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Surgery under Propofol Anesthesia Induced Behavioral Changes Associated With Increased Cerebral Apoptosis in Rats

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Abstract

It is not known whether prolonged propofol anesthesia and/or surgery are responsible for postoperative cerebral deterioration, including Alzheimer-like histological changes, apoptosis and cognitive dysfunction. Therefore, the aim of the present study was to use partial liver resection as a surgical rat model in middle-aged rats in order to distinguish postoperative cerebral changes brain from those in rats after propofol anesthesia without surgery.

In this randomized, controlled study, behavioral changes were investigated in n=36 rats (12- to 14-month-old) using the hole board test system and Morris water maze. Cerebral glycogen synthase kinase-3ß (GSK-3ß) and tau protein were analyzed using ELISA technique. Cerebral amyloid was determined using congo red staining with subsequent fluorescence analysis. Apoptosis in rat brain was analyzed using TUNEL test and caspase-3 immunhistochemistry.

Alzheimer-like specific histological markers were not markedly increased up to 28 days after propofol anesthesia without partial hepatectomy. In contrast, propofol combined with partial liver resection caused long-term deterioration in spatial cognitive behavior in the rats. These postoperative cognitive dysfunctions were associated with pronounced cerebral apoptosis and increased GSK-3ß.

We conclude that a surgical procedure in the form of partial hepatectomy, but not propofol anesthesia alone, induced persistent cognitive deterioration and increased apoptosis in middle-aged rats. Although apoptotic changes seem to be mediated via GSK-3ß, further studies are now necessary to investigate the underlying mechanisms and other potential pathogenetic factors for postoperative cognitive dysfunction.

Keywords: Postoperative cognitive dysfunction; Propofol anesthesia; Partial hepatectomy; Apoptosis; Alzheimer-like markers; GSK-3ß

Abbreviation: AD: Alzheimer's Disease; APP: Amyloid Precursor Protein; GSK: Glycogen Synthase Kinase; I.V.: Intravenously; MAPB:Mean Arterial Blood Pressure; PLR: Partial Liver Resection; POCD: Postoperative Cognitive Dysfunction; S.C.: Subcutaneously

Introduction

Postoperative Cognitive Dysfunction (POCD) is a common complication after major surgery, the underlying cause of POCD seems to be multi factorial and is unknown [1]. POCD affects different domains of patients' cognition, including verbal memory, language comprehension, abstraction ability, attention, and concentration, all of which show a high prevalence among the elderly [2].

Little information exists whether prolonged anesthesia alone or in combination with a surgical procedure (minor versus major) can induce or may trigger transient and persistent cognitive dysfunctions. Surgical stress and postoperative pain after tissue injury are known to modulate immune responses in patients undergoing surgery [3-5]. Otherwise, due to their lipid solubility, anesthetics enter the brain readily in high concentrations, dissolving into cellular membranes, penetrating organelles, and acting on cerebral receptors, second messenger systems, ion channels, and cytoskeletal components [3,6]. Until recently, it has been assumed that effects of anesthetics are reversible and non-toxic. However, a growing body of laboratory evidence suggests that anesthetics might induce cognitive dysfunction [7-9] and might be neurotoxic, especially in aged brains [10,11]. The aged and stressed brain often suffers from loss of neurons, reduced rates of neurogenesis, and synaptogenesis and accumulation of potentially toxic agents [12]. Thus, neural insults, such as oxidative stress or toxin exposure, can cause functional impairment owing to the reduced synaptic plasticity and increased vulnerability in the aged brain [13].

There is also a growing interest in the potential relationship between general anesthesia application and progression of Alzheimer's disease (AD), a mainly age-related disease. Indeed, AD patients appear to be particularly at risk of cognitive deterioration following anesthesia, and some studies suggest that exposure to anesthetics may increase the risk of AD [14].

Glycogen Synthase Kinase (GSK)-3ß is an important enzyme, which is changed e.g. during AD and can link the cerebral amyloid and tau cascades [15,16]. Furthermore, GSK-3ß is an important enzyme in neural systems, affecting gene expression, neurogenesis, synaptic plasticity, neuronal death, and survival [17]; the activation of GSK-3 has been described as being pro-apoptotic [18,19].

Among the anesthetics, propofol is an intravenous hypnotic agent which is widely used for the induction and maintenance of anesthesia. Interestingly, in vitro propofol application seems to accelerate tau

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hyperphosphorylation [20] and to interact with Abeta protein [21]. The effect of propofol on apoptosis is the subject of controversial discussion and seems to be dosage- and age-dependent [11,22,23]. Overdose propofol treatment over a prolonged period injures multiple cell types; however, the molecular mechanisms underlying this injury are still not known. In contrast, it was shown that propofol did not change APP protein and APP mRNA concentration in rat brain [24]. Given these conflicting results, the conditions under which propofol exposure results in neurodegenerative changes and cognitive impairment are still elusive. In addition to anesthesia, a surgical intervention is a stressful event which itself may represent a risk factor for cognitive dysfunction [25].

Therefore, the aim of the present study was to use a rat model of partial hepatectomy to distinguish postoperative cerebral changes relating Alzheimer-associated elected characteristics (i) after propofol administration and surgery from those with (ii) propofol alone and to compare these changes with (iii) a control group without surgery and without propofol. In order to simulate a typical middle-aged patient, studies were performed in 12- to 14-month-old rats.

Materials and Methods

The experimental protocol for the studies was approved by the appropriate review committee of the Medical Faculty of the University of Heidelberg (Germany) and complied with the guidelines of the responsible government agency and with guidelines laid out in the Guide for the Care and Use of Laboratory Animals.

Pilot study

Owing to the wide range of different rat propofol concentrations reported in the literature, a pilot study was conducted to investigate the effect of prolonged propofol anesthesia on Mean Arterial Blood Pressure (MABP) and arterial blood gases in the rats.

To measure MABP changes during a 2-hour propofol infusion (Harvard apparatus, Hugo Sachs Elektronik, March-Hugstetten, Germany) the Arteria femoralis and Vena jugularis were prepared (<8 min) in n=10 rats that were under sevoflurane (2.5 vol%, and nitrous oxide/oxygen, 70:30) using a rat-adapted mask. For propofol infusion, a longitudinal incision of about 1.5 cm was made in the right jugular vein to insert the catheter. Sevoflurane anesthesia was then stopped and propofol infusion started for 2 hours. Propofol (disoprovan®, 2%, Fresenius Kabi, Bad Homburg v.d.H., Germany) was administered at 40 mg kg-1hour-1 (constant rate: 0.02 ml/min) [20,26,27]. In all, about 2 ml of 2% propofol was given.

Decreases in MABP were monitored every 5 min after starting the propofol infusion. Blood gas analysis (pH, pCO_2, HCO_3-, base excess, hematocrit, hemoglobin, oxygen saturation) included glucose concentration measurement (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany) at the beginning of experiments and after 2 hours of propofol infusion. Any decreases in MABP and blood volume were compensated by administering about 2 ml propofol and an additional 2-3 ml of 0.9% NaCl solution.

Body temperature was measured rectally and maintained at 37˚C over the observation period using a heating blanket.

Main study

The aim of the main study was to investigate the short- and long-term effect of prolonged propofol anesthesia and of surgical intervention (partial hepatectomy) on rats' cognitive behavior and on neurohistological markers.

Animals

The studies were performed on adult 12- to 14-month-old male Wistar-HAN rats (JANVIER, Elevage Janvier, Le Genest St. Isle, France) weighing 663 ± 50g. They were housed alone in one cage in a temperature-controlled room at 22 ± 0.5˚C with a reversed day-night cycle (12 h:12 h, light on at 7 p.m.), and psychometrically tested during the dark period. Free access to food (Altromin, standard no. 1320, Lage, Germany) and water was allowed throughout the experimental period.

Anesthesia and surgery

Propofol anesthesia was performed as described for the pilot study. In addition, all rats received buprenorphin (temgesic®) in a concentration range of 0.05- 0.1 mg/kg s.c. 5 minutes before the end of the infusion / experiments for postoperative pain prevention.

Placebo rats were injected in the same way with sterile vehicle control for propofol (intralipid, Sigma RBI, St. Louis, MO). In these animals, a special catheter (30-cm silicone, which is connected to a polyethylene catheter) was inserted in the vena jugularis which, via a special rotation system. This catheter system made it possible to move the rats into their cages for 2 hours of placebo treatment.

After an experimental period of 120 min, catheters for propofol i.v. infusion and placebo were drawn, vessels were closed, wounds were disinfected and the rats were placed back into their cages. Rats remained under observation for 1 hour after surgery to exclude agitated reactions or pain.

Partial hepatectomy was performed in accordance with studies [28,29] under short-lasting sevoflurane (3.5 vol%, and nitrous oxide/oxygen, 70:30) using a rat-adapted mask. In the surgical procedure, the abdominal wall and peritoneum were opened and the inferior left lateral lobe, which accounts for about 3-5% of the total liver weight, removed after clamping and ligating the narrow pedicle with a 6-0 prolene suture. After placing the clamp around the base of the respective lobe, the liver tissue was dissected just above the branches of the instrument. Thereafter, the bleeding was stopped, the clamp was released, and the lesion was sutured.

The aim of the partial liver resection was to imitate a typical, but minimal, surgical intervention without any organ-specific deterioration such as changes in liver metabolism. Other surgical models, such as e.g. fracture fixation, are not adequate for the aim of the study because rats should be swim after surgical trauma.

Experimental scheme

To minimize rats' stress reaction to water maze testing, all animals were handled daily by the examiner (J.S.) for 1 week before the psychometric experiments began (Figure 1).

After a 5-day handling period, a total of 36 rats were randomly allocated to 3 groups with n=12 each: (I) controls (without partial liver resection surgery and with placebo infusion), (II) propofol group (anesthesia group), and (III) Partial Liver Resection (PLR) under propofol (surgery group).

Psychometric re-testing was performed on postoperative days 1, 3, 7, and 28, analogously to the psychometric training procedures performed before placebo/propofol anesthesia and partial liver resection. Rats' body weight was measured over the whole experimental period.

Psychometric Investigations

Modified holeboard testing

Non-associative spatial habituation to a new environment was evaluated using a modified holeboard test. A defined holeboard box (square closed-field area 70×70×40 cm) contained 16 holes in a 4x4 array on a flat surface was used. Each hole contained a metal cup (2 cm in diameter, 1.5 cm deep) which had a perforated bottom. The rats were placed into the starting box (20×20×20 cm) and allowed to enter the testing area to explore the holeboard. A trial started when the door was opened and ended after 5 min. Two consecutive trials were conducted on one day. After each trial, the box was cleaned with ethanol and water. The latency time (time from the start of the experiment until the visit of the first rewarded hole), the number of nose pokes and the total distance moved during the 5-min interval were determined for each rat by the AKS registration system (TSE Systems, Bad Homburg, Germany). This system measures the movement of the rat by registering contact with an infra-red light barrier in each hole.

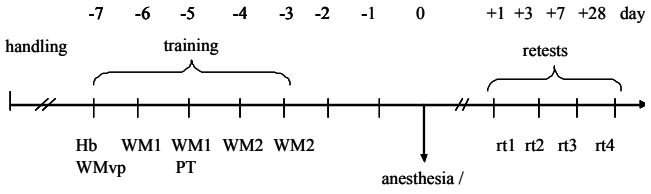
Morris water maze

The Morris water maze was used to assess rats' spatial cognitive abilities [30] before the injection period (training and testing) and after 3 days of injections (re-testing). All psychometric investigations were performed in a randomized order between 11 a.m. and 3 p.m., as has also been described in detail in previous studies [31,32].

Morris water maze procedure: Finding the platform was defined as staying on it for at least 4 s before the time frame of 75 s ended. Thereafter, rats were returned to their home cage, which was provided with a heat lamp for warming up and drying off. If the rat failed to find the platform in the allotted time, it was placed onto the platform for 20 s and assigned a latency of 75 s. between one trial and the next; the water was stirred in order to erase olfactory traces of previous swim patterns. The entire procedure took 5 consecutive days, each animal having three training trials per test and day, with a 30- to 40-min inter-trial interval.

Visible platform (cued trial) and training phase: The cued trial (three trials per rat) was performed on the first day to assess the rats' motivation to escape from the water and to evaluate their sensor-motor integrity. The platform was placed in the north quadrant and had a black and a visible cue (yellow striped flag at the top, 13 cm high, 3 cm in diameter, visible platform test). For the following two days (six trials, hidden phase), the platform was also located in the north quadrant, where it was now hidden from view. If the starting point and platform location were constant, the test was defined as WM1.

To compare learning abilities between the different animal groups in more detail, a more difficult learning paradigm was applied in which the platform location (east, south and west) was changed and equidistant starting points (west, north and east) employed. Thus, six trials with a hidden platform and changing positions were performed on two consecutive days (WM2).



Abbreviations: Hb: Holeboard; WM: Water Maze; vp: visible platform; PT: Probe Trial, rt: re test: Retest were performed on days 1, 3, 7, and 28 after surgery and include Morris water Maze testing for not visible platform with constant platform location with following probe trial and variable platform locations. Probe trial was performed only once, the mean of 3 trials per rat and per test was taken for retesting.

Figure 1: Experimental scheme.

Re-test: To compare memory abilities between the different experimental groups, a re-test with hidden constant (WM1) and hidden variable (WM2) platform positions was performed on the first postoperative day and on days 3, 7, and 28 after propofol and/or surgery. The mean value of three trials was calculated.

Probe trial: A probe trial was administered directly after the testing with the hidden platform in a constant position north with constant starting positions. Each rat was placed into the water diagonally from the target quadrant and for 75 s was allowed to search the water, from which the platform had been removed. The time spent searching for the platform in the former platform quadrant (north) was measured for each rat.

Final experiment

At the end of the 28-days experiments, arterial blood samples (0.5 ml) were taken in heparinized syringes under 3.5 vol% sevoflurane anesthesia and nitrous oxide/oxygen (70:30) and the glucose concentration was measured before the rats were sacrificed. Rats were sacrificed by decapitation in deep anesthesia under 3.0 vol% sevoflurane and nitrous oxide/oxygen (70:30). The time from the induction of anesthesia to decapitation was exactly 2 min in each rat. Brain was rapidly removed. In three rats per group one hemisphere was used for histological investigations the cerebral cortex and the hippocampus from the remaining hemisphere were used for biochemical analysis. Tissue was frozen in isopentane for 5 min. Until further biochemical and histological analysis, the rat tissue was stored at -80˚C.

Biochemical determination

For biochemical analysis, respective brain samples were homogenized by Potter homogenization in ice-cold lysis buffer, pH 7.4, comprised of 10 mmol/l Tris-HCl, 150 mmol/l NaCl, 1 mmol/l EDTA, and 1 mmol/l EGTA containing protease inhibitors (AEBSF 2 mM, aprotinin 0.3 µM, bestatin 130 µM, EDTA 1mM, E-64 14 µM, and leupeptin 1µM; Sigma, Munich, Germany). The lysates were centrifuged at 20,000 x g for 10 min at 2˚C to remove insoluble debris. Protein concentration in the supernatant was determined in triplicate using the Bradford protein assay.

Sandwich ELISA technique: The content of total and phosphorylated GSK-3beta was measured using the DuoSet IC ELISA (R&D Systems GmbH Wiesbaden, Germany).

To detect tau protein (total and phosphorylated) in rat brain, commercial tau ELISA kits were used (E02T0024, E02P0014, Shanghai BlueGene Biotech Co., Ltd., Shanghai, China). These kits are solid-phase, sandwich Enzyme-Linked Immuno-Sorbent Assays (ELISA) for quantitatively determining in vitro tau in brain using polyclonal antibodies as described by the manufacture. Thus, both ELISA assays were performed in accordance with the manufacturer's instructions.

Histological analysis: Histological investigations were conducted 28 days after partial liver resection and propofol infusion.

Cerebral amyloid: Cryoslices (6 µm) were made from rat brain hemispheres (Leitz 1720 Cryostat Microtome, Wetzlar, Germany). Modified protocols of haematoxylin and Congo red staining [33] were performed. The sections were coverslipped and observed under a

light and polarization microscope (OLYMPUS, Hamburg, Germany) at various magnifications (10x-400x) in pre-defined rat brain areas (subventricular zone, the basal ganglia, hippocampus and cerebral cortex).

Furthermore, slices were analyzed by fluorescence microscopy (Zeiss axioscop, Jena, Germany) according to red amyloid staining intensity using IMAGE J (version 1.45).

Apoptosis

Six-µm-thick cryoslices (n=6 per group) were analyzed for apoptotic changes using a TUNEL assay [FragEL TM DNA Fragmentation Detection Kit (QIA33 -1EA), Calbiochem, Merck Biosciences, Darmstadt, Germany]. Testing was conducted according to the manufacturer's instructions.

Caspase-3 staining was performed according to the manufacturer's instructions using monoclonal rabbit-cleaved caspase-3 antibody (ASP 175, 5A1E, #9664, 1:200, Cell Signaling Technologies, Danvers, MA USA). A respective negative control was performed in the same way but without antibody. As secondary antibody the HRP-conjugated goat anti-rabbit antibody (Dianova GmbH, Hamburg, Germany, and 1:1000) was used. All slices were stained with diaminobenzidine (DAB).

Apoptotic cells and caspase 3-positive cells were counted with a light microscope (Olympus BX43, 100-400x enlargements) in pre-defined tissue areas by an investigator blinded to the experimental conditions by using digital imaging software SpotBasic™ (Sterling Heights, MI, USA). Therefore, in hippocampal (-3.36 mm from bregma) and striatal layers (0.36 mm from bregma) apoptotic cells were counted in 10 (5 for each brain hemisphere: striatum, CA1-CA4 hippocampal areas, frontal cortex, parietotemporal cortex) different and predefined fields of interest for brown positive cells under a 200-fold magnification. Respective cell counts from all fields of interest were summarized and differences between the groups were determined using two-tailed, non-paired, Student's t-test. P-values<0.05 were considered statistically significant.

Statistics

Assuming a physiological important difference of 15% in Water Maze parameters, ≥10 rats were presumably required for each group of this study (alpha=0.05; 1-beta=0.8). Under this condition, a cohort of 10 rats per group would yield an 80% power to detect the aforementioned group difference escape latency or distance using LSD test at the 5% level of significance.

All measurements were performed by an independent investigator blinded to the experimental conditions. Results in tables and figures are expressed as mean (standard deviation, SD) or ± Standard Error of Means (SEM). Differences within or between normally distributed data were analyzed by analysis of variance (ANOVA) using SPSS 19.0 version (IBM, Chicago, USA) followed by post-hoc LSD-Fisher test. Statistical significance was set to the P<0.05 level.

Behavioral variables from the Morris water maze test were quantified with the EthoVision XT8.5 software (Noldus Information Technology, Freiburg, Germany), and mean values for each trial were calculated for each subject. The following parameters were calculated: (1) escape performance by time to find the platform (TTP=escape latency in seconds), (2) distance moved in the basin (path length in dm) during a trial, and (3) the swimming velocity (cm/sec).

For statistical analysis, the effect of training before and recovery after intervention on the results of the water escape task was assessed by using ANOVA with the repeated measure factor sessions (number of days). During re-testing, data in rats' acquisition of the water escape task was analyzed by using two-way ANOVA with factors 'surgery' and 'time'. For probe trial analysis in the water maze, within-group differences in the time spent in the north quadrant were analyzed by means of one-factor ANOVA followed by post-hoc LSD-Fisher test.

Results

Pilot study

After 2 hours of propofol infusion, the MABP was significantly (P<0.001) reduced from 109 ± 8 mmHg to 62 ± 4 mmHg. This reduction was associated with the following blood gas changes: pH: 7.28 ± 0.03; pCO_2: 60.7 ± 0.2 mm Hg; pO_2: 81.2 ± 13.2 mmHg; HCO_3-: 26.2 ± 1.6 mmol/L; Na+: 136 ± 7 mmol/L; K+: 4.0 ± 1.4 mmol/L; hematocrit: 40.3 ± 2.9; and glucose: 69 ± 14 mg/dL. Thus, propofol induced hypercapnia that was associated with blood acidosis.

Main study

In all, four rats died within 24 hours after the intervention, two rats died in anesthesia group and two rats died after partial liver resection. In the control group, no rat died during the experimental period.

The approximately 1-year-old rats had a mean body weight of 663 ± 50 g at the beginning of the experiments. Four weeks later, there were no significant differences in rat body weight between the groups: control group: 665 ± 56g, anesthesia group: 608 ± 34 g, and surgery group: 658 ± 62g (P>0.05).

In addition, blood glucose concentrations (mg/dl) were not significantly different between the control (98 ± 33), anesthesia (98 ± 31), and surgery (115 ± 11) groups.

Rats' spontaneous locomotor activity is unchanged in holeboard system: In order to analyze how rats were able to move spontaneously, the holeboard system was used. On the first postoperative day (24 hours after intervention), spontaneous locomotor activity tended to be reduced in all groups (P=0.08). No significant differences between the groups were observed either before or after surgery (Table 1).

Psychometric analysis in Morris water maze: The effect of surgery and the effect of pronounced propofol anesthesia on spatial memory were assessed in the Morris water maze test, a hippocampus-dependent spatial learning task in which rats are required to learn to locate an invisible (hidden) escape platform (constant or changing platform) in a pool of water [30].

Rats were trained over a time interval of 5 consecutive days to find a visible or a hidden platform in a water basin. The results of the training procedure are summarized in Table 2. The results of training showed an about 2-fold significant reduction (P<0.05) in time and distance to the platform in all performed tasks. Thus, all rats were able to fulfil the given tasks with comparable swimming velocities (P>0.05).

Partial hepatectomy reduced rat memory capacities: In order to compare rats' recovery abilities, post-operative re-tests were performed over 4 weeks after the intervention. Here, re-test performances and probe trial were analyzed on the first postoperative day and on days 3, 7 and 28 after intervention in control, anesthesia, and surgery groups.

Between post-operative day 1 and day 28 a significant reduction (P<0.05) in distance to hidden platform from 57.8 (56.3) [(means(SD)] to 32.3 (36.0) dm (-46%) and in time to platform from 26.4 (23.7) to 16.4 (16.4) sec (-38%) was obtained, respectively, if all groups were evaluated together. Thus, a principal trainability (learning capacity)

Before surgery Mean (SD)	Time to first hole (sec)	Number of rewarded food holes	Distance moved in holeboard (dm)
(1) control (n=12)	22 (21)	8 (1.5)	19 (5)
(2)Propofol (n=12)	35 (31)	6 (2.5)	14 (6)
(3) PLR + Propofol (n=12)	37 (28)	7 (4)	16 (8)
24 h after interventions			
(1) control (n=12)	14 (12)	5 (3)	11 (7)
(2) Propofol (n=10)	16 (15)	6 (3)	13 (6)
(3) PLR + Propofol (n=10)	19 (19)	6 (3)	16 (6)

Rat spontaneous activity was analyzed before and after interventions [(1) control: placebo anesthesia, (2) propofol anesthesia, or (3) PLR: Partial Liver Resection with propofol anesthesia] and is presented as mean (SD). No significant changes were obtained between the groups.

Table 1: Holeboard data.

	Visible platform		Hidden platform (WM1)		Hidden platform (WM2)	
	First trial	Last trial	First trial	Last trial	First trial	Last trial
Time to platform (sec)	63.2 (17.1)	31.4 (26.4)*	45 (25)	23 (21)*	31 (25)	21 (19)*
Distance to platform (dm)	88 (56)	50 (44)*	92 (59)	49 (39)*	78 (68)	47 (43)*
Velocity (cm/sec)	30.4 (5.8)	32.2 (8.1)	30.5 (5.3)	31.4 (9.1)	30.3 (8.5)	25.3 (12.4)

The training effect was determined before propofol anesthesia / surgery was done. The first and the last trial of a respective water maze task were represented as mean (SD) and compared each to other. A significant (*: P<0.05) improvement of time and distance to the platform was obtained. No significant changes in rat swimming speed (velocity) were observed.

Table 2: Morris Water Maze Training.

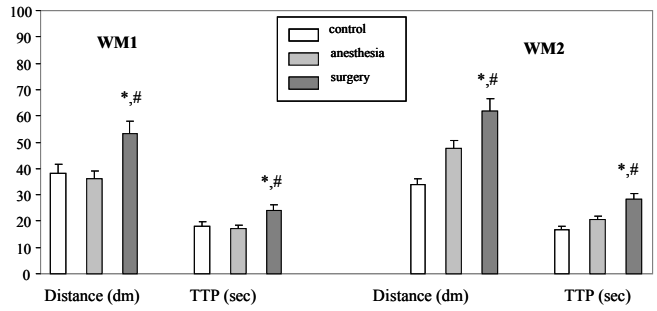


Figure 2: Water maze retest analysis

Because there were no significant differences according to factor 'time' results of all four Water Maze (WM) retests (rt) were summarized and presented as mean + SEM. Rats with a partial liver resection need more time and a longer distance to find the hidden platform from a constant starting point (WM1) or with changing starting points (WM2) (P<0.05: * versus control group, #: versus anesthesia group).

was preserved in all animals independent of conducted anesthesia or surgery procedure (data not shown). However, despite a marked improvement in the water maze parameters, significant differences between the groups were observed. Water maze re-testing analyses showed a significant (P<0.05) deterioration in the surgery group for time and path length to the platform compared to controls and propofol-injected rats (Figure 2). Thus, after partial liver resection rats need more time and a longer path length to reach the hidden platform with constant (WM1) and variable platform (WM2) locations.

Analysis of postoperative probe trial also demonstrated the impairment in the rats' memory after partial liver resection (Figure 3). After surgery in the form of partial liver resection rats spent significantly less time in the target quadrant north in the Morris water maze shortly after the platform was removed from the basin.

Alzheimer-like hallmarks: Four weeks after prolonged anesthesia and/or partial liver resection, no significant changes were observed in total and phosphorylated cerebral tau protein concentrations. Phosphorylated GSK-3ß [pg/mg protein] showed no significant differences between controls [294.4 (35.3)], propofol group [253.7 (33.4)], and after partial hepatectomy [268.3 (32.6)]. However, total GSK-3ß, as the active form of the enzyme, was significantly increased in the surgery group (Table 3).

Like for tau, no significant difference in cerebral vascular amyloid content (Table 4) was observed between the groups.

Memory deteriorations are associated with pronounced apoptosis: Four weeks after surgery and propofol administration, significant increased apoptosis was found in the brain of adult rats that had received partial hepatectomy (respective pictures: see Figure 4, a-f). TUNEL positive cells in rat brain were 3.5fold increased in the partial liver resection group compared to controls and 3fold compared to propofol group. In parallel, caspase-3 positive cells were 3.1fold significantly increased in the partial liver resection group compared to controls and 2.5fold to rats under propofol (Table 5).

Discussion

To our knowledge, this study provides evidence for the first time that, in middle-aged rats, partial hepatectomy under propofol anesthesia induces (i) long-term cognitive dysfunction and (ii) increased cerebral apoptosis as compared to propofol anesthesia alone and to placebo anesthesia.

As stated in the Introduction, a growing body of literature describes an association between major surgery and cognitive impairment in patients [1]. Although animal models have provided some insight into the mechanisms underlying neuronal loss and accumulation of neurotoxic proteins [34], the connection between lesions, symptoms, and causes of cognitive dysfunction remains difficult to unravel. A causal link between anesthetic-induced biochemical markers of neurotoxicity and the development of long-term cognitive dysfunction is the subject of controversial discussion [9] and seems to depend on the kind of anesthetics, administration time and dosage, and surgical intervention. Moreover, it was hypothesized that surgery, and not anesthesia, induced acceleration of ongoing endogenous neurodegenerative processes, including reduced cognitive abilities, and induced gliosis and Alzheimer-like markers [34]. In contrast to a recent paper of Li et

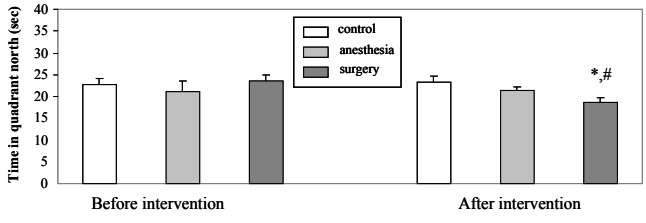


Figure 3: Probe trial

Because there were no significant differences according to factor 'time' results of all four retests were summarized and presented as mean + SEM. Rats with a partial liver resection (surgery group) spent less time in the north quadrant if platform was removed from basin (P<0.05: * versus control group, #: versus anesthesia group) after invention compared to testing results before intervention.

al. [35] our present data did not focused on the role of isoflurane and of neuroinflammation. Our present data showed the deleterious role of surgery compared to propofol anesthesia alone; nevertheless, ongoing studies on this field are required to gain new insight into the etiology/pathomechanisms of POCD.

Extending the findings of Wan et al. [34], we used the partial hepatectomy model [28,29] for investigation on aged rats. Wan et al. [34] demonstrated that partial hepatectomy is able to induce cerebral changes and otherwise it is minimally invasive. It is well known that hepatocytes have a great capacity for replication and are capable of re-populating the liver [35,36]. Indeed, in accordance with Wan et al., [34] we assume that rat liver function was not markedly disturbed if only <5% of liver tissue was resected. Therefore, it is unlikely that detected changes in our measured parameters are related to disturbed liver metabolism; however, we did not confirm this.

Mean (SD)	Total Tau (ng/mg protein)	phospho Tau (ng/mg protein)	phospho Tau / total Tau	GSK-3ß (pg/mg protein)
(1) Control (n=12)	0.050 (0.024)	0.019 (0.03)	25.87 (31.8)	1140.5 (180.6)
(2) Propofol (n=10)	0.060 (0.037)	0.029 (0.02)	33.1 (20.1)	1133.4 (152.1)
(3) PLR + Propofol (n=10)	0.058 (0.039)	0.012 (0.01)	17.0 (18.5)	1406.8 (210.3)*#

Cerebral tau characteristics and the glycogen synthase kinase (GSK) content are presented as mean (SD). Groups: (1) control: placebo anesthesia, (2) propofol anesthesia, or (3) PLR: partial liver resection with propofol anesthesia. Significant changes were obtained in GSK-3ß after surgery (group 3) in rat hippocampus as compared to group 1 (*: P=0.01) and to group 2 (#: P=0.02).

Table 3: Tau metabolism and GSK-3ß.

Amyloid content mean abr. intensity (SD)	Vessels 35 - 80 µm	Vessels 120 - 210 µm
(1) Control	72998 (47378)	573033 (289990)
(2) Propofol	56038 (36494)	456170 (240295)
(3) PLR + Propofol	57346 (25917)	444419 (150301)

Amyloid content in smaller (35-80 µm) and larger (120 – 210 µm) cerebral blood vessels was analyzed in rat brain using (n=3 per group) IMAGE J program, and is presented as abbreviated (abr.) fluorescence intensity as mean (SD). PLR: Partial Liver Resection

No significant changes were obtained between the groups

Table 4: Cerebral amyloid content.

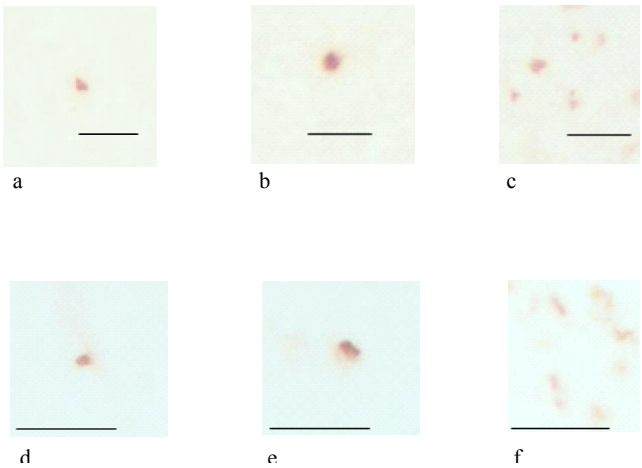


Figure 4: Apoptosis in rat brain

TUNEL- (upper line) and caspase-3 (lower line) positive cells and were elevated in controls (a, d), in animals with propofol anesthesia (anesthesia group b, e) and with after partial liver resection under propofol (surgery group c, f) 4 weeks after invention. Representative pictures of brown colored TUNEL (magnification 200 x) and caspase-3 positive cells (magnification 400x) are included. In contrast to pronounced apoptosis in rat brain after surgery (c, f), only isolated apoptotic cells were detected in control rats and animals without partial liver resection (a, b, d, e).

----------: 20 µm

The aim of the present study was to determine the cerebral effect of long-term propofol administration and to distinguish this effect from surgery-induced changes. From our pilot study we know that 2 hours of propofol administration at a concentration of 40 mg kg-1hour-1 [27] significantly reduced MABP to about 60 mm Hg at the end of the infusion period. From previous investigations we know that mean arterial blood pressure about 60 mm Hg was not significantly associated with marked deterioration in cerebral perfusion. Rats were able to compensate cerebral vessel occlusion and hypoperfusion [37]. Therefore, it should be excluded that possible differences in cerebral parameters between the groups are related to pronounced cerebral hypoperfusion.

Our Alzheimer-related histological data are consistent with previous reports [24] demonstrating that acute or long-term anesthesia with propofol in therapeutic and toxic concentrations does not affect amyloid metabolism in the rat brain. Furthermore, an additional partial hepatectomy did not cause any changes related to Alzheimer-like hallmarks in the rats, such as vascular amyloid staining or tau protein concentration in the present study. Therefore, it seems that injected anesthetics such as propofol have no effect on amyloid in wild-type animals, in contrast to inhaled anesthetics in rodent Alzheimer's Disease (AD) models [38]. A comparison of these studies further suggests that animals with pre-existing AD pathology appear to be more at risk for Alzheimer-like histopathological changes. However, the aim of our study was to investigate the effect of propofol anesthesia and partial hepatectomy in a middle-aged rat model without any genetic deterioration to simulate a typical middle-aged patient without marked cognitive decline who undergoes a typical surgery under propofol anesthesia.

Our data showed that partial hepatectomy induced short- and long-term disturbances in rats' spatial cognitive abilities up to 4 weeks after surgery. In parallel, partial hepatectomy triggered cerebral apoptosis in this study. TUNEL staining represents a tool for morphological diagnosis of apoptosis, detecting DNA fragmentation, so-called 'apoptotic bodies' and condensed chromatin. Furthermore, caspases play a distinct role in the apoptotic cascade [39]. Caspase 3 is one of the prominent effector caspases at the final level of the apoptotic

	TUNEL positive cells		Caspase -3	
	mean	SD	mean	SD
(1) Control	18	10	5	0.6
(2) Propofol	21	12	6	5
(3) PLR + Propofol	64*	25	15*	11
P-value	$P_{1/2}$<0.001		P_1=0.02; P2=0.04	

Data from n=6 slices per group were analyzed 28 days after surgical intervention. Partial liver resection significantly (*: P<0.05, P_1: PLR versus control, P_2: PLR versus 2) increased the number of TUNEL- and caspase-3 positive cells in rat brain compared to propofol group without partial hepatectomy (PLR) and compared to controls.

Table 5: Apoptosis in rat brain.

cascade and therefore represents a potential target for neuroprotective interventions [39].

Propofol alone, however, did not induce apoptosis in rat brain in the present study. This result is in contrast to other studies which used higher and up to toxic propofol concentrations instead of therapeutic dosages [40]. In a recent study in humans, it was shown that serum levels of TRAIL and caspase-3 were significantly elevated immediately postoperatively and then decreased significantly after 24 hours in a group of patients that received propofol [41]. Concerning propofol, however, it has neither been proven nor excluded in detail and in vivo that propofol can definitively induce cerebral apoptosis; however, it has been suggested that propofol attenuates intestinal epithelial apoptosis in rats by reducing ceramide production, a messenger for apoptosis [42]. In rabbit hearts, propofol can also prevent dopamine-induced apoptosis after ischemia, as assessed by immunoblotting of caspase-3 cleavage [43]. In addition, propofol could inhibit apoptosis in astroglial cells evaluated by cytotoxicity assay and caspase-3 activation [44]. In contrast, Siddiqui et al. [45] proposed that the anti-cancer effects of propofol on breast cancer cells significantly induced apoptosis; however, they studied propofol conjugates and not propofol itself. In another study conducted by Tsuchiya et al. [46], the authors found that propofol treatment could activate different caspases. This dissimilarity in results may be due to different markers used and to different study designs; apoptosis was often investigated after ischemia and reperfusion or in combination with other drugs that cause apoptosis via different mechanisms.

In the present study we demonstrated that cerebral apoptosis was not induced after propofol anesthesia alone, but only in combination with partial hepatectomy. Why this surgical intervention induced cerebral apoptosis, however, still remains speculative. In cardiac patients, it has usually been attributed to the adverse effects of surgery-related trauma, micro-embolization, temperature changes, levels of mean arterial pressure used, or changes in jugular bulb oxygen saturation with bypass [47]. We assume that partial hepatectomy -- as a kind of surgical trauma -- induced a pronounced glucocorticoid-mediated stress reaction. Although we did not measure the glucocorticoid concentration in the present study, it can be assumed that the glucocorticoid-mediated stress reaction may interact with the immune system; this phenomenon has been demonstrated in animals [32] and in humans [47] in some of our previous studies. It is well known that neurons may be damaged following exposure to high concentrations of corticosterone, leading to cognitive decline [48,49]. To investigate these mechanisms, studies are now ongoing in our lab on the role of stress and immune reaction after partial hepactectomy, also in comparison to other anesthetics.

Following an acute traumatic brain injury, neuronal cell death can be induced by caspase-dependent or -independent pathways [50,51]. Interestingly, it has been shown that GSK-3 is able to regulate differention-induced apoptosis of human neural progenitor cells [52]. Although GSK-3 was originally named for its ability to phosphorylate glycogen synthase and regulate glucose metabolism, this multi-functional kinase is presently known to be a key regulator of a wide range of cellular functions. Increased GSK-3 activity is believed to contribute to the etiology of chronic disorders such as AD, schizophrenia, diabetes, and some types of cancer, thus supporting the therapeutic potential of GSK-3 inhibitors. As GSK-3 is involved in multiple signaling pathways and has many phosphorylation targets, it should therefore not be surprising that GSK-3 has both pro- and anti-apoptotic roles [53]. For instance, GSK-3 over expression in vivo induces neuronal cell death and increases the number of reactive microglia in the brain of 3-month-old mice [54]. The results of our present study showed increased GSK content in rats after partial hepatectomy, supporting the hypothesis of a pro-apoptotic effect; however, the cause and effect relationship should be demonstrated in further studies (see below).

It was also shown that GSK-3 has been linked to behavioral effects in rodents [55]. Recent evidence suggests that GSK-3 plays a key role in certain types of synaptic plasticity, in particular a form of Long-Term Depression (LTD) that is induced by the synaptic activation of NMDA receptors [56]. Therefore, inhibiting GSK-3 activity via pharmacological intervention has recently become an important strategy for treating neurodegenerative and psychiatric disorders [57].

The present study has some limitations: (1) the results of animal research cannot be extrapolated to humans without any doubt. Therefore, the present results should be interpreted with care relating transferability to humans. (2) In order to conduct the planned manipulation to reach the study aim it was necessary to use two forms of anesthesia: a short sevoflurane and a long-lasting propofol anesthesia. Although sevoflurane anesthesia was comparable for all groups and short for catheter placement (<8min) and for decapitation procedure (<2 min) in contrast to propofol infusion (2 hours), a triggering role of sevoflurane is unlikely but cannot be completely excluded. (3) The stated possible pathogenetic link, that partial hepatectomy may increase cerebral GSK and apoptosis requires further substantiation by future research. Thus, other kinds of surgery should be evaluated, and the potential role of GSK it should be verified by the use of GSK inhibitors.

Conclusion

We conclude from the present animal study that prolonged propofol administration at clinically relevant doses did not induce changes in middle-aged rat brain. In contrast, partial hepatectomy induced cognitive deteriorations which were associated with increased cerebral apoptosis.

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