Chapter 6
General Discussion
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More elderly and otherwise vulnerable people are undergoing surgery every year, due to rising life expectancy and safer surgical procedures (Kotekar et al., 2018). Although the aim of these surgical procedures is to improve the health and well-being of the patients, this population tends to develop more complications after surgery. One of the complications after surgery, especially among older patients, is cognitive dysfunction (Rasmussen, 2006; van Harten et al., 2012). Up to 40% of the patients experience long-lasting postoperative cognitive dysfunction (POCD) (Evered et al., 2011; Hovens et al., 2013; Monk et al., 2008; Plas et al., 2017; Price et al., 2008). Moreover, symptoms of POCD, such as memory impairment, problems in information processing, concentration and executive functions and memory (Hovens et al., 2012; Krenk et al., 2010) are associated with poor outcomes, resulting in reduced quality of life and increased disability and dependency on social support and services (Dijkstra & Jolles, 2002; Hovens et al., 2012; Monk et al., 2008; Steinmetz et al., 2009; Vanderweyde et al., 2010). Although the pathophysiology of POCD still remains to be fully elucidated, it is considered that inflammation is a key player in the disease progress (Cibelli et al., 2010; Lin et al., 2020; Peng et al., 2013; Skvarc et al., 2018; Terrando et al., 2010; van Harten et al., 2012). Surgery induced inflammatory response triggers an activation of the local and systemic immune system, which in turn can activate the brain’s immune system. Subsequently neuroinflammation can lead to impaired neuronal and cognitive functioning (Barrientos et al., 2012; Barrientos et al., 2006; Dilger & Johnson, 2008; Wang et al., 2017; Yirmiya & Goshen, 2011; Zhu et al., 2018). Although this inflammation hypothesis is widely accepted, studies also showed that anti-inflammatory treatment alone is not sufficient enough to recover cognitive impairment in POCD (Mohammadian et al., 2019). More research is needed to elucidate the underlying mechanism of POCD and subsequently a step towards effective treatment needs the be made.

Therefore, the goal of the research culminated in this thesis was to investigate the mechanism of POCD and explore novel interventions for POCD in different rat models. Rats were used to study behavioral, metabolic and physiological changes after abdominal surgery. Moreover, we included different rat models and different therapies to enhance clinical relevance. As (neuro)inflammation is seen as major culprit of POCD, we hypothesized that anti-inflammatory treatment could prevent or alleviate POCD. In our first study (chapter 2) we tested if anti-inflammatory treatment with ibuprofen could prevent or alleviate POCD in young and aged rats. From this study we concluded that a single dose of ibuprofen preceding abdominal surgery in rats increased neurogenesis and cognition. Surprisingly, this was not attributable to the anticipated anti-inflammatory effect of ibuprofen, as ibuprofen even seemed to increase neuroinflammation. Moreover, the beneficial effects of ibuprofen were
more pronounced in young than in aged rats. As inflammation did not seem to be the primary target for treatment of POCD, in the next study (chapter 3) we included a more general anti-ageing drug, called J147 (Currais et al., 2015; Daugherty et al., 2018; Emmanuel et al., 2019; Goldberg et al., 2018; Larrick & Mendelsohn, 2018; Prior et al., 2013; Prior et al., 2016). J147 was effective in preserving behavioral and cognitive performance in young rats, but again this seemed not attributable to anti-inflammatory effects, nor to cell-metabolic effects. This seems contradictory to literature that suggests that J147 has anti-inflammatory and metabolic effects (Currais et al., 2015; Currais et al., 2019; Daugherty et al., 2018; Goldberg et al., 2020; Goldberg et al., 2018; Prior et al., 2013). Although these results are of importance to the POCD research, clinically it would be more relevant to study J147 in an increased-risk model, better mimicking the patient group that develops POCD. In chapter 4 and 5, effects of J147 were investigated in the potential high-risk Zucker rat model. Cognitive, (neuro)inflammatory and metabolic parameters were assessed, to conclude that Zucker rats appeared not more susceptible for POCD, but rather developed signs of postoperative depression. However, J147 could improve early recovery. In addition, positive effects on behavioral and metabolic parameters were seen after J147 treatment. In agreement with the previous studies (Oberman et al., 2022; Oberman et al., 2023), positive effects of J147 were not attributable to (anti-) inflammatory effect of J147, but metabolic processes could have played a role.

**Proof of principle: inflammation as key player in POCD?**

As (neuro)inflammation is seen as major culprit of POCD (Cibelli et al., 2010; Lin et al., 2020; Peng et al., 2013; Skvarc et al., 2018; Terrando et al., 2010; van Harten et al., 2012) we hypothesized that anti-inflammatory treatment could prevent or alleviate POCD. Neuroinflammation which is caused by surgery induced local inflammation can initiate POCD (Barrientos et al., 2012; Beloosesky et al., 2007; Bettcher et al., 2021; Wang et al., 2017; Yirmiya & Goshen, 2011; Zhu et al., 2018). Based on this literature, an anti-inflammatory treatment before surgery could attenuate the derailed inflammatory response and thereby prevent development of POCD. Therefore, in our first study in chapter 2 we tested the anti-inflammatory hypothesis for inhibiting POCD. As proof of principle, we tested a general anti-inflammatory drug, ibuprofen, in our previously established POCD model of young and aged rats (Hovens et al., 2014). As cognitive problems are the main symptom of POCD, we assessed cognitive performance in the second week after surgery (Hovens et al., 2014), to investigate POCD development under low and higher risk conditions. In agreement with the anti-inflammatory hypothesis, hippocampal neurogenesis and spatial memory were
improved after ibuprofen treatment. Moreover, other studies also showed that ibuprofen treatment could be beneficial for post-operative recovery and prevention of cognitive decline after surgery (Huang et al., 2018; Le et al., 2016; Xu et al., 2014). Surprisingly the beneficial effect of ibuprofen on cognition seen in our study, was not associated with increased, but rather suppressed (neuro)inflammation. This could suggest that suppression of inflammation may not provide the main target for treatment of POCD. However, although the type of (neuro)inflammatory markers and the time points of measurements were chosen carefully, it should be considered that this is not a full representation of the (neuro)inflammatory processes. Previously described by Hovens et al. in a rat model for POCD, neuroinflammation was elevated 1 week after surgery, subsided in the second week, and was declined at the third week (Hovens et al., 2014). This study underscores that the inflammatory response is a dynamic process, and choosing the right timing is crucial. In the studies presented in this thesis, neuroinflammation was assessed two weeks after surgery. Therefore, it could be that the neuroinflammation caused by the surgery, and the effects of treatment, may have changed the time course of the process as well.

As age is a major risk factor in development of POCD (Hua & Min, 2020; Moller et al., 1998), aged rats were also included in this study. In general, in aged rats effects seemed similar to those in young rats, though less pronounced. It is therefore likely that other factors, such as pre-operative health (Kapoor et al., 2019; Kotekar et al., 2018; Rundshagen, 2014; Sauër et al., 2009), also play a role in older patients that are at higher risk of developing POCD. Important conclusion drawn from this study were that a single injection of ibuprofen before surgery increased neurogenesis and improved hippocampus-associated aspects of POCD. However, in contradiction to our hypothesis, based on previous studies (Cibelli et al., 2010; Lin et al., 2020; Peng et al., 2013; Skvarc et al., 2018; Terrando et al., 2010; van Harten et al., 2012), the positive effects of ibuprofen seemed not directly associated with reduction of (neuro)inflammation. Therefore, we concluded that inhibition of (neuro)inflammation may not be sufficient to prevent POCD. Moreover, the inflammation hypothesis originates from a correlation that has been observed between (neuro)inflammation and cognitive decline in (pre-)clinical studies. Although often suggested as causal relationship, it could well be a reflection of a common denominator. We have reported the phenomenon that two strongly correlated factors seemed to be causal, but only shared a common denominator before in (neuro)inflammation associated with depression and myocardial infarction (Gouweleeuw et al., 2015; Gouweleeuw et al., 2021; Liu et al., 2013). This could explain why (neuro)inflammation is found increased in POCD, but anti-inflammatory treatment would not necessarily prevent POCD. Furthermore, an important observation is that effects observed in younger animals
may not directly predict the effects in older animals. We have observed this point before in previous POCD research (Hovens et al., 2021). This study on enriched nutrition in prevention of POCD, also emphasize that effects in young treated animals were substantially different from those in old rats. These observations may point to a different mechanism in young and old animals. As ageing is one of the most important risk factors for POCD in patients, it would be more clinical relevant to study POCD and potential treatments in an increased risk model.

**J147, a general anti-aging and anti-dementia drug, effective in POCD?**

**Healthy young Wistar rats**

We saw positive results with standard anti-inflammatory treatment using ibuprofen in a rat model for POCD. However, this was associated with higher rather than lower neuroinflammation (Chapter 2, Oberman et al., 2021). This stresses the notion that potentially successful treatment for POCD should combine anti-(neuro)inflammatory with other neuroprotective effects. Accordingly, we explored the drug J147, as it has anti-dementia, anti-inflammatory and general anti-ageing properties (Currais et al., 2015; Daugherty et al., 2018; Goldberg et al., 2020; Goldberg et al., 2018; Prior et al., 2013; Prior et al., 2016). Therefore, in chapter 3 the therapeutic potential of J147 was studied regarding prevention of POCD, and underlying mechanisms in young rats, by assessing cognitive functions, cell metabolism, (neuro)inflammation markers and neurogenesis. In rats that were chronically treated with J147, prevention of cognitive decline was seen, as both long and short term spatial memory was preserved after surgery. These results were in agreement with what we expected, as previous studies already showed the positive effects of J147 treatment on cognition in most common age-associated neuropathy models (Currais et al., 2019; Goldberg et al., 2020; Goldberg et al., 2018; Prior et al., 2013; Prior et al., 2016). Moreover, in this study we identified a potential anxiolytic effect of J147, as chronic J147 treated animals payed more visits to the center area and displayed more rearings, at similar distance moved in the open field test (Schoemaker & Smits, 1994). Similarly, previous studies showed an anxiolytic effect of J147 in an open field test (Pan et al., 2021) and in a forced swimming- and tail suspension tests (Lian et al., 2018). Although others have proven effectiveness of (sub)acute J147 treatment (Daugherty et al., 2018; Li et al., 2020), we could not show positive effects of acute intraoperative J147 treatment in our study. Nevertheless, as beneficial effects of J147 treatment were seen in these young rats, the next step was to investigate the mechanism of action of J147. As inflammation is hypothesized as one of the most important factors for
development of POCD and J147 could have effects on cell metabolism (Currais et al., 2019; Goldberg et al., 2020; Goldberg et al., 2018; Larrick & Mendelsohn, 2018), next to its anti-inflammatory effects (Currais et al., 2015; Daugherty et al., 2018; Prior et al., 2013), different parameters for peripheral- and neuroinflammation and cell metabolism were included. However, these factors did not seem to contribute to the mechanism of action by which J147 exerted its positive effects in this study. Although we saw no effects of J147 on (neuro)inflammation, it could well be that J147 shifted the time course of the cytokine response, rather than, or in combination with, altering the magnitude. Moreover, although IL-1beta and NGAL have been shown to be important indicators for POCD (Cibelli et al., 2010; Gouweleeuw et al., 2017), other markers, such as IL-6, are also markers that are associated with cognitive decline (Beloosesky et al., 2007; Hovens et al., 2014; Hovens et al., 2013; Yaffe et al., 2003). Although we did not see an anti-inflammatory effect of J147 on the measured markers, it does not exclude changes in other neuroinflammatory markers. Additional, a potential indicator for the mechanism behind J147 came from the food intake results. Reduced body weight loss after surgery with chronic J147 treatment, was not compensated for by increased food intake, hence pointing to metabolic changes.

Overall, from this study we concluded that chronic, but not acute, J147 treatment could prevent POCD. Chronic J147 preserved behavioral and cognitive performance in our rat model and could therefore be a stepping stone towards a novel therapeutic route in the treatment of POCD. Although the positive effects of J147 treatment were evident, the mechanism through which J147 acted were less clear.

**Zucker rats as a potential high risk model**
The results in chapter 3 showed that a more general anti-aging and anti-dementia drug called J147, could provide a potential effective intervention for POCD in young healthy rats. The next step would be to extend to a model that better represents the patients group that develop POCD, in order to heighten clinical relevance. The patient group undergoing surgery consists mainly of elderly patients with poor pre-operative health, which includes known risk factors for worse recovery after surgery (Misal et al., 2016) and development of POCD (Kapoor et al., 2019; Kotekar et al., 2018; Rundshagen, 2014; Sauër et al., 2009). These factors include hyperglycemia, hypertension, uremia and liver disease (Kapoor et al., 2019; Kotekar et al., 2018; Rundshagen, 2014; Sauër et al., 2009; Misal et al., 2016). Accordingly, we previously showed that worse presurgical conditions and advanced age resulted in more wide-spread cognitive decline and exaggerated neuroinflammation (Hovens et al., 2013; Hovens et al., 2015a, 2015b). However, in chapter 2 we showed that effects
observed in young rats do not necessarily predict effects in aged rats (Oberman et al., 2021). As commercial obtained aged rats are kept under optimal conditions, such as regulated climate conditions, pathogen free environment and optimal food, they may not best represent the elderly patient with poor health condition, that is prone to develop POCD. In this regard the Zucker rats may provide a relevant model for POCD research, as they display obesity, hypertension, hyperglycemia, low-grade (neuro)inflammation and mild cognitive dysfunction; all known risk factors for POCD (Aleixandre de Artiñano & Miguel Castro, 2009). Since early post-operative recovery is regarded as an important predictor for development of long-term complications, the aim of chapter 4 was to evaluate early postoperative recovery in Zucker rats and the effect of treatment with J147. To determine whether J147 can be effective in improving early postoperative recovery and thereby long-term complications, body weight loss (as preclinical parameter) and time to regain activity (as major clinical parameter) were assessed in this study. Despite the fact that we choose the Zucker rat model as a potentially better representation for patients that have a higher risk for developing post-surgical complications, surgery in these rats induced only relatively mild weight loss and disturbed activity. Although the Zucker rats indeed displayed risk factors for worse post-operative outcome, one prominent risk factor for postoperative complications in patients we could not include, namely age. The rats we used were 25-30 weeks old, since in older Zucker rats, spontaneous mortality steeply increases (Johnson et al., 1997), potentially biasing our results. Moreover most risk factor are already present at 6 months of age (Aleixandre de Artiñano & Miguel Castro, 2009).

Although Zucker rats did not displayed the expected early post-surgical complications, J147 treatment still had beneficial effects after surgery; it reversed the surgery induced prolonged time to first activity and improved diurnality. Supporting our results from chapter 3, again the beneficial effects of J147 were not associated with anti-inflammatory effects. Interestingly, similar effects on overall metabolism were seen; body weight remained the same at a lower food intake. All in all, these results showed that J147 may improve early post-surgical recovery and hence potentially reduces the risk of developing longer-term postoperative complications.

Results from chapter 4 indicated that J147 treatment had beneficial effects on early recovery after surgery, reflected in shorter time to regain activity and restored diurnality. Previously it was already shown that a delayed or incomplete recovery may result in more persistent postoperative complications, including cognitive decline and psychological problems (Fernandez-Bustamante et al., 2017; Gomes
do Carmo et al., 2021; Rasmussen, 2006; Tengberg et al., 2017). Moreover, delayed recovery can lead to prolonged hospital stay and subsequently delayed social activities, increased disability, dependency on social services, reduced quality of life and even death (Almashrafi & Vanderbloemen, 2016; Engelman et al., 2019; Goudzwaard et al., 2020; Pimentel et al., 2017; Wu et al., 2021). Therefore, it could well be that the beneficial effect of J147 on early recovery seen in chapter 4 could result in a lower development of long term complications, such as POCD. Considering the risk factors for POCD included in the Zucker rat model, we expected that Zucker rats would be more prone to develop POCD. As POCD is linked to a derailed inflammatory response (Cibelli et al., 2010; Lin et al., 2020; Peng et al., 2013; Skvarc et al., 2018; Terrando et al., 2010; van Harten et al., 2012) we expected to see increased (neuro) inflammatory markers, such as plasma IL-1beta and NGAL and more microglia activation. Moreover, if Zucker rats are indeed more prone to develop POCD, it was to be expected that Zucker rats would perform worse in the cognitive tests included in this studies. However, the Zucker rats did not develop substantial POCD as shown in Chapter 5. Therefore, we aimed to explore the Zucker rat model more in detail, including the potential risk factors for POCD. Indeed, Zucker rats expressed risk factors for POCD development, including impaired cognitive performance, high plasma triglycerides, obesity, decreased neurogenesis and low-grade systemic inflammation. However, similarly to what we saw in chapter 4, surgery did not seem to have the anticipated more extensive POCD in the Zucker rat, when compared to our previous studies in young (Oberman et al., 2021; Oberman et al., 2022) and aged rats (Oberman et al., 2021). Although surgery induced persistent weight loss, disturbances in day/night rhythm (diurnality), increased blood brain barrier (BBB) leakage and proteinuria, it did not induce POCD. Instead, surgery seemed to induce post-operative depression. In patient post-operative depression may include symptoms like persistent feelings of sadness, changes in appetite or weight, loss of interest or pleasure in activities, feelings of worthlessness or guilt, and thoughts of death or suicide (Ghoneim & O’Hara, 2016). As post-operative depression can occur in up to 60% of patients depending on type of surgery (Tegegne et al., 2022; Ghoneim et al., 2016) it is of importance to elucidated the cause for development of post-operative depression.

In addition, the effects of acute and chronic J147 treatment, regarding post-surgical cognitive function and (neuro)inflammation were measured. Since the underlying mechanisms of J147 are still not completely clear, other parameters such as effects on activity, day-night rhythm, anxiety/depressive-like behavior, neurogenesis, metabolism and BBB function were obtained. As the Zucker rat model did not show POCD, it limited the opportunity for testing the therapeutic potential of J147 on
cognitive dysfunction. Nevertheless, J147 treatment had beneficial effects on long-term spatial memory, diurnality, BBB integrity, anxiety, short term spatial memory, glucose sensitivity and proteinuria, underlining the multifactorial mechanism of J147. Similar to our results from the previous studies (Oberman et al., 2022; Oberman et al., 2023) described in chapter 3 and 4, no effects of J147 treatment were seen on (neuro)inflammation, indicating that other mechanisms were targeted to led to the observed effects.

The outcomes of the latter study indicated that Zucker rats indeed displayed risk factors for POCD however, surgery seemed to induce post-operative depression, rather than POCD in these rats. Since the main focus of the thesis was on (neuro) inflammation and cognitive function that were hippocampus related, more research would be needed, including other behavioral tests and different brain areas, to further elucidate on the effects of J147 on postoperative depression. Some evidence for anxiolytic effect of J147 in other animal models was already provided in our previous study in chapter 3, and other labs (Lian et al., 2018; Pan et al., 2021). However the link between J147 and anxiety in Zucker rats still needs to be clarified.

Although many parameters were included to investigate the underlying mechanism of J147, we could still not point out the actual mechanism of J147 involved in our studies. As all three studies conducted with J147, discussed in chapter 3-5, indicated a role for a potential altered metabolism, it would be worthwhile to further investigate the connection between J147 and metabolism. Moreover, since J147 showed a mixed beneficial effects of acute and chronic treatment it may suggest a combined treatment for optimal results.

**Future perspectives**

In conclusion, in the studies included in this thesis we showed that treatment with Ibuprofen and J147 could be effective in prevention of POCD in young healthy rats. However, in contrast to what we hypothesized, the positive effects of both treatments were not attributable to anti- (neuro)inflammatory effects. In addition to young healthy Wistar rats, we studied old healthy Wistar rats and Zucker rats in order to enhance clinical relevance. Both models reflected risk factors for developing POCD, reported in patients. Whereas aged rats indeed developed more extensive POCD, Zucker rats, despite displaying risk factors for POCD, did not develop cognitive decline after surgery. In aged rats, ibuprofen appeared not as effective as was seen in young rats. Similarly, effects of J147 in Zucker rats were not predicted
by the effects in young healthy rats. This was partly explained by the observation that Zucker rats did not develop POCD, hence leaving little scoop for prevention. However, similar to the studies in young rats, treatment in both increased risk rats did not relate to anti-inflammatory effects.

Our findings support the idea that (neuro)inflammation may rather be a common denominator with cognitive dysfunction in POCD than reflecting a causal relationship. Therefore, other factors may play an important role in development of POCD. Indicated by the studies presented in this thesis, metabolic changes could play a part in the development of POCD. Therefore, future research should focus on untangling the factors that initiate POCD to be able to develop a rational for effective treatment for POCD.

Moreover, as the pathophysiology of POCD is not fully elucidated, it is also important to focus on other treatments that could protect neurons from the POCD pathophysiology. In line with this, we explored in follow-up studies (Oroszi et al., 2022; Keijzer et al., 2022), protection of neurons against the devastating effects of neuroinflammation in combination with interfering with the derailed inflammatory response. A candidate target could be provided by the net-like protective collagen sheets structures around the cell body and dendrites of neurons, also known as perineuronal nets (PNNs). These specialized substructures of the brain extracellular matrix (ECM) consist of low amounts of fibrillar collagens (Krishnaswamy et al., 2019; Reichardt & Tomaselli, 1991). PNNs play an important in neuronal functioning, and therefore could be an important factor in cognitive dysfunction (Wingert & Sorg, 2021). In the same POCD model as used in the present thesis, surgery affected cognition (Oroszi et al., 2022), which was linked to the loss of protective collagen sheets around the hippocampal neurons (Keijzer et al., 2022). One way of combining anti-inflammatory effects with neuronal protection through collagen could be obtained by active exercise (Gleeson et al., 2011; Smith et al., 2015). Active exercise is known to have positive effects on mental and physical health. Moreover, it has anti-inflammatory effects (Gleeson et al., 2011) and positive effects on PNNs (Smith et al., 2015). Although exercise may provide a beneficial intervention after surgery, POCD develops after surgery in a period where patients could still experience pain and immobility from the surgical procedure, and therefore unable or unmotivated to perform exercise. Therefore, another therapeutical intervention, sharing the positive effects of exercise but with less physical load was explored as well. Whole-body vibration (WBV) has been indicated as a passive alternative for active exercise (Oroszi et al., 2020; Sitjà-Rabert et al., 2012). Similar to exercise, WBV can affect collagen in the brain (Keller et al., 2013) and could therefore be protective.
in POCD. Indeed, both exercise as well as WBV normalized the reduced collagen expression after surgery (Keijzer et al., 2022). Therefore, it may be the collagen that protected against the cognitive decline after surgery. Moreover, both exercise and WBV had positive effect on cognition after surgery, however, without effects on neuroinflammation (Oroszi et al., 2022). This supports the notion to investigate interventions that are neuroprotective, for example targeting perineuronal collagen, improving mitochondrial function, and inhibiting BBB leakage. In that regard, J147 a general anti-ageing a dementia drug, affecting different mechanisms, including metabolic and BBB leakage may bring us one step closer to an effective treatment for POCD.

**Conclusion**

Overall, the outcomes of this thesis clearly showed that POCD is a multifactorial disease. Although (neuro)inflammation may be indicated to play a key role in the development of POCD, results from the present thesis suggested that (neuro) inflammation alone may not provide a relevant target for treatment. The observation that effects of interventions in young healthy rats, that may help to explain underlying mechanisms, but do not predict effects in models with added risk factor for POCD in patients, such as age and presurgical health, strengthen the point of choosing an animal model for testing treatment very carefully. The results obtained in the Zucker rat model, that despite presence of risk factors, showed almost no POCD, but indications of postoperative depression, further challenges experimental studies into these conditions, and could narrow the gap between preclinical and clinical research on POCD.
References


Chapter 6


