The effects of abdominal surgery on post operative cognitive dysfunction.

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Abstract

Post operative cognitive dysfunction is a disorder which affects learning and memory after surgery and can persist for long periods of time. Due to aging and high number of performed surgeries it primarily affects the elderly and their quality of life after treatments.

The aim of this study was to analyse the effects of abdominal surgery on learning and memory in three month old wistar rats. Although POCD has long term clinical effects this study only aimed to analyse the animals' performance in the short term, up until three weeks after surgery. The animals' performance was assessed by using behavioural tests aimed at learning, memory and attention.

Results showed no effects of anaesthesia on cognitive function but did show a decrease in learning and memory in treated animals that lasted up to two weeks. Treated groups also showed a decrease in spatial memory that lasted up to two weeks. Because POCD is a characterized as a long term dysfunction it would be expected that cognition would be impaired for longer periods of time. This however was not found.

Introduction

In a society which continuously tries to improve the duration of life and its quality many people, especially the elderly, receive surgery. *Post operative cognitive dysfunction (POCD)* can occur after surgery and can cause deficits in learning ability and memory. This could mean that in some cases surgery can have deleterious effects on the very quality of life it was aimed to improve (*Monk et al 2011)*

Effects of surgery on cognition.

Any patient who has been subjected to surgery will need time to recover from the surgery as well as the accompanying anaesthesia. Short term effects of surgery may induce delirium. Which can affect the patient's health, mental status and cognition. Long term effects are found in patients with POCD. These patients suffer from various cognitive deficiencies such as: impaired learning and memory. Due to the difficulty of distinguishing effects of delirium from effects of POCD it has proven challenging to point out the onset of POCD (*Monk et al 2011, Hartholt et al 2012, Hovens et al 2013)*. Some clinical studies found an incidence of POCD between 5 and 15%, however estimates have been made that up to 40% of patients could suffer from POCD 1 year after surgery (*Hartholt et al 2012, Moller et al 1998).* There are several factors which contribute to the incidence of POCD such as: The type of surgery, age, pre-surgical afflictions and the level of inflammation (*Krenk et al 2010)*.

Anaesthesia and POCD

Anaesthesia could play a part in the development of POCD, although results have been ambiguous. It has been shown that anaesthetics can induce apoptosis *in vivo* and neuronal decline*. (Hudson et al 2011).* This is an important research topic due to population aging. Estimates have been made that by 2051 40% of all anaesthesia will be administered to the elderly *(Silbert et al 2011).* In human studies findings with regards to the effects of central anaesthesia on POCD are inconclusive, some studies find different effects when the type of surgery changes (*Ologunde et al 2011)* whereas others do not (*Silbert et al 2011)*. And therefore it would be difficult to state that anaesthesia itself could induce POCD. This situation would benefit from further elucidation of the mechanism behind the development of POCD.

Mechanism

Even though a definitive mechanism for the pathology of POCD has yet to be established, some suggestions have been made. Surgery will invariably induce inflammation and the effects of inflammation on learning and memory has been researched. Peripheral inflammation can activate CNS microglia and release cytokines in the brain (*Hovens et al 2012*). This can release pro-inflammatory agents and have different effects on the brain. For example IL-1 and IL-6 have been found to cause detrimental effects on learning and memory, alternatively release of cytokines could also be induced by stress (*Yirmiya et al 2011)*. Clinical studies have found pre existing disease, age and duration of hospital stay to be risk factors which can also increase the effects of POCD *(Krenk et al 2010).* Even though POCD could occur after many different types of surgery there could also be specific symptoms which only occur after specific types of surgery *(Silbert et al 2011).* In POCD research there is a relatively high amount of clinical data and animal trial data, however there seems to be a lack of studies which monitor cognition over time and thus the development of POCD. This opens up the opportunity for more research which might help in elucidating the mechanism behind POCD.

The aim of this study is to use behavioural tests to map learning and memory performance in rats, during the first three weeks after surgery. This could contribute to the problems regarding development of POCD by measuring animals' performance over the cause of time and might also show differences between POCD and delirium.

Methods

The goal of this study was to ascertain the effects of surgery on inducing POCD. The surgical procedure of choice was an abdominal surgery. The reasoning behind this was to approximate the everyday procedures performed in a division of the *University medical center Groningen,* which is linked to this research program.

Animals

This study was conducted using 3 month old male Wistar rats (N=72). These were grouped into Control (n=8), LPS (N=8), one hour anaesthesia (N=8), animals tested one, two and three weeks after surgery (N=8, N=8, N=8) and animals tested one, two and three weeks after surgery coupled with an intraperitoneal (i.p) LPS injection (N=8, N=8, N=8). LPS was injected into the abdominal cavity in a 1ng/g bodyweight concentration. The goal of the LPS only group would be to establish whether just the LPS injection can bring on cognitive effects. Likewise the anaesthesia only group was used to check the impact of anaesthesia on cognitive function. All animals were weighed daily, when an animal's weight reached <85% of baseline this would be deemed as excessive weight loss resulting in exclusion from experiments and humanely ending its life.

Procedure

The entire abdominal surgical procedure was performed under 3-4% sevoflurane-O2 anaesthesia, sevoflurane was chosen because of its clinical use in the *UMCG* hospital.

Surgery

The animals were anesthetized and a blood sample was taken from their tail vein. After blood sampling the abdomen was opened and the intestines were exteriorized. This allowed for clamping of the *upper mesenteric artery*. The *upper mesenteric artery* branches from the aorta and supplies the intestine with blood. This artery was clamped for 30 minutes, suppressing blood flow to induce damage to the intestine in order to raise an inflammatory response. Anaesthesia only animals were anesthetized for one hour (the amount of time a surgery would take) in order to ensure that all animals who underwent anaesthesia would be subjected to it for similar amounts of time

Canulation

In order to take daily blood samples an indwelling cannula was installed in the jugular vein. During this 30 minute period a jugular vein canulation was performed. After canulation and the 30 minutes had expired the clamp was removed, the LPS groups received a 1ng/g bodyweight concentration LPS dosage sprayed on top of the intestine before closing the animal up.

Sacrifice

Blood samples were taken using heartpunction and animals were sacrificed using transcardial perfusion under pentobarbital anaesthesia (2.5ml). This allowed for the salvage of the animals brain without blood. Urine and CSF samples were also taken. Half of the rats' brain was dissected into prefrontal cortex, cortex, hypothalamus, thalamus, hippocampus, striatum, brain stem and cerebellum and stored at -80 degrees Celsius. The other half was fixated by immersion in 40% formaldehyde.

Behavioural tests

To determine the effects of surgery the following behavioural tests were used in order to quantify the animals' anxiety, memory and mental flexibility.

All behavioural experiments were performed in the same room with controlled temperature ~21 degrees Celsius and ~50% humidity. Experiments were recorded by camera and tracked using ethovision.

Open Field

In order to assess the effects of the surgical procedure on activity and willingness to explore in rats the open field test was used. This experiment puts the rat in a square box in a lit room, and allows the animal to roam and explore the novel environment freely for 5 minutes. The entire experiment is recorded using Ethovision, to determine how much time the animals spends at sides and centre of the box. The assumption is that less anxious animals are more eager to explore the box and the more anxious animals stick to the sides of the box.

Behaviour associated with anxiety is done scored using Eline. In this experiment ambulation, sniffing and rearing are used as markers for explorative behaviour while freezing and resting account for immobile behaviour. Apart from those, grooming is also scored.

Novel object and location recognition

After the open field test the animals are habituated to the box which was used to perform the novel object recognition test. Each animal got 2 rounds of habituation each lasting 3 minutes.

The day after the habituation they underwent the novel object test (*fig. 1a*). An animal was placed in the novel object box for 3 minutes. After the initial 3 minutes, 2 identical objects were introduced. 2 different sets of objects were used: A set of hand held plant sprayers and a set of blocks. The animal was exposed to the identical objects for 3 minutes. After 3 minutes both objects were taken out and cleaned with 70% ethanol. Then one of the old objects and a new one were introduced for another 3 minutes. After the 3 minutes of exposure to the novel object both objects were taken out and cleaned. The same objects were introduced again; however the "newest" object was moved to a novel location. Between rats the entire box was cleaned using 70% ethanol.

Novel object testing uses the animals' propensity to explore unknown objects, in order to asses memory capacity. If the animal can remember an old object it would be likely to explore the new one (*fig. 1ab)*. If it is unable to recall the old object it may spend similar amounts of time exploring both objects. The novel location test (*fig. 1c)* is a modification which attempts to test the animals' spatial memory (*Antunes et al 2012*).

Data collection was done by recording the sessions and manually scoring time spent on each object and on resting and grooming behavior. Ethovision could also have been used to gather data by recording the amount of time the animal spends in the area of the object. Because not all time spent around the object is also used to explore the object the method of recording the animals' location and manually scoring exploratory behavior was preferred.

When animals would spend too little time exploring either object they were excluded from data. Originally this was established at <5% of time spent on the objects, however upon data analysis this threshold would omit such amounts of animals that it would make analysis impossible. Therefore only animals who spent <4% of the 3 minute sessions exploring either objects were removed from the data set.

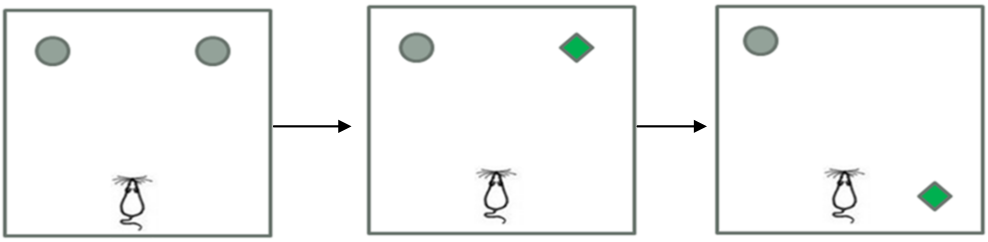

Fig 1, Novel object test for short term memory and novel location test for short term spatial memory.
1a, left, animal spends three minutes with 2 identical objects.
1b, middle, both objects are removed and cleaned, one object is replaced by a novel object.
1c, right, both objects are removed and cleaned

Morris water maze

The Morris water maze test is used to assess memory and memory flexibility. This is judged by the animals' ability to find a submerged platform in a round tub filled with water. The layout of the tub is as follows: the tub is dealt into quarters giving it four different zones, one of these zones contains the platform and the walls have four different icons posted up which could allow the animal to distinguish the different zones by the different icons. This experiment is divided into training phases and test phases and runs over the course of three days.

On the first day the animals are trained in finding the platform. Within one minute of time they are allowed to search for the platform starting in a random zone (except for the zone which contains the platform). When they find the platform they are allowed to remain on the platform for ten seconds, in case they are unable to locate the platform within the allotted 60 seconds they are guided towards the platform and remain there for 10 seconds. On the first day of training each animal gets 3 sessions consisting of 3 attempts to find the platform. The attempts are made from randomized zones. Analysis of training sessions is used to show the ability of the animals to learn the platforms location

On the second day of training each animal receives 2 training sessions consisting of 3 attempts to find the platform. Before these 2 sessions the animals are tested. During the test the platform is removed and Ethovision is used to measure the time spent in the zone which had previously contained the platform. The testing phase of the experiment is used to estimate the animals' capacity to remember the platform location.

On the third day the animals undergo a second test. After the second test they are subjected to one session of reversal training where the platform is placed on the opposite side of the original training sessions and the animals get 4 one minute attempts to find the platform. After the reversal training the animals are tested again. This is aimed at testing cognitive flexibility by testing the animals' ability to adapt to the new platform location

.Analysis

Comparison of groups was done by ANOVA. p<0.05 was deemed significant. To measure significance of effects over time during Morris water maze trials, repeated measures ANOVA was used. Post hoc testing was done using bonferroni.

During the analysis some of the video footage turned out to be blurry, although it did not make analysis impossible it did make it more difficult to distinguish between behaviour.

During the analysis of open field data no distinction was made between time spent in corners or time spent along the edge of the box.

Results

The open field test was performed to assess the levels of anxiety between groups. No differences were observed in time spent along the edges of the arena between control, anaesthesia and LPS groups (*fig 2a)*.

When examining the treatment groups the group tested one week after surgery showed a slight increase in the percentage of time spent along the edge of the arena. However, no significant differences were observed (*fig 2b*).

Neither the two or three week post-surgery groups nor the surgery animals treated with LPS(*fig 2c)* displayed any significant differences in the percentage of time spent along the edge of the arena when compared to healthy controls .

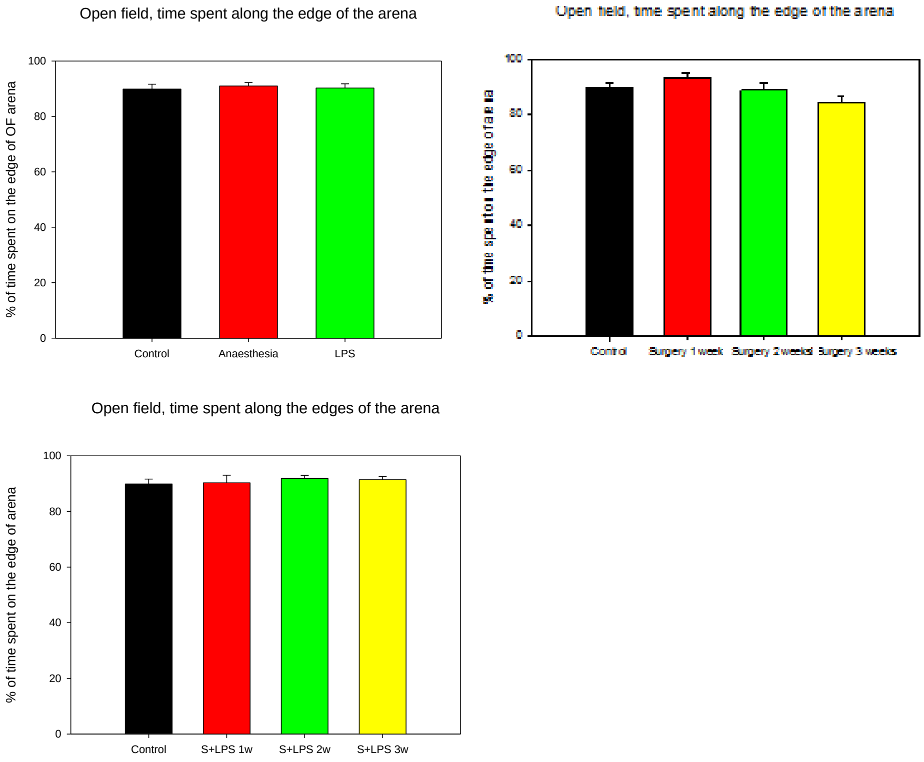

Fig 2.The open field test, bars represent average (SEM) percentage of time spent along the sides of the arena.
Fig. 2a top left, control (n=8) animals did not perform differently compared to LPS (n=8) and anaesthesia (n=8) treated animals.
Fig. 2b top right, control animals (n=8) compared to animals tested one, two and three weeks post surgery (n=8, n=7, n=8 respectively).
Fig. 2c bottom left, Healthy control animals(n=8) shown next to animals tested one, two and three weeks after abdominal surgery with subsequent LPS dose of LPS denominated in the graph as S+LPS(n=8, n=8, n=8) .

During the open field test, behaviour was also assessed. Anxiety was quantified as the cumulative score of immobile behaviour meaning: Freezing and scanning. There was a significant increase in anxious behaviour in the LPS group, one week post-surgery and one week post -surgery + LPS groups. These differences were not observed in the two and three week groups (*fig 3)*.

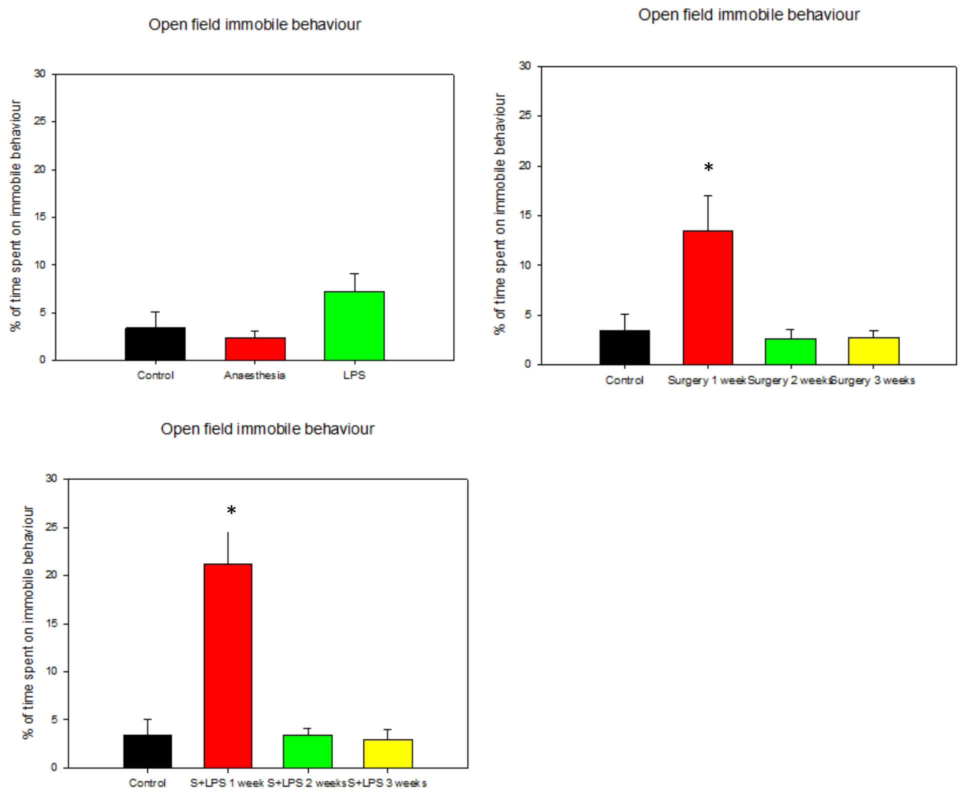

Fig 3. Behaviour in the open Field. Bars represent percentage of time spent on immobile behaviour.
Fig 3a, top left. LPS (n=8) animals show a significant increase in immobile behaviour compared to healthy controls (n=4). Anaesthesia (n=8) does not impact the behaviour significantly.
Fig 3b, top right. Animals tested 1(n=4) week after surgery displayed a significant increase in immobile behaviour compared to healthy controls (n=4). This difference is not observed in the two (n=6) and three week (n=7) groups.
Fig 3c, bottom left. During the first week after S (S+LPS) *animals show an increase in immobile behaviour.*

The novel object recognition and location tests were conducted to test memory and spatial memory. Trials with less than 4% (2,4 seconds) Total object interaction were excluded from the groups because these animals did not seem interested in the objects. When comparing the control, anaesthesia and LPS groups no difference can be observed in the habituation phase nor the novel object and novel location (*fig 4abc)*.

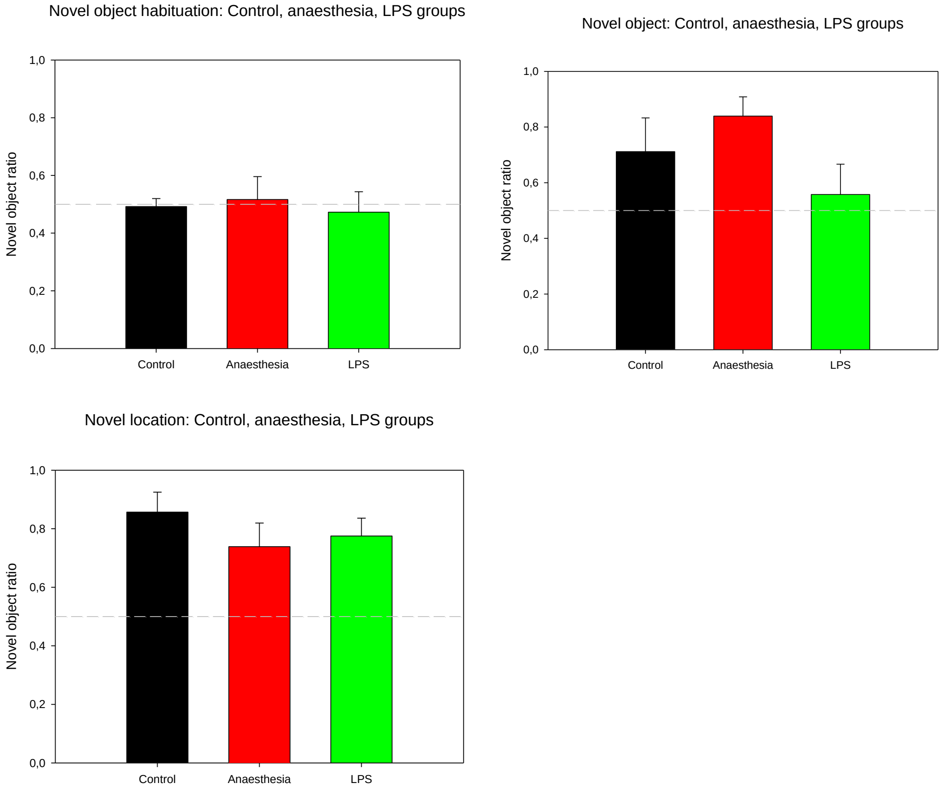

Fig 4. Novel object habituation, exploration and location data comparing control, anaesthesia and LPS groups. Novel object ratio is used which equals: time spent on novel object/novel+old object. Reference line placed at 0.5 which is the expected result from random chance.

Fig. 4a, top left. Object habituation, anaesthesia (n=6) and LPS (n=8) groups do not differ from control (n=6).

Fig 4b, top right. The novel object recognition shows no significant difference between the control (n=5), anaesthesia (n=5) and LPS animals (n=7)

Fig 4c, bottom left. The novel location test did not show a significant impact of the anaesthesia (n=5) or LPS (n=5) treatment compared to control (n=5)

In the case of the surgery groups there is no significant difference in the observed habituation period or novel object exploration. However, the novel location test displays a significant difference between the control group, and both the one and two weeks post-surgery groups ($p<0.05$ in both cases) (*fig 5abc)*.

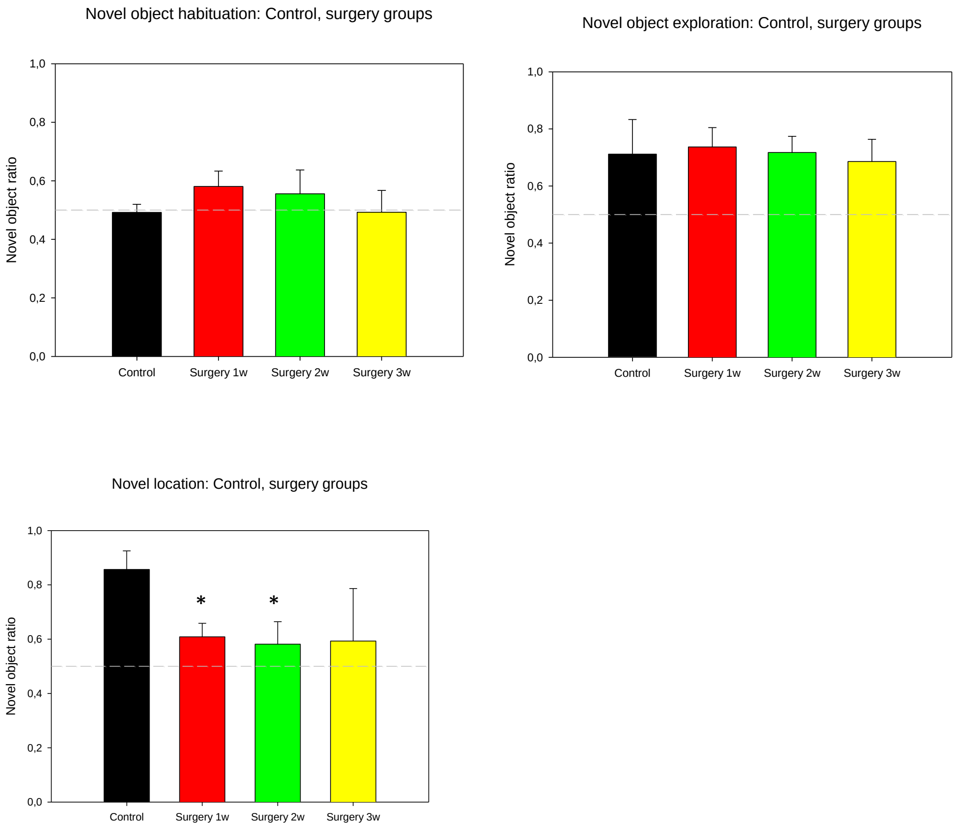

Fig 5. Novel object habituation, exploration and location data comparing the control and surgery groups using novel object ratio which equals: time spent on novel object/novel+old object. Reference line placed at 0.5 which is the expected result from random chance.

Fig 5a, top left. The object habituation shows no difference between one (n=8), two (n=6) and three (n=8) weeks post-surgery groups as compared to healthy controls (n=6).

Fig 5b, top right. In the novel object exploration the one (n=6), two (n=5) and three (n=6) week groups did not differ from the control group (n=5).

Fig 5c, bottom left. The one (n=7) and two (n=5) week post- surgery groups showed a significant decrease (p<0.05) in novel location ratio compared to control animals (n=5). Three (n=5) week groups did not show a significant difference.

The rats that underwent surgery followed by LPS did not differ significantly from each other (*fig 6abc)*. Although, the novel object exploration does show decreased levels of attention towards the novel object particularly in the two and three weeks post-surgery groups compared to healthy control animals (*fig 6b)*.

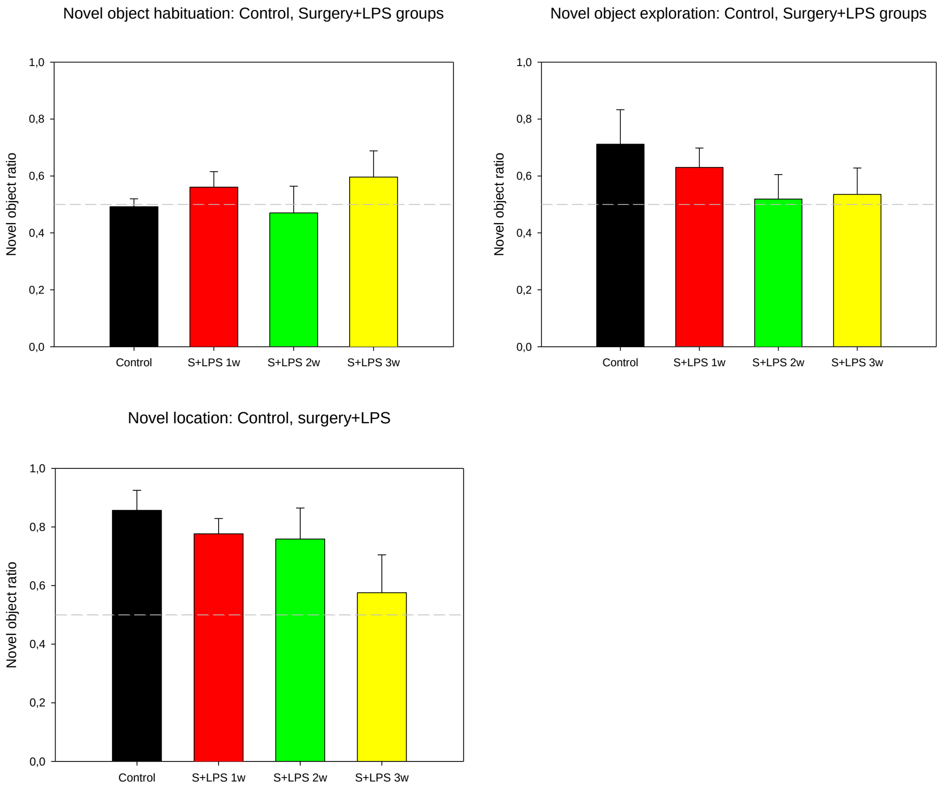

Fig 6. Novel object habituation, exploration and location data comparing the control and (S+LPS) groups using novel object ratio which equals: time spent on novel object/novel+old object. Reference line placed at 0.5 which is the expected result from random chance.

Fig 6a, top left. Object habituation data shows no significant difference between control (n=6) groups and groups tested one (n=8), two (n=7) and three (n=6) weeks post surgery and LPS treatment.

Fig 6b, top right. Novel object data shows no significant difference between the one (n=5), two (n=6) and three (n=6) weeks post surgery with LPS groups. Although LPS treated groups show less interest in the novel object compared to control (n=5) animals, they not differ significantly

Fig 6c, bottom left. Novel location test did not yield significant results when comparing the control (=5) group to one (n=5), two (n=4) and three (n=5) weeks post S+LPS animals. The treated animals do exhibit a lower level of attention towards the novel located object.

The animals showed an interest in the objects throughout all the tests, but when going from novel habituation to novel object to novel location. Their interest declined significantly when comparing the Total time spent with objects during the object habituation and the novel location test (fig 7).

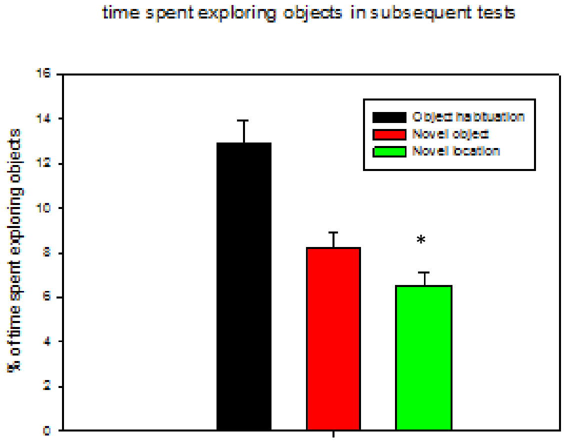

Fig 7. Animals displayed a significantly lower level of attention to the objects during the novel location test compared to the object habituation (p<0.01).

During the Morris water maze training, the animals were subjected to five training sessions. There were no significant differences between the control (n=8), anaesthesia (n=8) and LPS (n=8) groups. All the animals were able to learn the platform location as evidenced by a significant effect of the amount of training sessions on the time until reaching the platform for all groups (p<0.01)(*fig. 8*).

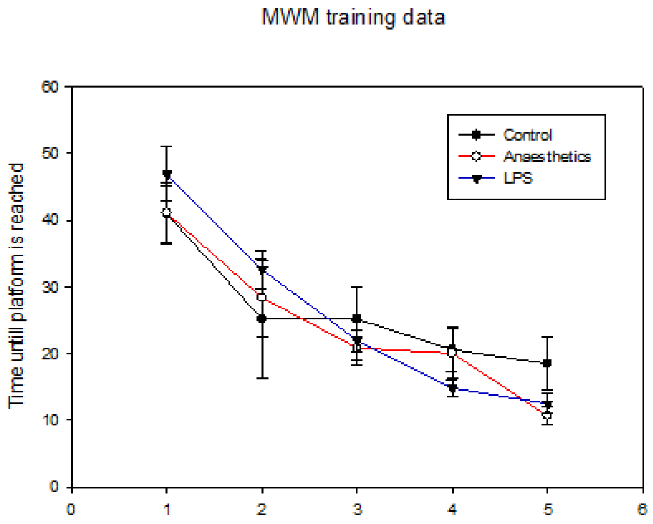

Fig 8. Data gathered from the Morris water maze training showed no effect of treatment when comparing the anaesthesia (n=8) and LPS (n=8) groups to control animals (n=8). All groups showed a significant decrease in their escape latency (p<0.01)

When comparing the control groups (n=8) to animals tested one (n=8), two (n=8) and three (n=8) weeks post-surgery, there is a significant difference (p<0.05) in performance during the second training session with regards to the control and one week post-surgery groups. As with the previous groups the animals who underwent surgery were able to learn the platforms location as their time to reach the platform decreased significantly (p<0.01) (*fig. 9).*

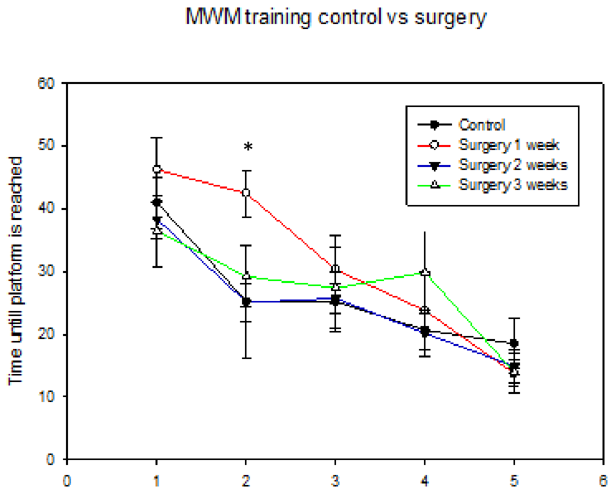

Fig. 9 Morris water maze training data comparing the control animals to animals tested one (n=8), two (n=8) and three (n=8) weeks pos surgery. Animals tested one week post surgery took significantly longer to find the platform during the second training (p<0.05) compared to control. All groups improved escape latency significantly from the first till the fifth training session (p<0.01).

The animals treated with LPS after surgery showed no significant difference when comparing the control group (n=8) to one (n=8), two (n=8) and three (n=7) week post surgery with LPS groups. As with the other treatment groups, abdominal surgery coupled with LPS did not impair the animal's ability to learn the platform location. (*Fig. 10)*

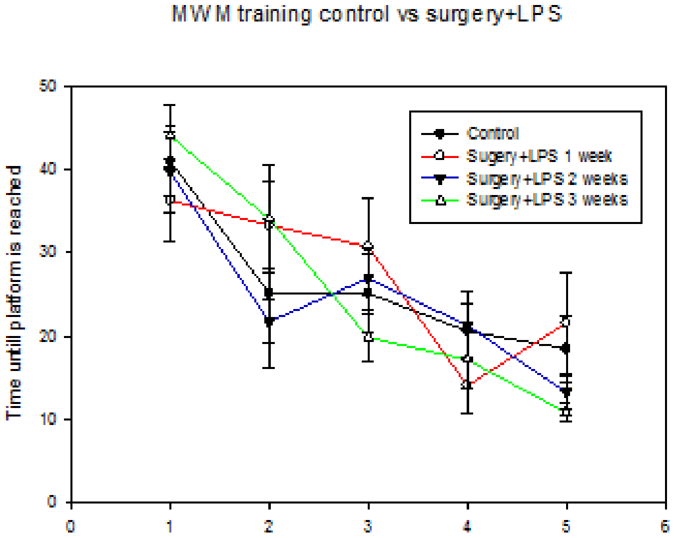

Fig. 10 Morris water maze training data comparing the control group (n=8) to the animals who underwent abdominal S+LPS and were tested one (n=8), two (n=8) and three (n=7) weeks afterwards. No significant difference was noticed between the treatment groups but the animals were able to improve their time significantly (p<0.01).

When assessing whether or not the animals have learned the location of the platform the percentage of time spent in the zone the platform was in during the training is used (zone 4+5). Animals who underwent anaesthesia (n=8) or LPS (n=8) did not differ significantly from control (n=8) animals with regards to the percentage of time spent in the correct zone (*fig. 11).*

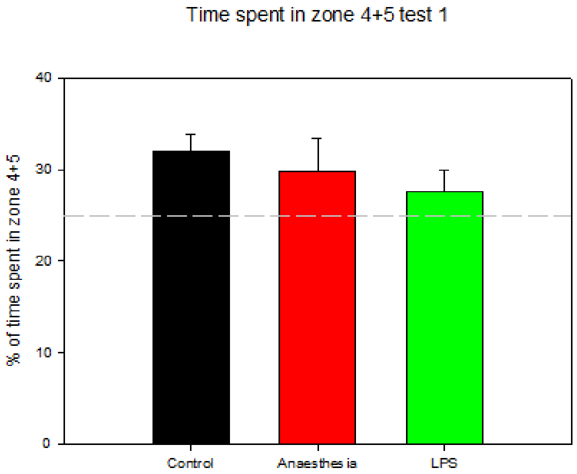

Fig. 11. Anaesthesia (n=8) nor LPS (n=8) animals showed a significant difference in the time spent in the correct zone during the First test compared to the control group (n=8).

When comparing the control group to the one(n=8), two(n=8) and three(n=8) weeks post surgery groups, there is a significant decrease in performance between control and both the one and two week groups which is not observed in the three week groups.(*fig 12)*

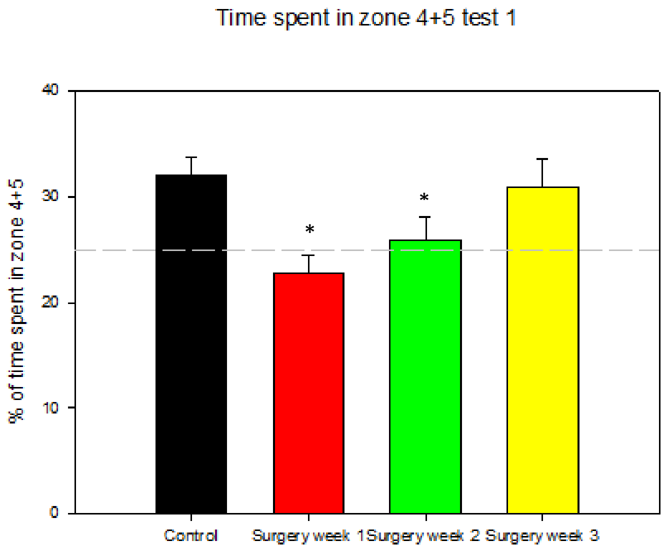

Fig. 12 Animals test one (n=8) and two (n=8) weeks after surgery performed significantly worse (p<0.05) than control (n=8) animals when it came to remembering the platform location on the first test session. After three weeks (n=7) the difference is gone

In the surgery with LPS groups the animals tested two weeks(n=8) after surgery spent significantly less time in the correct quadrant compared to controls(n=8) ($p<0.05$) the one(n=8) week group also spent less time in the correct quadrant but this difference was not significant(*fig. 13*).

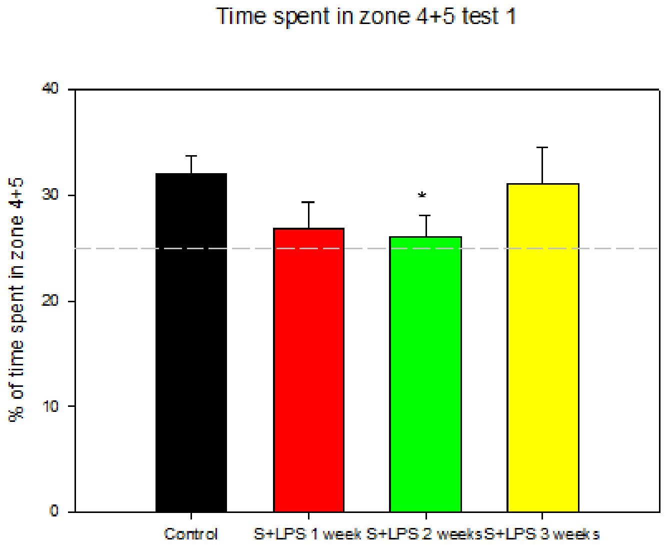

Fig. 13 Control, one (n=8), two (n=8) and three (n=7) weeks post S+LPS groups. The two weeks group showed a significant decrease ($p<0.05$) in performance compared to control.

During the second test anaesthesia or LPS groups did not differ from control animals (*data not shown*). When comparing the one, two and three weeks post surgery groups there is a significant increase in performance by the 2 weeks post surgery group.

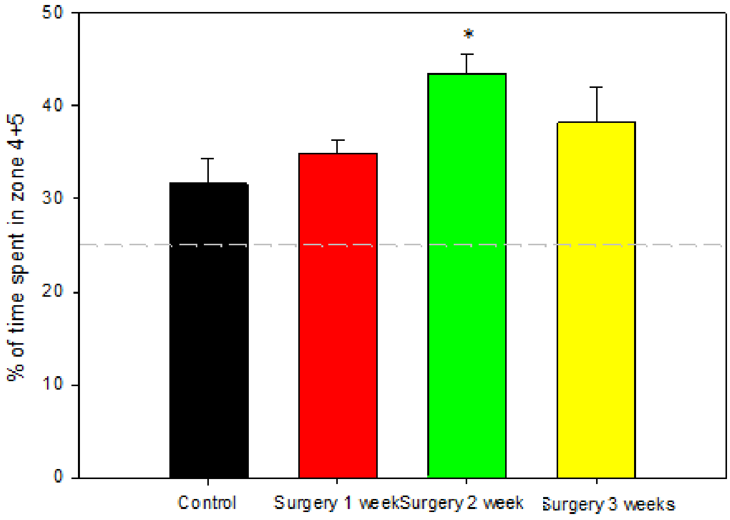

Fig. 14 Time spent in the correct quadrant during the second test. Animals tested two weeks after surgery showed a significant increase in performance (p<0.05).

The animals who underwent abdominal surgery with LPS performed somewhat better than controls however no significant differences were observed (*data not shown)*.

The last part of the Morris water maze experiment was the reversal test aimed at memory flexibility. The animals show no capacity to adapt to the new location of the platform (*data not shown)*.

To monitor the animals' health they were weighed daily for the full duration of the experiments, if they would lose more than 15% of their weight that would be considered too much. Minimum weights were compared to a 100% baseline. Although all animals who underwent abdominal surgery lost weight none of them came close to 15% body weight loss (fig 13).

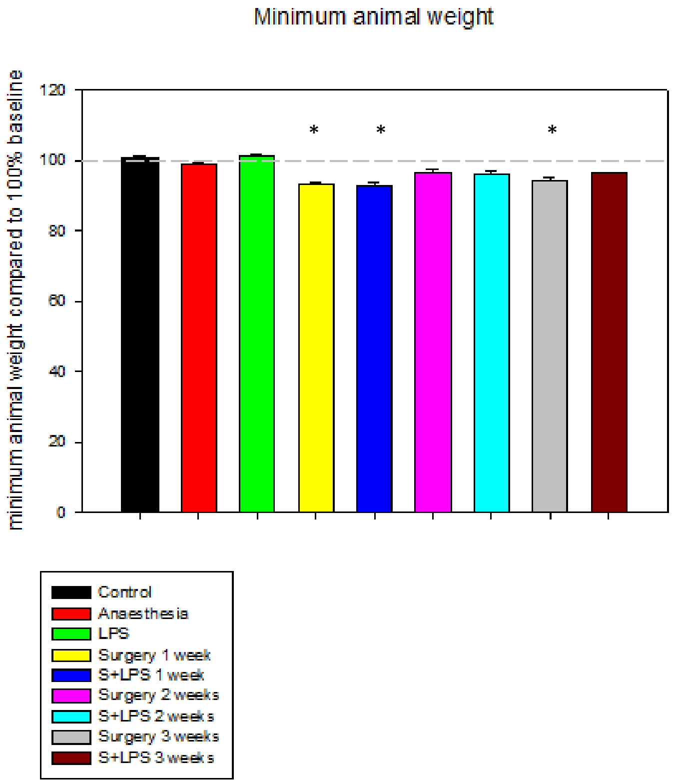

Fig. 15 Minimum animal weight as a percentage of baseline weight. Some animals lost weight, however none of them reached the critical level of 85%

Discussion

The aim of this study was to look into the short term effects of POCD. This was done by performing abdominal surgery on rats and subsequently subjecting them to behavioural tests. The open field test did not show any significant result with regards to the animals' behaviour. Novel object location data showed a significant decline in interest in the relocalized object in animals tested one and two weeks after surgery compared to controls. The Morris water maze training did not show an impaired ability to learn the location of the platform though the group tested one week after surgery did perform worse compared to control animals during the second training session. During the first Morris water maze test the animals tested one and two weeks post surgery performed significantly worse than control animals when it came to remembering the platform location. This difference was also present in the animals that underwent surgery with subsequent LPS, but was only significant with regards to the animals operated two weeks after surgery. Surgery did induce some derogatory effects on the animals' weight implying that there was some degree of impact on the animals' condition, In none of the conducted experiments the data indicated a difference between control, anaesthesia and LPS animals. This is in line with literature on cognitive function with regards to these compounds (*Kilicaslan et al 2013)*. The sevoflurane anaesthesia alone would not be expected to affect the animals' health (*Peng et al 2012, Eberspächer et al 2009*). And the dosage of LPS was chosen such that on its own it would not affect the animals' performance. When LPS was added to surgery this again did not produce any significant effects, this might indicate that the dosage was too low. To further extend the control groups it would be possible to create anaesthesia with LPS group in order to assess their combined effects.

Open field data

When looking at the data gathered from the open field test there seems to be some increase in the time spent along the edge of the box in all groups involving surgery. This could mark an increase in anxiety, however none of the groups showed a significant increase. Increased levels of anxiety could imply the presence of a depression like state, after surgery anxiety and stress could occur and stress has been linked to the release of cytokines which could play a part in the mechanism behind POCD (*Yirmiya et al 2011*, *Gomes et al 2012,boufleur N et al 2012, Glenn MJ et al 2012)*.

_In the behavioural data there is an observed increase in immobile behaviour in the open field but this change has disappeared by the second week after surgery. Unfortunately the video recording for some of the open field behaviour data were too blurry to make out detailed behaviour but it was still clear enough to observe the animals' location and movements

Novel object data.

The novel object test leaves more to be discussed. First off there were 36 data points which were omitted. This was even the case when only excluding animals that spent less than four percent of their time investigating the object, whilst the original plan was to exclude all animals that spent less than five percent of their time doing so. This implies a diminished interest in the objects when the habituation, novel object and novel location tests are performed in succession.

Because of the number of animals who did not spend enough time interacting with the objects it may be interesting to look at the total percentage of time spent with both objects across all three tests. Looking at this data there is a significant decrease in interest when comparing the habituation phase to the novel object phase ($p<0.01$). This is to be expected because at the point of habituation the animals are unfamiliar with both objects, but the total interest also decreases from novel object to novel location tests. Although this decrease is not significant ($p=0.06$), this might indicate that after spending 6 minutes with two objects and 9 minutes roaming the box there is a decrease in curiosity towards the objects. This could skew the data because of the reduced group size and thus lower statistical power.

In the test data there is no significant difference between the groups during habituation or novel object introduction. But when the novel location test was performed a decrease in the interest towards the novel located object was observed when looking at the one and two week post surgery groups. This could be the sign of impaired spatial memory on the short term after surgery (Bello-Medina et al 2013). The three weeks after surgery group did not exhibit a significant difference from control animals, however the standard error in this group is very high. This means a larger variance in the group when it comes to the animals' interest in the novel location. This also holds true for patients afflicted by POCD. Even though most people recover within months, there are some who suffer from long term cognitive decline (*Lombard et al 2010)*.

These results are intriguing because they give insight into the animals' spatial memory performance which is linked to the hippocampus (*Pilly et al 2012).* Other research also found impaired spatial memory but these findings were limited to short term effects (*Lin et al 2011, Wang et al 2012*). This would therefore make the data suitable to compare to e.g. an immunohistochemistry staining, to check for microglia activation (e.g. IBA-1) (*Nakamura et al 2013*) or protein analysis from areas within the hippocampus to monitor the activity of cytokines post surgery (e.g. il-1 and il-6).

A way to remedy this may be to use a design more akin to *Jacoby et al 2012.* In this study the animals were introduced to the objects and allowed to explore for 5 minutes similar to our design, but they ran the setup again 24 hours later and switched the object locations whereas we switched the objects immediately during the same day. Maybe a one day interval between object introduction and presenting the object at a novel location would be enough to revitalize the animals' eagerness to explore the objects. The downside to using a design like this is that it tests the animals' memory performance over a longer period of time and thus long term memory, in contrast to the design used in this study which tests short- term memory.

Another problem concerning the novel object recognition test is the manner in which to collect the data. The data shown was collected by hand which incurs problems with observer bias. Although the only solution to this is to have guidelines to help determine whether an animal is exploring or not (*Grayson et al 2007*) it would still be difficult for the observer to claim to be free of bias.

Morris water maze data

The Morris water maze training showed that all treatment groups were capable of finding the platform and increasing their performance over the period of five training sessions. The animals that were tested one week post surgery performed worse than control animals at the point of the second training session. The groups who underwent abdominal surgery with LPS did not differ significantly compared to control.

The second part of the Morris water maze test which tested how much time the animals would spend searching for the platform in the correct quadrant showed that there is a significant decrease in performance for as long as two weeks after surgery. In groups which were treated with abdominal surgery and LPS there was a decrease in performance during the first week, but this was not significantly worse than control animals and are similar to surgery only groups. This could mean that the LPS dosage was insufficient. The two weeks after surgery with LPS group did show a significantly worse performance than control. But this effect did not persist through animals tested three weeks after surgery. These results show there are short term but non persistent effects of surgery on memory.

On the second test the animals tested one week after surgery performed unexpectedly well, significantly better than control animals. However when comparing the first and second test the control animals do not seem to be improving over time while the treated animals do. A possible explanation for this could be that the treated animals needed more time to learn the test and improved their performance at a slower pace compared to control animals which might have learned the procedure to reach the platform in the first test.

Finally, the reversal test showed no difference in the ability to adapt to the new location of the platform between treatment groups, in fact none of the animals were able to learn the new location of the platform which is why the data was not shown. The inability to learn the test may indicate that the animals were unable to perform the test with limited training.

Conclusion

In summary, the longest cognitive effects found were 2 weeks after surgery in the treated groups. When LPS was paired with the surgery, however we did not find the expected results. We expected the LPS to simulate complications and maybe affect the animals' performance in some way. During the tests this did not seem to be the case. Although the surgery with LPS groups did perform significantly worse than control in some tests, they did not seem to be performing any worse than the regular surgery group. The experiments described were able to show some cognitive effects but not to the extent that would be expected from an affliction like POCD. This is also hindered by the prevalence of POCD. In humans the estimates vary from 10 to 40% of patients (*Hartholt et al 2012, Coburn et al 2012*). Therefore it would be optimistic to believe that all animals who have been operated on are also affected by POCD. A second difficulty when looking at short term effects is the separation between POCD and the effects of a delirium after surgery, because both induce similar effects but delirium occurs short term whilst POCD persists for a longer period of time(*Krenk 2012 et al*).

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