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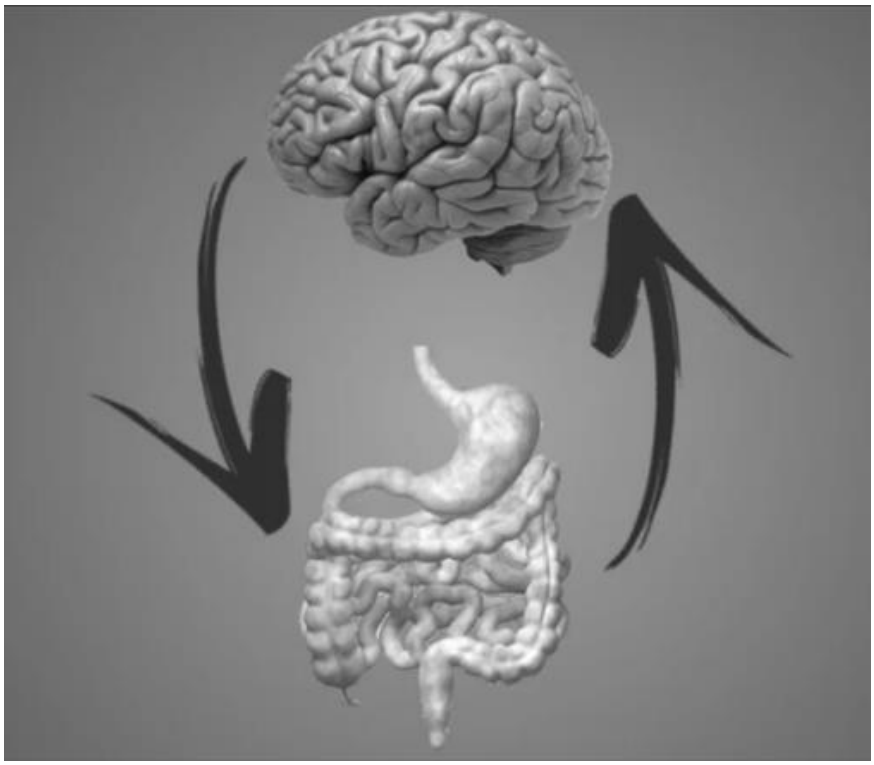
The Role of the Gut-Brain Axis on the Development of Postoperative Cognitive Dysfunction (POCD)

Bachelor's Thesis



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ABSTRACT

Postoperative cognitive dysfunction (POCD) is a disorder that impairs cognitive functioning as a consequence of surgery or anesthesia. The symptoms caused by POCD can seriously limit daily activities of the patient. A possible underlying mechanism of POCD is the gut-brain axis. The gut-brain axis is a bidirectional communication system between the gut and the brain via multiple pathways. Information about the intestine is sensed by enteric innervation via vagal afferent fibers, while the central nervous system (CNS) affects the gut by efferent fibers. Surgery can cause compositional changes of the gut microbiota, leading to the upregulation of inflammatory cytokines, such as TNF-a and IL-1. Besides that, surgery can induce damaged cells to release high molecular group box 1 protein (HMGB1) that can initiate the inflammatory NF-kB signalling pathway in bone marrow derived monocytes (BMDMs), which in turn release pro-inflammatory cytokines. The upregulated inflammatory mediators, together with the dysbiosis, impair the intestinal epithelial barrier, ultimately leading to a leaky gut. As a consequence, all kind of bacteria and toxins escape the gut lumen and enter the bloodstream. The increased pro-inflammatory cytokines in the circulation, together with the presence of mast cells and serotonin, are contributing to the breakdown of the blood-brain barrier (BBB). Once the BBB is disrupted, BMDMs can flow into the CNS, releasing inflammatory cytokines. Subsequently, this will lead to the activation of microglia, further amplifying the neuroinflammation. Neuroinflammation can lead to neuronal damage and cognitive impairments underlying POCD. Thus, there are sufficient mechanisms existing which represent the role of the gut-brain axis in the development of POCD. Potential treatments for POCD patients involve vagal stimulation and the use of probiotics, which intervene with the inflammatory reactions and the dysbiosis, respectively.

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POSTOPERATIVE COGNTIVE DYSFUNCTION (POCD)

Postoperative cognitive dysfunction (POCD) is a disorder which affects predominantly the cognitive functioning that establishes following surgery or anesthesia (Safavynia & Goldstein, 2019). The symptoms of POCD are reduced attention, impaired memory, difficulties with information handling, and changes in personality and mood. This array of symptoms could seriously deteriorate the living standards of the patients, which in turn can give rise to other psychological and physiological disorders, leading to even more problems (Lin et al., 2020). The duration of these symptoms differ from only a few days after surgery, till even years, between patients. The incidence of POCD lies between the 9 and 46%. Postoperative recovery is impaired, and the morbidity and mortality is increased in patients with POCD.

The main risk factor for developing POCD is advanced age. Furthermore, there are some other preoperative factors which may also contribute to the development of POCD. One of them is the physical state or preexisting frailty of the patient (Lin et al., 2020). In addition, chronic inflammation states like atherosclerosis, diabetes or other metabolic syndromes, as well as preoperative depression, have also been indicated as potential risk factors for POCD (Safavynia & Goldstein, 2019). Perioperative factors such as intraoperative hypotension, anesthesia method, and surgery time, and postoperative factors like infection, also determine the development of POCD (Lin et al., 2020).

However, the definition of POCD is still under debate, and the methods to measure POCD are not generally among researchers (Lin et al., 2020). When the definition is not clear, it is hard to accurately identify patients with POCD. Although, there are made up guidelines for the identification of POCD, which focusses mostly on the cognitive reduction and deficits in neurocognitive domains such as attention and psychomotor speed (Safavynia & Goldstein, 2019). Additionally, the actual contributions of the anesthesia and surgery on POCD is difficult to interpret, since different types of surgery all have their own influence on developing POCD. Moreover, high-risk surgery often goes hand in hand with longer anesthetic times, which makes it hard to indicate the correct cause of POCD, and to determine the difference in impact of anesthesia and surgery.

One of the potential mechanisms of POCD is the gut-brain axis (Su, 2021). The gut-brain axis is a communication system, mediated by neuronal, immunological, and hormonal signals, between the gut and the brain. The changing of composition of the gut microbiota and other components after anesthesia and surgery seems to interfere with this communication system, thereby stimulating the development of POCD (Agustí et al., 2018).

THE GUT-BRAIN AXIS

Studies concerning the gut-brain axis has grown exponentially last decade (Miller, 2018). Nutrition scientists, neurologists and microbiologist have experienced that separate bodily areas seem to interact intensively. The scientists revised their understandings and came to the conclusion that our brains, guts, nervous systems, and behavior are far more interconnected than thought. This is largely due to the found influence of the gut microbiota on well-being. Around the nineteenth-century, doctors blamed the gut for their consequences on the mind, while doctors at the start of the twentieth century blamed the mind for its effects on the gut. Nowadays, the focus is on the connection between these two bodily organs.

The gut-brain axis is connecting the gut and the brain and is formed by the central nervous system (CNS), the enteric innervation, the HPA-axis, and the intestinal microbiota (Agustí et al., 2018). Dysregulation of the gut-brain axis is associated with (non-)psychiatric and metabolic diseases, which can be caused by an imbalance of the gut microbiota (dysbiosis). The gut and the brain have a bidirectional communication system. One the one hand, the gut gives signals to the CNS via the extrinsic innervations of the gastrointestinal (GI) tract through spinal and vagal fibers, while, on the other hand, the CNS signals the gut by sending efferent fibers to the GI tract. The HPA-axis is involved in the limbic system and is regulating the stress response. Furthermore, the HPA-axis regulates various body processes, such as bowel and immune functioning, and emotions and mood.

There are several factors which influences the gut-brain axis. First, gut microbiota seem to have a strong influence on the communication of the gut-brain axis. These microorganisms interact with the ENS and the intestinal cells, and even with the CNS through different pathways (Carabotti et al., 2015). A change in the composition of the microbiota has an effect on these interactions, which may give rise to multiple disorders. Furthermore, excessive inflammation reactions caused by the alterations in the microbiome can negatively influence the gut-brain axis. High amount of pro-inflammatory cytokines can disrupt barriers which are important of the maintenance of proper functioning of the gut-brain axis. Additionally, the vagus nerve (VN) mediates the bidirectional connection of the gut-brain axis. The VN has protective mechanisms and modulates for instance intestinal homeostasis, and is therefore also one of the factors that affect the gut-brain axis.

Thus, sufficiently enough mechanisms by which the gut-brain axis is affected, and therefore can be disturbed. A disturbed gut-brain axis have detrimental effects on mental health. Potentially, the consequences will build up till neuroinflammation and cognitive impairments. POCD is one of these results of a disturbed gut brain axis. The role of the gut-brain axis on the development of POCD is under discussion lately and will be further reviewed in the next section.

THE INFLUENCE OF THE GUT-BRAIN AXIS ON DEVELOPING POCD

Gut microbiota changes after surgery

A collection of the microorganisms in the GI tract, called the gut microbiota, represent the biggest microorganism community in our body, and is essential for several body mechanisms and development (Morais et al., 2021). The gut microbiota is important for nutrient metabolism, since it synthesizes short-chain fatty acids (SCFAs) and vitamins for use of the host. In addition, they are necessary for the developing of the immune system and the intestinal mucosal, and are providing a physical barrier to block incoming pathogens with support of antimicrobial compounds (Sekirov et al., 2010). Most importantly, the intestinal microbiota affect the brain development. Studies have shown that alterations in the gut microbiome can lead to several deficit in brain functions, like working memory (Collins et al., 2012).

As discussed before, the microbiome plays an important role in body processes. Despite the fact that the microbiome is localized in the intestinal tract, it can affect brain functioning via the gut-brain axis. Surgery/anesthesia are one of the factors that have an impact on the gut microbiome (Jiang et al., 2019). Intestinal dysbiosis is often seen in patients after surgery or anesthesia. In their study, they investigated what the influence is on the gut microbiome. They

did not observe any difference in the alpha-diversity, which refers to the local scale of the microbial species, between the groups. However, the beta-diversity, which reflects the variation between the groups, showed that the surgery/anesthesia group contained other types of microbial species in comparison with the control group. Especially, the surgery/anesthesia group consists of five types of species that are increased and five that are decreased significantly.

Subsequently, quantitative analysis revealed which microbial species may be responsible for the causation of POCD. Two types of bacteria, *Lachnospiraceae* and *Ruminococcaceae*, demonstrated the highest probability of contributing to the impairments caused by surgery/anesthesia (Liang, 2019). Another study by Zhan et al. (2018) indicated that mice with deficits in learning and memory (deficient mice) also include an abnormal microbial composition compared with control mice. The deficient mice showed alterations in the bacteria *Firmicutes, Bacteriodales* and *Parasutterella*, which are associated with cognitive dysfunction, thereby may having an influence on the development of POCD. Overall, these studies suggest that the abnormalities in the microbiome may give rise to the establishment of POCD.

Moreover, an increased level of *Lachnospiraceae* and *Ruminococcaceae* in the gut microbiome after surgery/anesthesia is associated with an impaired hippocampus (Beilharz et al., 2016). The increase of these bacteria will lead to an increase in inflammation-related hippocampal genes, like NfkB1, IL-1B and TNF-a. Furthermore, the death of other bacteria can stimulate an overproduction of lipopolysaccharides (LPS), thereby leading to inflammation and cognitive impairments (Jiang et al., 2019). Surgery and anesthesia go often hand in hand with inflammation by amplification of pro-inflammatory cytokines (Safavynia & Goldstein, 2019). As a response to the surgery, damaged cells will release damage-associated molecular patterns (DAMPs). High molecular group box 1 protein (HMGB1) is such a DAMP, and will bind to receptors (TLR4 and RAGE) on bone marrow derived monocytes (BMDMs).

Safavynia and Goldstein (2019) observed an increased level of hippocampal HMGB1 expression after surgery and anesthesia, which is linked to cognitive deficits (Li et al., 2013). This is supported by the findings of Lin et al. (2014) that an elevation of HMGB1 are found in patients with POCD after surgery. After HMGB1 have bound to TLR4 and RAGE, NF-kB will be activated and cause upregulation of the pro-inflammatory cytokines TNF-a, IL-1 and IL-6. This will in turn lead to further upregulation of NF-kB, inducing upregulation of cyclooxygenase 2 (COX-2), and HMGB1, thereby causing a positive feedback loop, strengthening the inflammatory response. The increase of pro-inflammatory cytokines and POCD are frequently associated in both animal (Hem et al., 2016) and human research (Ji et al., 2013).

Enteric innervation

Enteric bacteria and their products, like SCFAs, support the intestinal epithelial barrier by regulating cell growth and differentiation (Pellegrini et al., 2018). The gut microbiota also ensures the maintenance of the immune tolerance and shapes the immune responses. Several studies suggest that the gut microbiota have an important role in the function and development of the enteric nervous system (ENS). The ENS consists of the submucosal and myenteric plexus, from which the latter showed a reduction caused by an abnormal microbial composition. Besides that, the ENS regulates digestive functioning and it cooperates with the CNS by (para)sympathetic pathways. The important component of the ENS, the enteric glial cells (EGCs), is involved in either the epithelial barrier integrity and the maintenance of the ENS. Even here, gut microbiota are involved in the regulation of the colonization of the EGCs,

thereby influencing epithelial barrier integrity and ENS maintenance. Taken together, altered gut microbiota, malfunctioning of the intestinal epithelial barrier, triggering inflammatory/immune cells and alterations in the ENS, are all factors which can contribute to the development of various neurological disorders. (Fung et al., 2017).

The gut contains a big surface and is therefore an important sensory organ (Breit et al., 2018). The sensory information of the gut, including the microbiota, is sensed by afferent fibers of the VN and delivered to the brain. The VN is capable of sensing signals from the microbiome by direct mechanisms, such as SCFAs products (Lal et al., 2001) and TLR4 expression (Goehler et al., 1999). Raybould (2010) has shown that the vagal chemoreceptors are most likely involved in the connection between the microbiome and the brain through sensing gut hormones and/or SCFAs. Vagal afferents are interacting with gut endocrine cells, called the interface of gut chemosensing (Bonaz et al., 2018). Enteroendocrine cells (EECs) modulate GI functions and interact with vagal afferents by releasing hormones or directly through serotonin release. Moreover, microbiota can signal EECs by TLRs that recognize bacterial products like LPS. Consequently, the EECs are important in the singalling and detection of the microbial composition which can regulate GI functioning by the interaction with the vagal afferent fibers. For instance, when there is a dysbiosis, the proper communication through the vagal fibers between the gut and the brain can be disturbed (Foster & McVey Neufeld, 2013).

As known, the communication between the gut microbiota and the brain is bidirectional. The vagal afferent fibers activate the vagal efferent fibers. Looking into the vagal afferent fibers to the brain, the microbiota in the gut can regulate the inflammatory pathway by either inhibiting or activating the VN, thereby being pro- or anti-inflammatory (Bonaz et al., 2018). Several studies showed an anti-inflammatory pathway (Martelli et al., 2016; Wang et al., 2003). For instance, both ACh release at vagal efferents and a vago-sympathetic pathway to the spleen caused an inhibition of the TNF-a release by macrophages. Next to the inhibition of intestinal inflammation by the VN, it also does have another protective role through enhancing the tight junctions and reducing the intestinal epithelial permeability (Zhou et al., 2013). Stimulation of the VN leads to an increase of the tight junction proteins ZO-1 and occludin, and an inhibition of the NF-kB activity in the intestine, both contributing to a healthy gut barrier functioning.

Leaky gut induces microbial translocation

The intestine contains of a barrier to protect the internal environment and block the entry of toxins. This barrier is supported by several components, including cytokines. When there is an abnormality present in one of those components, the barrier functioning will be reduced and cause an increased intestinal permeability, called a leaky gut. A leaky gut allows commensal bacteria and toxin to escape the gut lumen, which can give rise to inflammation and even tissue damage when there is an translocation into the peripheral circulation (Mu et al., 2017).

Thus, when the intestinal epithelial permeability is increased, translocation of microorganisms from the lumen into the circulation can take place (Brenchley & Douek, 2012). This flow from the GI tract into the systemic circulation could give harmful effects, such as activation of the immune system. The tight junctions in the intestinal epithelial barrier can also be a cause of the microbial translocation. Enterotoxins, which are expressed by pathogenic bacteria, can disrupt the tight junctions. Additionally, the regulation of tight junction can be a cause, since an important component of tight junctions, claudin, can be modulated by immune regulators. Yang & Rosenberg (2011) demonstrated that alterations in the tight junctions is involved in the development of POCD after surgery. A possible consequence of the microbial translocation

and the altered tight junctions can be an increased production of inflammatory cytokines like TNF-a and IL-1, and HMGB1. Excessive production of this inflammatory agents may lead to elevated systemic inflammatory responses, which is even more detrimental than the bacterial infection itself. The immune modulator TNF-a has a huge impact in the barrier functioning, because it can stimulate apoptosis in epithelial cells and increase the permeability by cytoskeleton rearrangements an tight junction functioning.

Serotonin and cytokines inducing blood-brain barrier breakage

A key neurotransmitter that is involved in the gut-brain axis is serotonin (Szőke et al., 2020). Serotonin is produced in the enterochromaffin cells (ECs) and is involved in several important functions in the GI tract, such as signalling and secretion (Kelly et al., 2015). Moreover, it is shown that serotonin plays a role in the regulation of the intestinal permeability. Coates et al. (2004) demonstrated that serotonin has an pro-inflammatory role in intestinal inflammation. Consequently, the inflammation leads to a reduction of SERT, which is responsible for the inactivation of serotonin, and a raised EC cell numbers, thereby resulting in an increase of serotonin availability which can act proinflammatory (Terry & Margolis, 2017). The increase of serotonin in the circulation has also been seen after metabolic and traumatic insults to the CNS (Winkler et al., 1995). Furthermore, it is proven that serotonin has an influence on the maintenance of the physical barrier which block the entry of toxic compounds into the brain, called the blood-brain barrier (BBB) (Szőke et al., 2020). Studies (Winkler et al., 1995 & Brust et al. 2000) stated that serotonin induce breakdown of the BBB, and that the cerebral endothelial cells modulate the metabolism and removal of available serotonin. This supports the fact that the serotoninergic regulation is present in the cerebral circulation, influencing the BBB functioning.

Besides that serotonin has negative effects on the BBB, proinflammatory cytokines also increase the BBB permeability (Safavynia & Goldstein, 2019). The BBB separates blood from the brain by an capillary endothelium consisting of tight junctions and transmembrane proteins, such as claudins and occludins (Rempe et al., 2016). Pathological disruption of either the endothelium, tight junctions or basements membrane of the BBB, damage the barrier functioning. The cytokines does this through increasing matrix metalloproteinases (MMPs) and COX-2, enabling the cytokines to enter the CNS. The proinflammatory cytokines TNF-a and IL-1 can increase the rate of COX-2 in neurovascular endothelial cells, which targets the BBB permeability and induces the synthesis of local prostaglandins (Safavynia & Goldstein, 2019). There is a presence of IL-1, IL-6 and TNF-a found in hippocampal areas and cerebrospinal fluid (CSF) after surgery, supporting the effects of a BBB disruption. The increased level of cytokines in the CNS due to BBB leakage is related to cognitive dysfunction, thus being a possible underlying cause of POCD.

Interestingly, there are found other abnormalities in the brain and circulation of POCD patients. CNS-specific proteins like neuron-specific enolase (NSE) and S100B are found in the plasma (Peng et al., 2013), and immunoglobulin G (IgG) is present in brain regions, such as hypothalamus and hippocampus, after surgery (He et al., 2012). In addition, mast cells possibly represent an important immune signalling connection to the brain during inflammation (Zhang et al., 2016). The mast cells are located at the CNS, near microglia and neurons, and produce several inflammatory agents, including nitric oxide and cytokines. Therefore, mast cells have the potential to negatively affect the BBB, neurons, and microglia. Besides the production of cytokines and nitric oxide, mast cells also secrete serotonin and TNF-a, which also have an inflammation potential as discussed previously, and thus contributing to BBB disruption. It is observed that surgery leads to upregulation of mast cells in the brain,

giving rise to the inflammation and rupture of BBB, consequently causing cognitive impairment and potentially POCD.

Next, circulating BMDMs can enter the CNS because of the disrupted BBB and enhancing neuroinflammation through microglial activation and cytokine expression (Safavynia & Goldstein, 2019). BMDMs executes this, when existing in the CNS, by secreting pro-inflammatory cytokines by stimulating NF-kB transcription and activating microglia, even more amplifying the neuroinflammation. Recruitment of BMDMs into the CNS is mediated by the interaction of the cell surface receptor chemokine receptor 2 (CCR2) and the chemokine monocyte chemo-attractant protein 1 (MCP-1). Degos et al. (2013) showed that the hippocampus expresses MCP-1, which attracts CCR2-expressing cells translocating over the deficient BBB, after surgery. This finding indicates that there is postoperative BMDM recruitment into the hippocampus, which is associated with postoperative memory dysfunction, as present in POCD (Degos et al., 2013).

Microglial activation

Other immune regulators that are present in the CNS, are microglia. These type of neuroglia comprise about 5-10% of the total CNS cells and are able to residence and self-renew there throughout life (Frost & Schafer, 2016). Microglia respond and work protective against injury and stress, and monitor health, by reacting to dangerous signals, synthesizing key components of neurons, and eliminate debris from tissue (Lin et al., 2020). Inflammation and BBB disruption promote the differentiation of these microglia into their activated states, M1 and M2. The M1 phenotype is pro-inflammatory and has phagocytic properties, whereas the M2 phenotype is associated with remodeling and repair and is anti-inflammatory (Safavynia & Goldstein, 2019). Not unexpectedly, pro-inflammatory mediators in the gut-brain axis like LPS and TNF-a promote the phenotypical change of the microglia into the M1 type. Further, a study by Zhang et a. (2016) demonstrated that surgery induce degranulation of mast cells in CNS, following microglial activation.

The activation of microglia leads to the continuously upregulation of pro-inflammatory cytokines, thereby strengthening neuroinflammation and contributing to the development of POCD. Several animal models (Clausen et al., 2008 & Terrando et al., 2010) have shown that activated microglia promote the release of TNF-a, IL-1B, and HMGB1. Besides that, they upregulate CCL2 and MCP-1, which also have been associated with cognitive malfunctioning by the further entry of BMDMs into the CNS. Subsequently, BMDMs themselves can promote differentiation of microglia into the M1 phenotype through IL-1 and TNF-a expression. Taken together, microglial activation is a potential mechanism in developing POCD. An increased level of activated microglia in POCD observed in aged mice supports this hypothesis (Hovens et al., 2015).

Furthermore, activated microglia in the CNS release reactive oxygen species (ROS) in response to S100B and HMGB1 (Heneka et al., 2014). An increased level of oxidative stress and markers of oxidative damage, such as depleted antioxidants, have been found frequently after surgery and may contribute to the development of POCD (Skvarc et al., 2018). Moreover, surgical insult can increase the levels of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in the CNS, which generates superoxide during stress (Safavynia & Goldstein, 2019). Thereafter, the formed superoxide can generate other ROS, inducing damage on neuronal tissues. Indeed, this oxidative stress potentially cause neuroinflammation by dysregulation of BBB functioning, consequently, allowing the entry of oxidative species into the CNS (Skvarc et al., 2018). Presence of ROS in combination with pro-inflammatory cytokines cause a toxic

milieu wherein neuronal functioning is impaired. In the end, a degenerative positive feedback loop will be created where injured neurons stimulate the release of ROS from microglia, and activated microglia in turn release HMGB1, further worsening the injury. However, evidence suggests that oxidative stress, following surgery, alone could contribute to POCD (Netto et al., 2018).

Neuroinflammation causing cognitive dysfunction

The pro-inflammatory cytokines, which contributes to the CNS inflammation, have a negative impact at the neurotransmitter regulation in the hippocampus, eventually leading to neuronal damage and cognitive impairment underlying POCD (Safavynia & Goldstein, 2019). The hippocampus is the centre of memory formation, which is supported by a process called long-term potentiation (LTP). Since the hippocampus consists of a large amount of cytokine receptors, it is susceptible to a large quantity of pro-inflammatory cytokines like TNF-a and IL-1 in processes like neuroinflammation (Rachal Pugh et al., 2001). When the cytokine receptors are stimulated in such high amount, it can cause glutamate toxicity and ultimately disruption of the LTP process. Next to this, TNF-a can slow down inhibitory neurotransmission, disturbing the balance between inhibitory and excitatory, thereby contributing to glutamate toxicity in the hippocampus. This toxicity is seen in neurodegenerative diseases, among which POCD (Lin et al., 2020).

Due to the defects in the hippocampus, especially memory functions, but also visuospatial, language and attention, could be affected in patients with POCD (Hovens et al., 2012). Hippocampal damage will especially lead to problems with long-term memory, while other cognitive (dys)functions are devoted to other brain areas. Brain areas involved in POCD include many regions, like frontal, cerebral, temporal, and parietal, with each their own contribution in declined brain functioning. Presumably, taking into consideration the role of the gut-brain axis and the additional neuroinflammation, the impairments in POCD are not caused by single beforementioned brain areas, but are rather induced by damage in multiple areas, or even the whole brain. However, there are large methodological differences in the methods to determine POCD and the characterization of patients with POCD, which makes it hard to state proper conclusions about the cognitive deficits in POCD.

INTERVENING IN THE PATHOGENESIS OF POCD

Vagal stimulation and anti-inflammatory drugs

A possible underlying mechanism of POCD is inflammation. Activation of pro-inflammatory cytokines have been associated to different aspects in the gut-brain axis, such as the microbial translocation and CNS inflammation. Nevertheless, these cytokines are also limiting the degree of inflammation by regulating the vagal reflex arc, known as the cholinergic anti-inflammation pathway (Safavynia & Goldstein, 2019). In this pathway, the DAMPs, which are released due to surgery, are sensed by vagal afferents, which will project on the brain. From this point, vagal efferent will connect to the splenic nerve, via the celiac ganglion, and synthesize ACh. Subsequently, newly synthesized ACh can activate circulating macrophages which express a7 nicotinic ACh receptors (a7 nAChRs), thereby inactivating NF-kB and thus causing a reduced cytokine release (Saxena & Maze, 2018). Furthermore, vagal stimulation is responsible for the secretion of anti-inflammatory cytokines IL-4 and IL-10, from which the former is promoting the phenotypical switch of microglia into the M2 type (Ghia et al., 2007). Taken together, the vagal fibers are involved in inhibiting and limiting the inflammation

reactions which may occur in the gut-brain axis. Anticholinergic drugs that decrease the hippocampal TNF-a and IL-1B levels are therefore a possible intervention for POCD.

Additionally, there are other anti-inflammatory treatments for POCD, among which COX-2 inhibitors. COX-2 is an enzyme which can increase the permeability of BBB and the formation of inflammatory prostaglandins, inducing enlarged inflammation (Engblom et al., 2002). Because of these mediators of neuroinflammation, COX-2 inhibitors are a potential therapy for POCD patients. Besides this, pro-neuronal treatments which promote neuronal health prior to surgery, are also candidates for treating POCD. Dexmedetomidine is one of them and is involved in norepinephrine inhibition in the hypothalamus (Hu et al., 2018). Further, it is shown that this receptor antagonist is responsible for enhancing HMGB1 resolution via vagal mechanisms. Hence, this medication supports the maintenance of the BBB and prevents hippocampal inflammation and surgery-induced cognitive dysfunction, such as POCD.

Another intervention for POCD which concerns vagal mechanisms is about the composition of nutritional intake. A study by De Haan et al. (2010) have shown that lipid-rich nutrition has positive effects regarding the prevention of POCD. The large amount of lipids in the diet improves the intestinal barrier functioning and prevent enterocyte damage. This is done through stimulation of the autonomic nervous system via activation of cholecystokinin-receptors (CCK-r). Therefore, the vagus-mediated pathway is the underlying mechanism which give rise to the effects of lipid-rich nutrition, preventing the development of systemic inflammation that may give rise to neuroinflammation or even POCD. In addition, this intervention showed to inhibit the activation of mast cells, which also have been seen to potentially induce activation of inflammatory agents and disturb the BBB and microglia. Likewise, the inhibition of mast cells goes via CCK-r activation, thereby contributing to the vagal anti-inflammatory pathway.

Probiotics

The gut microbiome is an important component of the gut-brain axis and show alterations after surgery/anesthesia. The variety of gut microbiota in POCD patients is far less compared to healthy individuals. Li et al. (2018) observed that in total 24 bacterial species were significantly changed in the microbiome of POCD patients. As mentioned before, abnormal microbial composition is associated with POCD, and alterations in specific gut bacteria may be contributors in the development of POCD. A way to increase the health of the gut microbiota is through probiotics. Probiotics are living microorganisms which contribute to intestinal microbial balance, giving health benefits to the host when administered in sufficient amounts. Moreover, they improve immune functioning and alleviate GI symptoms. Akbari et al. (2016) have demonstrated that probiotics have an influence on the CNS and behavior via the microbiota-gut-brain axis. They show promising results through protecting cognitive functioning and improving depressive or anxiety behavior after surgery in Alzheimer's disease patients and healthy individuals, respectively. Since dysbiosis has an important role in the pathogenesis of POCD, perioperative probiotic treatment could be used as a potential intervention for this condition.

DISCUSSION

The gut-brain axis is a broad concept, since it is influenced by multiple factors and it affects whole body processes. A main factor that influences the gut-brain axis is the gut microbiome composition. After surgery or anesthesia, the composition of these gut microbiota can be

altered. This intestinal dysbiosis causes upregulation of surgery induced inflammatory cytokines, such as IL-1B and TNF-a, and pathogenic factors like LPS. Besides the inflammation reaction caused by surgery, the operation itself will cause damage to cells which then release DAMPs, such as HMGB1. Thereafter, HMGB1 will bind to TLR4 and RAGE at BMDMs, thereby activating NF-kB that cause further upregulation of pro-inflammatory cytokines IL-6, IL-1, and TNFa. These cytokines will in turn lead to the increase of COX-2 and HMGB1 expression, again strengthening the inflammatory response.

Gut microbiota are also partly responsible for the maintenance of the intestinal epithelial barrier. Pathogenic bacteria in the gut and the upregulated cytokines can affect the tight junction in the intestinal epithelial barrier. Another component that is involved in the functioning of the intestinal barrier is the ENS. The microbiome interacts bidirectional with vagal afferent and efferent fibers, thereby sensing signals between the gut and brain. Dysbiosis can cause a disturbance in this communication of vagal fibers. The reduced communication between the gut and the brain, in combination with the excessive inflammatory upregulation and serotonin production in the ECs, could disrupt the barrier functioning in the intestine, ultimately leading to a leaky gut. Consequently, all kind of toxins and bacteria are escaping the gut lumen, entering the blood.

Furthermore, the increased pro-inflammatory cytokines in the circulation, together with the increased availability of serotonin and mast cells, could disrupt the BBB. When the BBB is disturbed, circulating BMDMs can enter the CNS, releasing again inflammatory cytokines. The increased level of inflammatory mediators in the CNS will promote the activation of microglia into their M1 phenotype. This type of microglia further raise the amount of inflammatory cytokines and HMGB1 in the CNS, and lead to an increased level of oxidative stress by the release of ROS. All these factors induce an toxic milieu in the CNS, further amplifying neuroinflammation. The pro-inflammatory cytokines are able to localize in hippocampal areas and the CSF. The presence of these cytokines in the brain will disturb the LTP process, causing cognitive impairments in for instance memory and attention, and even changes in personality and mood. All of these impairments are of frequent occurrence in POCD.

Thus, in turn to answer the main question, the gut-brain axis do show serious connections with the development of POCD (figure 1). Several studies confirmed this relationship and particular address the role of the gut microbiota, intestinal epithelial barrier, enteric innervation and BBB maintenance in the development of POCD. Changes or damage to these components have frequently been demonstrated to enhance the change of developing POCD. Further research is needed to indicate the precise influence of the gut-brain axis on developing POCD.

Potential interventions for POCD involve vagal stimulation and the use of probiotics. The former is a mechanism to inhibit the inflammation reactions, whereas the latter fight against dysbiosis. Both concepts are observed frequently in the pathogenesis of POCD, thus should give appropriate effects in the prevention of POCD. In my opinion is the anti-inflammatory treatment through vagal mechanisms the most promising in preventing this condition. Vagal nerve fibers are connecting the intestinal, including the microbial composition, with the brain. Therefore, the VN is of importance in detection of any abnormalities that are present somewhere in the gut-brain axis, and act directly via signalling afferent and efferent fibers through for instance neurotransmitter release. However, further research is needed in order to confirm this direct connection to validate it as suitable treatment for POCD.

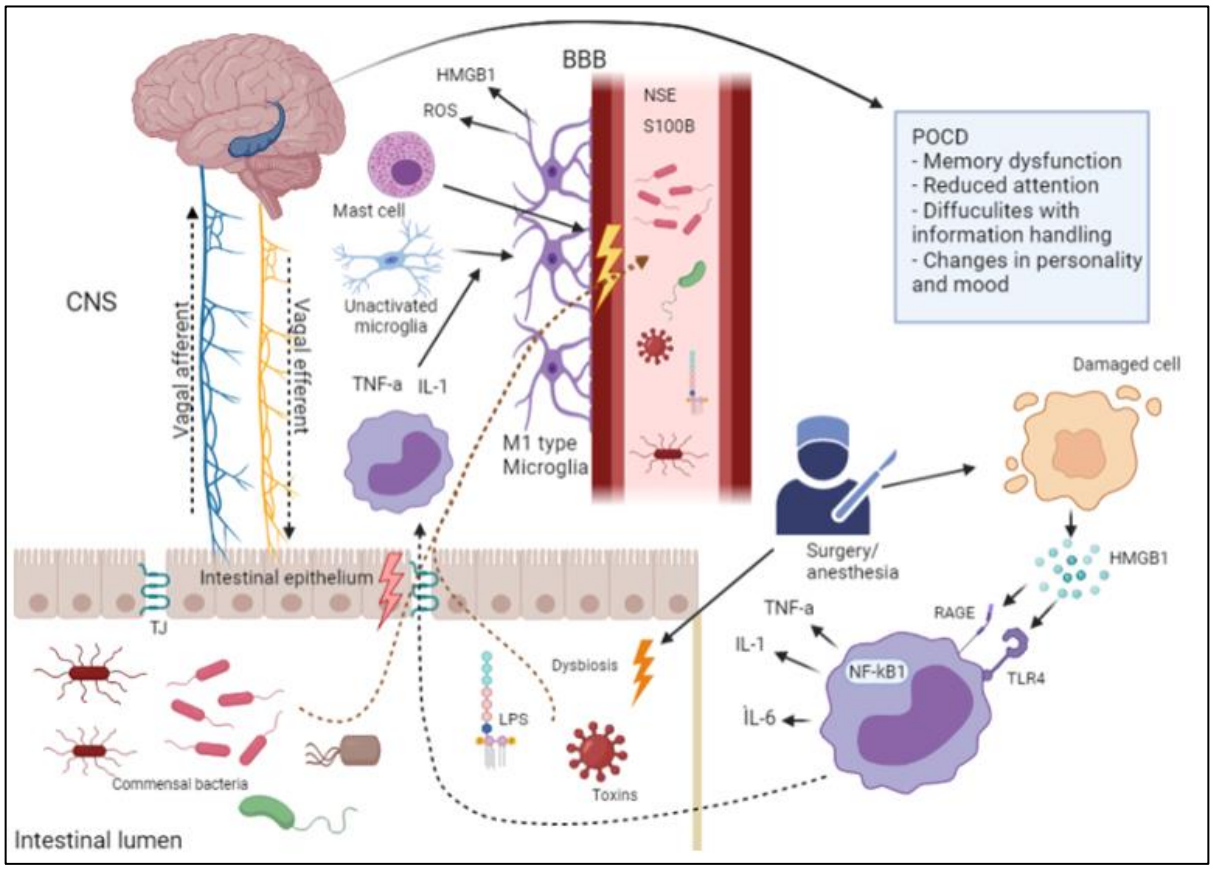

Figure 1: The role of the gut-brain axis on the development of POCD. *Surgery/anesthesia cause damaged cells, which in turn release HMGB1. HMGB1 binds to receptors (RAGE and TLR4) of a bone marrow derived monocyte (BMDM). This leads to the activation of the NF-kB signalling pathway, that causes a release of the pro-inflammatory cytokines TNF-a, IL-1 and IL-6. These cytokines, together with the surgery/anesthesia-induced dysbiosis (orange lightning bold), lead to TJ breakdown in the intestinal epithelium (red lightning bold), allowing microbial translocation of commensal bacteria, LPS, and toxins into the circulation (brown dashed line). In addition, there are found other abnormalities in the bloodstream, such as NSE and S100B. Meanwhile, sensory information of the intestine, including the microbiota, is sensed by vagal afferent fibers of the vagus nerve and delivered to the brain, where it activates vagal efferent fibers that in turn influences the intestine. The elevated level of pro-inflammatory cytokines in the circulation, together with the presence of mast cells, will induce the breakdown of the blood brain barrier (BBB). Consequently, BMDMs will flow into the CNS, releasing pro-inflammatory cytokines in there, leading to the activation of microglia and differentiating into their M1 type. This phenotype releases HMGB1 and ROS, which will, together with the activated microglia and increased level of cytokines, lead to a toxic milieu in the CNS. This toxic environment will give rise to brain damage, especially in the hippocampus (blue area), causing cognitive dysfunctions that characterize POCD. HMGB1, high mobility group protein B1; RAGE, receptor for advanced glycation end-products; TLR4, Toll-like receptor 4; NF-kB1, nuclear factor kappa B subunit 1; TNF-a, tumor necrosis factor alpha; IL-1, interleukin-1, IL-6, interleukin-6; LPS, lipopolysaccharide; TJ, tight junction; ROS, reactive oxygen species; NSE, neuron-specific enolase; S100B, S100 calcium-binding protein B*

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