To study the development and underlying mechanisms of these postoperative behavioral changes, young (3 months) and aged (18–20 months) male rats were subjected to abdominal surgery followed by behavioral testing during a period of 6 weeks. Microglia activation (IBA-1) and neurogenesis (DCX) were immunohistochemically determined. In separate experiments, the effects of anesthesia and the cytokine response (IL-6) following surgery were evaluated.

Increased age was associated with changes in affective behavior, decreased cognitive flexibility and increased microglia activation as well as increased weight loss and plasma IL-6 following surgery. No effects of surgery on cognition were observed at either age. However, aged rats displayed long-term changes in affective behavior and had increased microgliosis in the CA1 hippocampal region following surgery. Microglia activation following surgery was positively correlated to parameters of behavior and spatial learning.

These findings support the hypothesis that elderly patients have an increased behavioral and (neuro)inflammatory response to surgery and these factors may be related.

1. Introduction

Cognitive and mood changes can develop gradually as a consequence of normal or pathological aging, but can also develop as a result of physical or psychological trauma. Surgery is one such event that can lead to persisting changes in cognitive functioning or mood (Borsook et al., 2010; Rasmussen, 2006; van Harten et al., 2012). Cognitive decline following surgery has been termed postoperative cognitive dysfunction (POCD). Transient cognitive decline following surgery occurs in 19% to 25% of aged patients. Persisting POCD, on the other hand, is seen in at least 10% of the elderly patients and can have a substantial impact on independence and quality of life (Newman et al., 2007; Rasmussen, 2006). Similarly, symptoms of depression and fatigue can develop after surgery and may persist for a long period (Borsook et al., 2010; Nickinson et al., 2009; Rubin et al., 2004; Tsapakis et al., 2009). In line with these observations in patients, preclinical studies have demonstrated behavioral changes following surgery in rodents, which correspond to clinical symptoms of cognitive decline, depression and fatigue (Cibelli et al., 2010; Dai et al., 2011; Fidalgo et al., 2011; Wan et al., 2010; Zhang et al., 2011). However, preclinical research has mainly focused on short-term postoperative changes, which may not necessarily represent the persisting POCD as found in human patients (Hovens et al., 2012). Moreover, preclinical studies usually focus on one aspect of behavior, such as spatial memory (Cibelli et al., 2010; Terrando et al., 2010; Wan et al., 2007), cognitive flexibility (Rosczyk et al., 2008) or anxiety (Dai et al., 2011). Therefore, knowledge of the postoperative changes in cognition and affective behaviors that reflect aspects of mood (e.g., anxiety, exploration and interest) is still limited.

Tissue damage due to surgery leads to local inflammation. This local inflammatory response is paralleled by systemic and brain inflammation (Beloosesky et al., 2007; Cibelli et al., 2010; Tang et al., 2011). Interestingly, (neuro)inflammation has been associated with changes in cognition, affective behavior and fatigue (Capuron and Miller, 2011; Lotrich et al., 2011; Yirmiya and Goshen, 2011). Age-related alterations of the immune regulation can exacerbate behavioral changes induced by neuroinflammation in the elderly (Dilger and Johnson, 2008;

Abbreviations: AUC, area under the curve; BLA, basolateral amygdala; BSA, bovine serum albumin; CA1, cornu ammonis region 1; CA 3, cornu ammonis region 3; CFC, contextual fear conditioning test; DCX, doublecortin-X; DG, dentate gyrus; DGib, dentate gyrus inner blade; EDTA, ethylenediaminetetraacetic acid; EP, elevated plus maze; IBA-1, ionized calcium binding adaptor molecule 1; IL-6, interleukin 6; MWM, Morris water maze; MWM-T, MWM test; NRS, normal rabbit serum; PFC, prefrontal cortex; POCD, postoperative cognitive dysfunction; PVP, polyvinylpyrrolidone; SMA, sensory motor area; TX, Triton-X; YM, Y-maze spatial reference task; YM-R, Y-maze reversal task; YM-S, spontaneous alternation in the Y-maze.

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Wynne et al., 2009). Accordingly, elderly patients seem especially at risk for developing surgery-induced changes in cognition and mood (Newman et al., 2007; Nickinson et al., 2009).

The aim of the current study is to investigate long-term changes in affective behaviors and cognition following surgery and their relation with neuroinflammation using a model of abdominal surgery in young and aged rats. We hypothesize that surgery will influence cognitive performance and affective behavior to a greater extent in aged rats compared to young rats, and that these changes are associated with increased neuroinflammation.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats (originally HsdCpb:WU) of 3 months (young, n = 20) and 18–20 months (aged, n = 20) were obtained from a colony of the Semmelweis University (Budapest, Hungary). The choice for this age category was based on previous findings from our research group, showing that in this rat strain a natural decline in cognitive or physical performance does not occur before 24 months of age (unpublished data). Animals were housed in groups of 2 or 3 under a controlled environment (20 ± 2 °C and 50 ± 10% humidity, 12:12 light:dark cycle) with ad libitum access to chow and tap water. All experiments were approved by the local animal ethics committee (DierExperimentenCommissie, Groningen, The Netherlands).

2.2. Experimental design

2.2.1. Experimental protocol 1

Young and aged rats (n = 10) underwent abdominal surgery. Rats that remained naïve served as age-matched control. Several aspects of cognition and affective behavior were assessed during a 6 week period (Fig. 1). Except for the contextual fear conditioning test, which was performed at the end of the experimental protocol, the behavioral tests were not considered to be aversive and outcomes were thus not expected to be influenced by previous tests. Tests were performed during the dark phase.

2.2.2. Experimental protocol 2

To investigate the effects of anesthesia and analgesia per se on cognition and affective behavior, aged rats (n = 5) received only anesthesia and analgesia and underwent behavioral testing in parallel with the animals in Experimental protocol 1.

2.2.3. Experimental protocol 3

To investigate the effects of age on the cytokine response after surgery, young (n = 8) and aged (n = 8) rats were subjected to abdominal surgery, and received a permanent indwelling jugular vein catheter during the surgical procedure, for stress-free timed blood sampling.

2.3. Weight parameters

Animals were weighed twice weekly at the end of the light phase. Maximal weight loss was determined and expressed as percentage of mean bodyweight in the week before surgery (baseline).

2.4. Surgical procedures

2.4.1. Abdominal surgery

Rats were anesthetized with sevoflurane-O_2 and received 0.003 mg/kg buprenorphine s.c. The intestines were exteriorized and the upper mesenteric artery was clamped for 15 min (see also: Supplemental methods). After clamp removal, the intestines were placed back and the abdominal wall and skin were closed separately by sutures. Anesthesia control animals (Experimental protocol 2) received 1 h of sevoflurane-O_2 gas anesthesia and 0.003 mg/kg buprenorphine s.c.

2.4.2. Jugular vein catheter

A silicon catheter (0.95 mm OD) was placed in the right jugular vein as described before (Steffens, 1969, see also: Supplemental methods) for repeated stress free blood sampling. A PVP/heparin solution was used to prevent blood clot formation in the catheter between sampling.

2.5. Behavioral tests

A figure depicting the behavioral tests is shown in the Supplemental materials and methods.

2.5.1. Morris water maze

Baseline cognitive performance and retrieval of pre-surgically acquired memory were determined in the MWM (Morris, 1984) as described before (Seigers et al., 2009). A round water-filled maze (140 cm ID, 26 ± 1 °C) was surrounded by visual cues and divided in 4 quadrants. An invisible platform was placed 1 cm below the water surface in quadrant 4 (target quadrant). MWM training was performed on 4 consecutive days, consisting of two trials in which the rat was randomly placed in quadrants 1, 2, or 3. The trial stopped when the animal found the platform and sat on it for 10 s. The rat was guided manually to the platform if it failed to find the platform within 3 min. Average escape latency to the platform for each training day was determined to assess learning performance. The day after the last training and at postoperative days 7 and 35, spatial memory was tested. Before the test, the platform was removed from the pool. The rat was placed in quadrants 1, 2 or 3 and the time in each quadrant and swimming speed were tracked for 1 min with Ethovision 3.0 (Noldus Information Technology, Wageningen, The Netherlands). Time spent in the target quadrant was determined to assess memory retrieval. Each test was followed by one training trial to avoid confounding effects of the MWM test on subsequent performance in the MWM.

2.5.2. Y maze

Y-maze tasks were performed in a PVC Y maze with arms (90 ∗ 17 ∗ 20 cm) converging at 120° angles that were designated A, B and C. Working memory was assessed with the spontaneous alternation test (Lalonde, 2002). The rat was placed in the center of the Y maze and the number of arm entries and alternations during an 8 minute period were recorded. Entrance in a new arm distinctive from the two previously entered arms was considered an alternation. The number of alternations, expressed as percentage of total entries, was used as a measure for working memory.

The influence of surgery on spatial learning and memory formation was determined using a Y-maze reference task (Havekes et al., 2006)

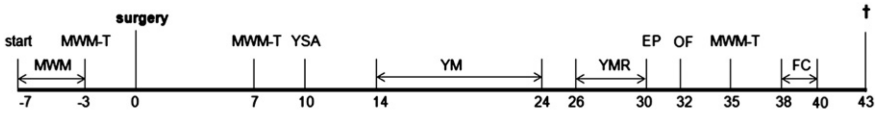

Fig. 1. Experimental design. A timeline is shown with time in days. MWM = Morris Water Maze learning, MWM-T = Morris Water Maze test, YSA = spontaneous alternation Y maze, YM = Y-maze learning, YMR = Y-maze reversal learning, OF = open field, EP = elevated plus maze test, CFC = contextual fear conditioning test, † = sacrifice.