Neutrophil gelatinase-associated lipocalin and microglial activity are associated with distinct postoperative behavioral changes in rats

Gouweleeuw, Leonie; Hovens, Iris B; van Leeuwen, Barbara L; Schoemaker, Regien G

Published in:
Behavioral Brain Research

DOI:
10.1016/j.bbr.2016.11.023

Publication date:
2017

Behavioural Brain Research 319 (2017) 104–109

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Neutrophil gelatinase-associated lipocalin and microglial activity are associated with distinct postoperative behavioral changes in rats

Leonie Gouweleeuw[a,*,1], Iris B. Hovens[a,b,1], Barbara L. van Leeuwen[b], Regien G. Schoemaker[a]

[a] Department of Molecular Neurobiology, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands
[b] Department of Surgery and Surgical Oncology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands

HIGHLIGHTS

- We explored the link between postoperative NGAL and behavior in a POCD rat model.
- Plasma and hippocampal NGAL were increased postoperatively.
- Plasma NGAL is associated with postoperative spatial learning impairment.
- Microglial activity is associated with reduced postoperative exploratory behavior.
- NGAL may be a sensitive marker connecting the peripheral inflammatory state to POCD.

ARTICLE INFO

Article history:
Received 16 September 2016
Received in revised form 4 November 2016
Accepted 9 November 2016
Available online 16 November 2016

Keywords:
Neutrophil gelatinase-associated lipocalin
Microglia
Neuroinflammation
Postoperative cognitive dysfunction
Behavior

ABSTRACT

Neutrophil gelatinase-associated lipocalin (NGAL) has recently gained interest as a marker for neuroinflammation and associated behavioral dysfunction. We aimed to explore the link between NGAL and behavior in a rat model of postoperative cognitive dysfunction (POCD).

Material collected in two previous studies on POCD was analyzed and associated with outcomes for exploratory behavior and spatial learning. Plasma and hippocampal NGAL and microglial activity were analyzed. Pearson's correlations and backward linear regression were performed to study the associations between behavioral parameters, NGAL concentrations, and microglial activity.

Plasma and hippocampal NGAL were increased following surgery. Plasma NGAL was associated with impaired spatial learning only, microglial activity was associated with exploratory behavior only, while hippocampal NGAL was associated with both behavioral aspects. Spatial learning was best predicted by a model containing plasma NGAL concentrations and hippocampal microglial activity.

NGAL may serve as a sensitive marker in connecting the peripheral inflammatory state to POCD, while postoperative changes in exploratory behavior are better reflected by hippocampal neuroinflammation. These findings warrant further exploration in the role of NGAL in development of postoperative behavioral deficits.

Acute systemic inflammatory events have often been associated with changes in cognition and behavior. A striking example is the observed association between surgery-induced inflammation and postoperative cognitive dysfunction (POCD) in both clinical and rodent studies [1–4]. These studies implicate communication between the periphery and central nervous system, in which systemic inflammation can result in neuroinflammation, influencing neuronal functioning [5].

Neutrophil gelatinase-associated lipocalin (NGAL) has recently gained attention as marker for inflammatory processes associated with behavioral changes [6]. Classically known as biomarker for renal damage, it has become clear that NGAL may reflect inflammatory processes in a variety of tissues, including the brain [7,8]. Circulating levels of NGAL were associated with cognitive and mental dysfunction in chronic inflammatory conditions, including Alzheimer's disease and depression [7–9]. In addition to its

* Corresponding author.
E-mail addresses: l.gouweleeuw@rug.nl (L. Gouweleeuw), i.b.hovens@rug.nl (I.B. Hovens), b.l.van.leeuwen@umcg.nl (B.L. van Leeuwen), r.g.schoemaker@umcg.nl (R.G. Schoemaker).
[1] Both authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.bbr.2016.11.023
0166-4328/

anti-bacterial properties [10,11], NGAL can cause microgliosis and astrocytosis and sensitize microglia and neurons to pro-inflammatory cytokines and apoptosis [7,12]. A potential role of NGAL in periphery to brain communication was supported by upregulated NGAL levels in the brain following systemic inflammation [10,13]. Thus, NGAL may play a role in the propagation of neuroinflammation and neuronal dysfunction after a systemic inflammatory event.

Recently, we showed increased systemic and central NGAL concentrations after cardiac and abdominal surgery in a rat model for POCD [14]. In another study, we showed that plasma and hippocampal NGAL levels were associated with exploratory behavior in a rat model for heart failure after myocardial infarction [15]. To further explore the potential association of NGAL with postoperative neuroinflammation and behavioral outcomes, we analyzed NGAL in plasma and hippocampal tissue from two of our previous POCD studies. We hypothesized that systemic and hippocampal NGAL levels correlate with microglial activation, spatial memory impairment, and altered exploratory behavior after surgery.

To investigate the association between NGAL concentrations and neuroinflammation with behavioral parameters, we analyzed plasma samples, hippocampal tissue, and behavioral data obtained during two previous studies in male Wistar rats. The first study (experiment 1) compared inflammatory markers and behavior 6 weeks after surgery in healthy young (3 months) and aged (20–21 months) rats (n = 8/group) [16]. The second experiment (experiment 2) compared inflammatory markers and behavior 2 weeks after surgery in aged rats (18 months) that were either healthy or had experienced an infection prior to surgery (n = 12/group) [17]. All experiments were approved by the local animal experiment and welfare committee (DierExperimentenCommissie, Groningen, the Netherlands). For a detailed description of the experimental procedures, we refer to Hovens et al. [16,17]. Briefly, under sevoflurane anesthesia and analgesia (0.01 mg/kg Temgesic), rats received major abdominal surgery including exteriorization of the intestines and clamping of the upper mesenteric artery for 15 (experiment 1) or 30 (experiment 2) minutes. In experiment 2 the rats were additionally equipped with a jugular vein catheter during the surgical procedure. Control animals did not receive surgery or anesthesia.

Behavioral tests were performed in the dark phase, each test was performed once per rat. Open field behavior was recorded for 5 min and time spent on exploration behavior (walking, sniffing and rearing) was used as a measure of interest in the environment [18]. Spatial learning was measured as area under the curve (AUC) for either the percentage of incorrect trials over 9 training sessions in a reward-driven Y-maze spatial learning protocol or the average escape latency over 5 training sessions in the Morris water maze. This yielded one outcome for spatial learning, with increased AUC indicating decreased spatial learning performance.

At sacrifice, blood samples were collected via cardiac puncture, rats were perfused with saline, and brains were collected. Blood was centrifuged and plasma was collected and stored at −80 °C until further analysis. From one hemisphere of each brain the hippocampus was dissected, snap frozen, and stored at −80 °C. Hippocampi were homogenized in a 50 mM Tris-HCL buffer containing 150 mM NaCl, 0.002% Tween-20, and protease inhibitor (Complete Mini, Roche Diagnostics, Indianapolis, USA), sonicated for 5 s twice and centrifuged. Supernatant was collected and diluted to 5 mg/ml protein based on a Bradford assay. NGAL concentrations were determined using the RAT NGAL ELISA kit (BioPorto, Hellerup, Denmark). Plasma samples were diluted 10.000 times and hippocampal supernatant was diluted 10 times in the provided dilution buffer, after which the ELISA analysis was performed according to manufacturer's instructions.

One hemisphere of each brain was emersion fixed with 4% paraformaldehyde for at least 3 days, dehydrated with 30% sucrose, deep frozen, and cut into 30 μm thick sections. Microglia in sections containing the dorsal hippocampus were stained against ionized calcium-binding adapter protein 1 (IBA-1) and morphologically characterized as described previously [19]. Briefly, sections were pretreated with H_2O_2, incubated for 3 days at 4 °C with 1:2500 rabbit-anti-IBA-1 (Wako, Neuss, Germany) in 1% BSA, 0.1% Triton-X at 4 °C, incubated for 1 h with 1:500 goat-anti rabbit secondary antibody (Jackson, Wet Grove, USA) in 1% BSA, incubated for 1 h with avidin-biotin peroxidase complex (Vectastain ABCkit, Vector, Burlingame, USA) and diaminobenzidine labeled. Images were acquired by bright-field microscopy (Leica, 100× magnification) of the dentate gyrus inner blade (DGib), cornu ammonis (CA) 1 and CA3 region, in 3 sections per rat. Using Image Pro Plus software (Media Cybernetics), the total cell body size to total cell size ratio (%) was determined as measure for microglial activity. Microglial activity outcomes of the DGib, CA1, and CA3 were averaged per brain section to yield an average activity outcome for the hippocampus.

For each experiment plasma and hippocampal NGAL concentrations, hippocampal cell body to cell size ratios, and the AUC of the spatial learning paradigm were expressed as percentage of young (experiment 1) or healthy (experiment 2) control rats. Data are displayed as mean ± SEM. Group differences in plasma and hippocampal NGAL concentrations were determined using two-way ANOVA followed by Tukey post-hoc analysis. Pearson's correlation coefficients were determined for experiment 1 and 2 separately and combined. Correlations were determined between 1) microglial activity and NGAL concentrations, 2) spatial learning and exploratory behavior, and 3) these inflammatory parameters and behavioral parameters. Finally, to determine which inflammatory parameters best predicted the behavioral outcomes, backward linear regression was applied with spatial learning and exploratory behavior as the dependent variables and plasma and hippocampal NGAL concentrations and hippocampal microglial activity as independent variables.

Table 1 shows the hippocampal microglial activity and the included behavioral parameters for the experimental groups in study 1 and 2. Fig. 1 displays plasma and hippocampal NGAL concentrations. In experiment 1, there was a significant effect of surgery on plasma NGAL concentrations ($F_{1,32} = 4.54$, $p = 0.042$, Fig. 1A) six weeks after surgery, but no significant effect of age or interaction age*surgery. Similar effects were seen for hippocampal NGAL concentrations ($F_{1,31} = 6.96$, $p = 0.014$, Fig. 1B), as well as a trend for an effect of age ($F_{1,31} = 4.00$ $p = 0.056$). In experiment 2, two weeks after surgery, rats that had experienced an infection had significantly increased plasma NGAL concentrations ($F_{1,32} = 20.89$, $p = 0.000$, Fig. 1C) compared to rats that were healthy, and rats that underwent surgery had significantly increased plasma NGAL concentrations ($F_{1,32} = 7.20$, $p = 0.012$) compared to non-surgical controls. Additionally there was a significant infection*surgery interaction effect ($F_{1,32} = 6.58$, $p = 0.016$); only rats that experienced an infection prior to surgery had significantly increased plasma NGAL concentrations compared to the other groups. Hippocampal NGAL concentrations were significantly affected by infection status ($F_{1,42} = 17.32$, $p = 0.000$, Fig. 1D) only.

When the data of experiment 1 and 2 were combined, there was a significant correlation between plasma and hippocampal NGAL concentrations ($r = 0.489$, $p < 0.001$). Hippocampal ($r = 0.260$, $p = 0.030$) but not plasma NGAL concentrations ($r = 0.034$, $p = 0.790$) were significantly correlated with hippocampal microglial activity. Separate analysis of experiment 1 and 2 showed similar correlations, only the statistical significance of the correlation between hippocampal microglial activity and NGAL was lost. There was no significant correlation between spatial learning performance and exploratory behavior ($r = -0.078$, $p = 0.494$). In the data of experiment 1 and 2 combined, plasma NGAL concentrations were significantly correlated with spatial learning (Fig. 2A) but not

Table 1
Behavior and microglia activity in the experimental groups.

	Rat-model POCD (1)				Rat-model POCD (2)			
	Healthy young		Healthy aged		Healthy aged		Infection aged	
	C	S	C	S	C	S	C	S
Microglial activity	100 ± 5	109 ± 8[b]	133 ± 11[a]	166 ± 9[a,b]	100 ± 3	102 ± 3	107 ± 3[a]	122 ± 4[*,a]
Spatial learning	100 ± 6	94 ± 10	96 ± 11	98 ± 9	100 ± 7	116 ± 8[b]	136 ± 6[a]	165 ± 8[a,b]
Exploration	90 ± 2	88 ± 2	64 ± 5[a]	49 ± 7[a]	91 ± 2	86 ± 3[b]	80 ± 3[a]	66 ± 4[a,b]

Data are represented as Mean ± SEM. Microglial activity in the hippocampus and the behavioral test outcome for spatial learning are expressed as percentage of the healthy control group. The behavioral test outcome for exploration behavior is expressed as percentage of time spent on exploration in the open field test. C = non-surgical control, S = abdominal surgery.

[*] $p < 0.05$ compared to own control (adapted from [16,17]).
[a] Main effect of age/infection $p < 0.05$.
[b] Main effect of surgery $p < 0.05$.

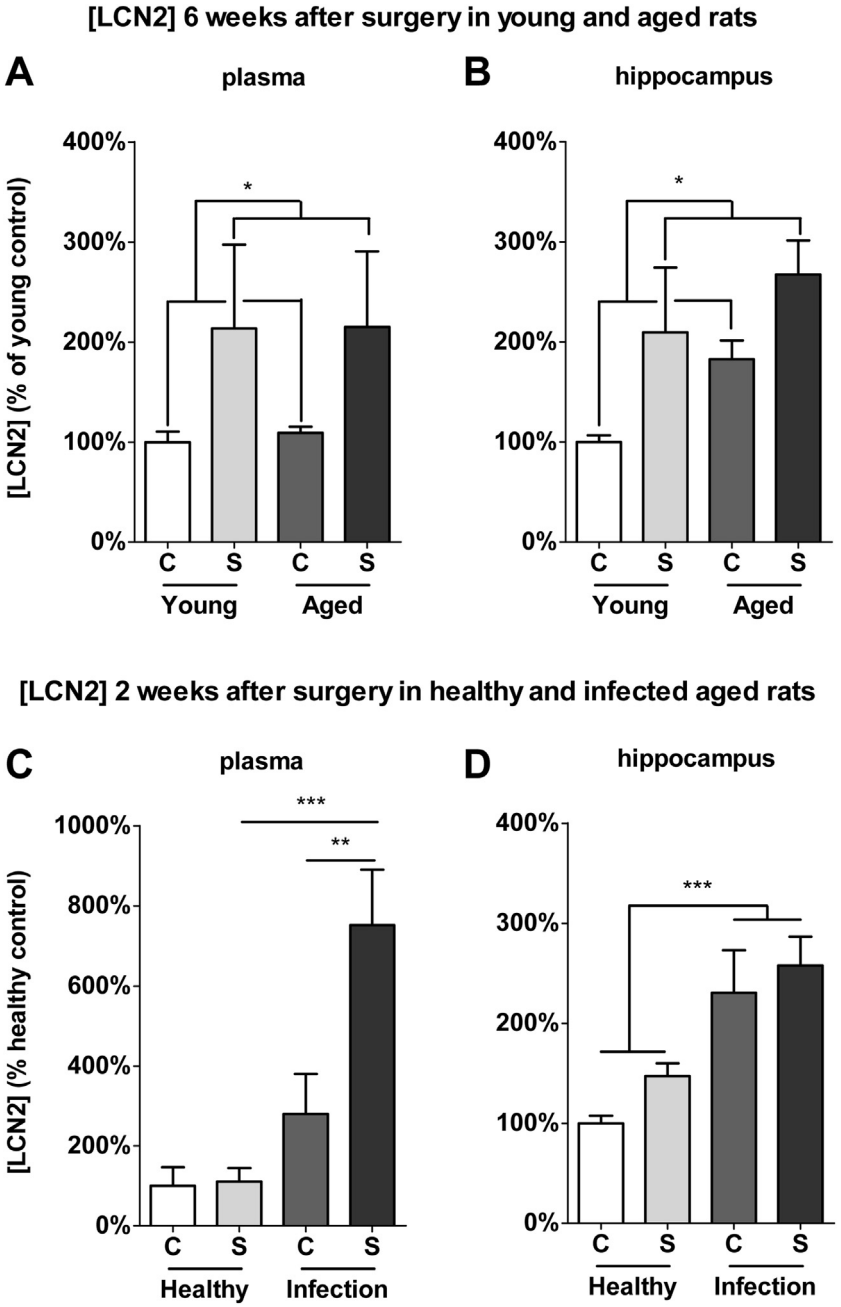

Fig. 1. Plasma and hippocampal NGAL concentrations (mean + SEM). A + B = NGAL concentrations in young and aged rats 6 weeks after a surgical intervention (S) or no surgery as control (C). C + D = NGAL concentrations 2 weeks after a surgical intervention (S) or no surgery as control (C) in aged rats that were either healthy or had undergone an infection. NGAL concentrations are expressed as percentage of young or healthy control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

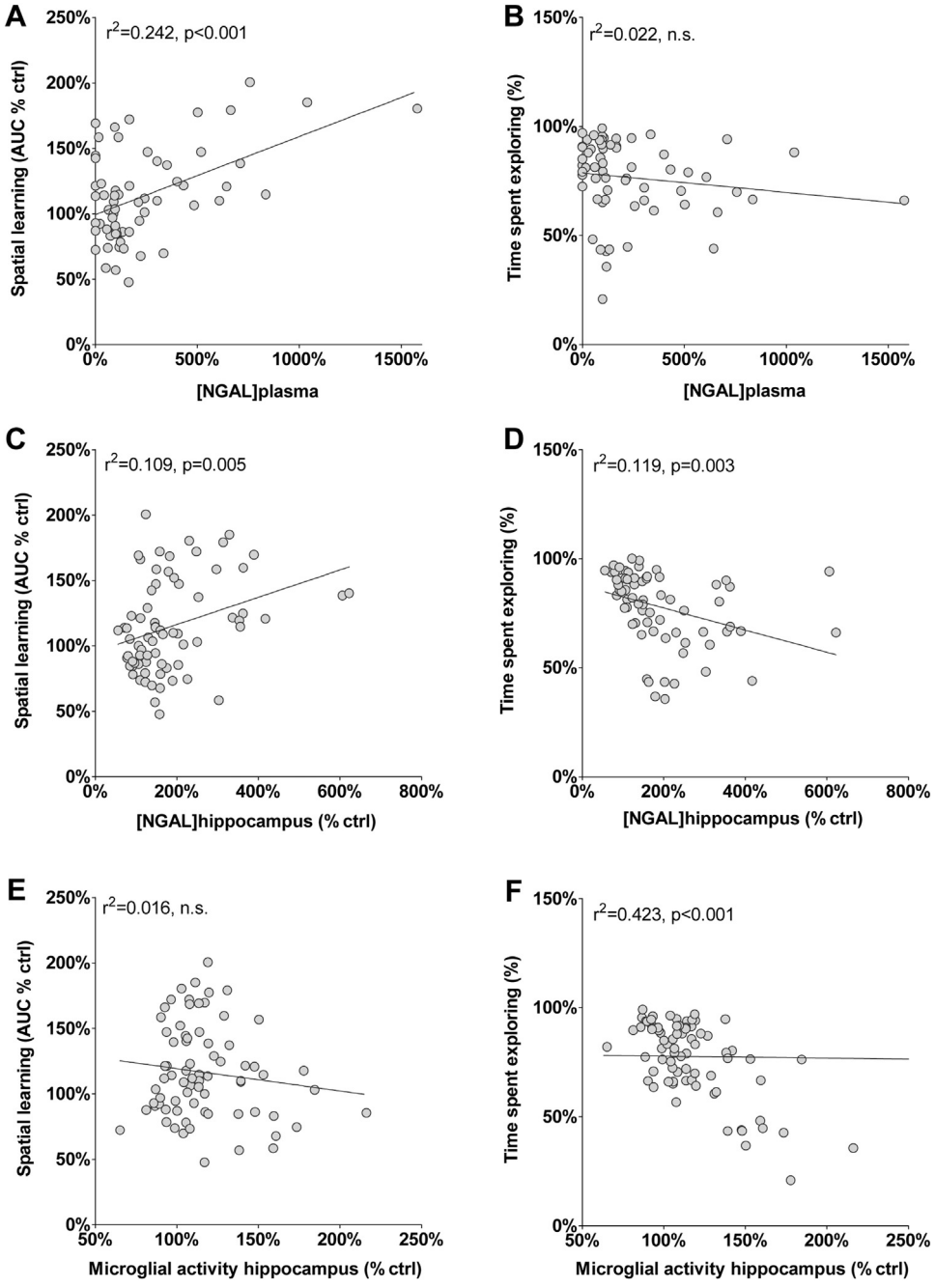

Fig. 2. Correlation between inflammatory and behavioral parameters. Correlation between the behavioral outcomes spatial learning as percentage of the area under the curve of young or healthy control (AUC% ctrl) (A,C,E) and exploratory behavior as percentage of time spent on exploration (exploration%) (B,D,F) and A,B) plasma NGAL concentrations as percentage of young or healthy control ([NGAL]plasma (%ctrl)), C,D) hippocampal NGAL concentrations as percentage of young or healthy control ([NGAL]hippocampus (%ctrl)), and E,F) hippocampal microglial activity as percentage of young or healthy control. Pearson's r^2 are shown.

exploratory behavior (Fig. 2B), hippocampal NGAL concentrations were significantly correlated with spatial learning (Fig. 2C) and exploratory behavior (Fig. 2D), and hippocampal microglial activity was significantly correlated with exploratory behavior only (Fig. 2F and G). Analysis of experiment 1 and 2 separately, showed similar associations between the inflammatory and behavioral parameters. In experiment 1, spatial learning was significantly correlated with plasma NGAL ($r = 0.373$, $p = 0.035$) and hippocampal NGAL ($r = 0.384$, $p = 0.033$), and exploratory behavior was significantly correlated with microglial activity ($r = -0.672$, $p < 0.001$). In experiment 2, spatial learning was significantly correlated with plasma NGAL ($r = 0.471$, $p = 0.007$) and hippocampal NGAL ($r = 0.368$, $p = 0.018$), and exploratory behavior was significantly correlated with plasma NGAL ($r = -0.483$, $p = 0.005$), hippocampal NGAL ($r = -0.464$, $p = 0.002$), and microglial activity ($r = -0.573$, $p < 0.001$).

Backward linear regression showed that spatial learning was best predicted by a model containing plasma NGAL concentrations and hippocampal microglial activity ($F_{3,55} = 13.41$, $p < 0.001$). In this model, worse spatial learning ability was significantly associated with increased plasma NGAL concentrations (standardized $\beta = 0.544$, $p < 0.001$) and there was a trend for an association with hippocampal microglial activity (standardized $\beta = -0.192$, $p = 0.088$). Exploratory behavior was best predicted by a model

containing hippocampal microglial activity and hippocampal NGAL concentrations ($F_{3,55} = 20.60$, $p < 0.001$). In this model, exploratory behavior was negatively associated with hippocampal microglial activity (standardized $\beta = -0.5473$, $p = 0.000$) and there was a trend for an association with hippocampal NGAL concentrations (standardized $\beta = -0.202$, $p = 0.060$).

Systemic NGAL may be a sensitive marker for inflammation-induced behavioral changes. This hypothesis was tested using data from two studies in a rat model for POCD, representing effects of time, age and prior infection on POCD. We correlated plasma and hippocampal NGAL levels, as well as hippocampal microglia activity with the behavioral parameters spatial learning and exploratory behavior.

Except for the healthy rats 2 weeks post-surgery, all rats showed increased systemic and hippocampal NGAL levels following a surgical intervention. Whereas age did not significantly influence NGAL levels, an infection prior to the surgical intervention did cause a long-lasting increase in both plasma and hippocampal NGAL concentrations. Notably, the increased NGAL levels in experiment 2 were still present when systemic concentrations of interleukin-6 and interleukin-12 had returned to control levels [17]. These findings indicate that NGAL may sensitively reflect inflammatory processes for an extended period after surgery that pro-inflammatory cytokines do not.

The strong correlation between plasma and hippocampal NGAL levels is in accordance with previous findings [15] and suggests that systemic NGAL is paralleled by NGAL signaling in the brain. However, neither plasma nor hippocampal NGAL levels were consistently correlated to hippocampal microglial activity, suggesting microglial activity and NGAL levels may reflect different (inflammatory) processes after surgery, or may have a different time line. In contrast, previous studies showed that NGAL could contribute to microglial activation [12], and that microglia are capable of producing NGAL upon inflammatory stimuli [7,12]. Alternatively, NGAL levels could reflect iron accumulation after surgery. Research indicated NGAL has an important role in iron-trafficking both under septic conditions and in aseptic tissue [10,11]. Interestingly, a recent study in a rat model for POCD reported increased iron levels, having toxic properties in the brain [20].

Correlation analysis showed an association of spatial learning with plasma and hippocampal NGAL levels, but not hippocampal microglial activity. Additionally, backward linear regression analysis indicated that plasma NGAL concentration is the main predictor for spatial learning activity. These findings suggest that NGAL concentrations may indeed be a marker for surgery-induced memory impairment. Moreover, NGAL concentrations may be a better predictor for spatial memory performance than hippocampal microglial activity. However, an extensive review of Yirmiya and Goshen [21] strongly indicates an involvement of hippocampal neuroinflammation, including microglial activity, in memory dysfunction. As we previously showed a correlation of microglial activity in the CA1 with postoperative spatial memory performance, microglial activity in specific hippocampal subregions, rather than whole hippocampus, may play a role in postoperative spatial memory impairment [16]. Finally, a difference in time course of behavioral changes and inflammatory parameters in the hippocampus [22] may provide an explanation as well.

Exploratory behavior in the open field was correlated to both hippocampal NGAL concentrations and microglial activity, but not to systemic NGAL concentrations. Backward linear regression analysis showed that exploratory behavior was significantly predicted by hippocampal microglial activity, but we also found a trend for an effect of hippocampal NGAL levels. These results suggest that spatial learning, representing cognitive behavior, and exploratory behavior, representing interest in environment may be regulated differently. While the correlations were calculated on the experiments combined, we found similar correlations in each of the two experiments separately.

This analysis of data from two previous studies provides a first indication of NGAL as a potential marker or mediator of postoperative cognitive impairment. We found evidence that NGAL is increased postoperatively and associated with postoperative behavioral outcomes. However, using material from previous studies limits optimal experimental design to the current research question. Further experiments are therefore necessary to give a definitive answer about the potential involvement of NGAL in mediating postoperative behavioral change. However, taken together, our results suggest that NGAL may serve a role in connecting the peripheral inflammatory state to POCD, while postoperative behavioral changes are better reflected by hippocampal neuroinflammation. As NGAL is a rather stable marker, measurable in tissues and plasma, it could provide a contribution to cognitive risk assessment after physiological trauma in the future.

Acknowledgements

This work was supported by funding of the University of Groningen, the Netherlands. We thank Jan Bruggink and Wanda Douwenga for their technical support.

References

[1] L. Peng, L. Xu, W. Ouyang, Role of peripheral inflammatory markers in postoperative cognitive dysfunction (POCD): a meta-analysis, PLoS One 8 (2013) e79624, http://dx.doi.org/10.1371/journal.pone.0079624.
[2] R.M. Barrientos, A.M. Hein, M.G. Frank, L.R. Watkins, S.F. Maier, Intracisternal interleukin-1 receptor antagonist prevents postoperative cognitive decline and neuroinflammatory response in aged rats, J. Neurosci. 32 (2012) 14641–14648, http://dx.doi.org/10.1523/JNEUROSCI.2173-12.2012.
[3] N. Terrando, A.R. Fidalgo, M. Vizcaychipi, M. Cibelli, D. Ma, C. Monaco, M. Feldmann, M. Maze, The impact of IL-1 modulation on the development of lipopolysaccharide-induced cognitive dysfunction, Crit. Care 14 (2010) R88.
[4] M. Cibelli, A.R. Fidalgo, N. Terrando, D. Ma, C. Monaco, M. Feldmann, M. Takata, I.J. Lever, J. Nanchahal, M.S. Fanselow, M. Maze, Role of interleukin-1beta in postoperative cognitive dysfunction, Ann. Neurol. 68 (2010) 360–368.
[5] I.B. Hovens, R.G. Schoemaker, E.A. van der Zee, E. Heineman, G.J. Izaks, B.L. van Leeuwen, Thinking through postoperative cognitive dysfunction: how to bridge the gap between clinical and pre-clinical perspectives, Brain Behav. Immun. 26 (2012) 1169–1179, http://dx.doi.org/10.1016/j.bbi.2012.06.004.
[6] L. Gouweleeuw, P.J.W. Naudé, M. Rots, M.J.L. DeJongste, U.L.M. Eisel, R.G. Schoemaker, The role of neutrophil gelatinase associated lipocalin (NGAL) as biological constituent linking depression and cardiovascular disease, Brain Behav. Immun. 46 (2015) 23–32, http://dx.doi.org/10.1016/j.bbi.2014.12.026.
[7] P.J.W. Naudé, C. Nyakas, L.E. Eiden, D. Ait-Ali, R. van der Heide, S. Engelborghs, P.G.M. Luiten, P.P. De Deyn, J.A. den Boer, U.L.M. Eisel, Lipocalin 2: novel component of proinflammatory signaling in Alzheimer's disease, FASEB J. 26 (2012) 2811–2823, http://dx.doi.org/10.1096/fj.11-202457.
[8] P.J.W. Naudé, U.L.M. Eisel, H.C. Comijs, N.A. Groenewold, P.P. De Deyn, F.J. Bosker, P.G.M. Luiten, J.A. den Boer, R.C. Oude Voshaar, Neutrophil gelatinase-associated lipocalin: a novel inflammatory marker associated with late-life depression, J. Psychosom. Res. 75 (2013) 444–450, http://dx.doi.org/10.1016/j.jpsychores.2013.08.023.
[9] P.J.W. Naudé, J.A. den Boer, H.C. Comijs, F.J. Bosker, M. Zuidersma, N.A. Groenewold, P.P. De Deyn, P.G.M. Luiten, U.L.M. Eisel, R.C. Oude Voshaar, Sex-specific associations between Neutrophil Gelatinase-Associated Lipocalin (NGAL) and cognitive domains in late-life depression, Psychoneuroendocrinology 48 (2014) 169–177, http://dx.doi.org/10.1016/j.psyneuen.2014.06.016.
[10] T.H. Flo, K.D. Smith, S. Sato, D.J. Rodriguez, M.A. Holmes, R.K. Strong, S. Akira, A. Aderem, Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron, Nature 432 (2004) 917–921, http://dx.doi.org/10.1038/nature03104.
[11] G. Bao, M. Clifton, T.M. Hoette, K. Mori, S.-X. Deng, A. Qiu, M. Viltard, D. Williams, N. Paragas, T. Leete, R. Kulkarni, X. Li, B. Lee, A. Kalandadze, A.J. Ratner, J.C. Pizarro, K.M. Schmidt-Ott, D.W. Landry, K.N. Raymond, R.K. Strong, J. Barasch, Iron traffics in circulation bound to a siderocalin (Ngal)-catechol complex, Nat. Chem. Biol. 6 (2010) 602–609, http://dx.doi.org/10.1038/nchembio.402.
[12] S. Lee, J. Lee, S. Kim, J.-Y. Park, W.-H. Lee, K. Mori, S.-H. Kim, I.K. Kim, K. Suk, A dual role of lipocalin 2 in the apoptosis and deramification of activated microglia, J. Immunol. 179 (2007) 3231–3241, http://www.ncbi.nlm.nih.gov/pubmed/17709539 (Accessed 29 July 2015).
[13] F. Marques, A.-J. Rodrigues, J.C. Sousa, G. Coppola, D.H. Geschwind, N. Sousa, M. Correia-Neves, J.A. Palha, Lipocalin 2 is a choroid plexus acute-phase

protein, J. Cereb. Blood Flow Metab. 28 (2008) 450–455, http://dx.doi.org/10.1038/sj.jcbfm.9600557.
[14] I.B. Hovens, B.L. van Leeuwen, M.A. Mariani, A.D. Kraneveld, R.G. Schoemaker, Postoperative cognitive dysfunction and neuroinflammation; cardiac surgery and abdominal surgery are not the same, Brain Behav. Immun. 54 (2016) 178–193, http://dx.doi.org/10.1016/j.bbi.2016.02.003.
[15] L. Gouweleeuw, I.B. Hovens, H. Liu, P.J.W. Naudé, R.G. Schoemaker, Differences in the association between behavior and neutrophil gelatinase-associated lipocalin in male and female rats after coronary artery ligation, Physiol. Behav. 163 (2016) 7–16, http://dx.doi.org/10.1016/j.physbeh.2016.04.040.
[16] I.B. Hovens, R.G. Schoemaker, E.A. van der Zee, E. Heineman, C. Nyakas, B.L. van Leeuwen, Surgery-induced behavioral changes in aged rats, Exp. Gerontol. 48 (2013) 1204–1211, http://dx.doi.org/10.1016/j.exger.2013.07.011.
[17] I.B. Hovens, B.L. van Leeuwen, C. Nyakas, E. Heineman, E.A. van der Zee, R.G. Schoemaker, Prior infection exacerbates postoperative cognitive dysfunction in aged rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 309 (2015) R148–R159, http://dx.doi.org/10.1152/ajpregu.00002.2015.
[18] R.G. Schoemaker, J.F. Smits, Behavioral changes following chronic myocardial infarction in rats, Physiol. Behav. 56 (1994) 585–589.
[19] I.B. Hovens, C. Nyakas, R.G. Schoemaker, A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: cell body to cell size ratio, Neuroimmunol. Neuroinflamm. 1 (2014) 82, http://dx.doi.org/10.4103/2347-8659.139719.
[20] L.-N. An, Y. Yue, W.-Z. Guo, Y.-L. Miao, W.-D. Mi, H. Zhang, Z.-L. Lei, S.-J. Han, L. Dong, Surgical trauma induces iron accumulation and oxidative stress in a rodent model of postoperative cognitive dysfunction, Biol. Trace Elem. Res. 151 (2013) 277–283, http://dx.doi.org/10.1007/s12011-012-9564-9.
[21] R. Yirmiya, I. Goshen, Immune modulation of learning memory, neural plasticity and neurogenesis, Brain Behav. Immun. 25 (2011) 181–213.
[22] I.B. Hovens, R.G. Schoemaker, E.A. van der Zee, A.R. Absalom, E. Heineman, B.L. van Leeuwen, Postoperative cognitive dysfunction: involvement of neuroinflammation and neuronal functioning, Brain Behav. Immun. 38 (2014) 202–210, http://dx.doi.org/10.1016/j.bbi.2014.02.002.