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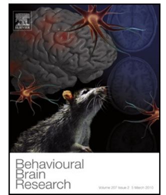

Research report

Isoflurane in the presence or absence of surgery increases hippocampal cytokines associated with memory deficits and responses to brain injury in rats

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HIGHLIGHTS

- Isoflurane exposure resulted in significantly less freezing in the contextual fear conditioning test.
- The addition of laparotomy with isoflurane caused no further deficits in cognition.
- Early after isoflurane exposure changes in serum and hippocampal cytokines were divergent but by 9 days were aligned

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ABSTRACT

Evidence from experimental animal studies convincingly argues for a role of pro-inflammatory cytokines due to surgical trauma in causing postoperative cognitive dysfunction. However, other studies have shown exposure to 2–4 h of isoflurane anesthetic without surgical trauma can also impair cognitive function. We aimed to determine cytokine changes over time following isoflurane exposure in the presence and absence of surgery and examine subsequent cognitive function. Male rats were exposed to isoflurane (1.8%, 4 h) with or without laparotomy or control conditions and tested in a contextual fear conditioning paradigm 8 days later. On day 9 rats were perfused, serum and hippocampal samples were collected and 24 cytokines were analysed. Groups of rats exposed as above were killed 6 or 48 h after isoflurane exposure to examine early cytokine changes. Isoflurane exposure resulted in significantly less freezing in the contextual fear conditioning test ($F_{(2,31)} = 6.11$, $P = 0.006$) and addition of laparotomy caused no further deficits ($P > 0.05$). At 6 h post isoflurane exposure an immunosuppressive response was observed in the serum while hippocampal cytokines were largely unchanged. These finding suggest isoflurane alone causes inflammatory changes and cognitive deficits. The addition of a laparotomy had a negligible effect. Early after isoflurane exposure changes in serum and hippocampal cytokines were divergent but by 9 days were aligned. At this time cytokines associated with memory deficits and brain injury processes were significantly elevated in serum and brain.

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1. Introduction

Cognitive deficits [1] after anesthesia and surgery, known as postoperative cognitive dysfunction (POCD) occur in around 10% of all surgical patients and 40% of patients over 65 years of age [2]. The causes are ill defined however there is persuasive evidence from experimental animal studies that surgical trauma-induced inflammation can result in neuroinflammation and cognitive deficits [3–5]. The pro-inflammatory response to surgery is well documented [6–9] but only relatively recently has it become recognised that systemic inflammation can cause CNS inflammation and ensuing cognitive dysfunction through activation of the innate immune system [1,10]. Maze and co-workers have demonstrated that surgi-

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cal trauma-induced inflammation results in cognitive impairments in rats after splenectomy [11] and in mice subjected to orthopedic surgery [4,12,13]. Induction of surgery 30 min after training in a hippocampal-dependent fear conditioning paradigm resulted in elevated systemic and hippocampal levels of pro-inflammatory cytokines within 6 h including IL-1β [11,12], TNFα [3,11,13] and IL-6 [3,13] and subsequent cognitive impairment in the fear conditioning test [12,13]. Further evidence for surgical-induced neuroinflammation in contributing to the cognitive dysfunction was provided when memory deficits and cytokine concentrations were reduced or completely abrogated by specifically inhibiting IL-1β through IL-1ra or through genetic intervention (IL-1R−/− mice), immune intervention with anti-TNFα [14] or general inhibition of inflammation using the anti-inflammatory agent minocycline [4]. Peripheral blockade of TNFα limited IL-1 release, neuroinflammation and cognitive deficits in a mouse model of surgery-induced cognitive decline suggest IL-1β and IL-6 act downstream of TNFα [3]. Taken together these data strongly support surgery-induced inflammation playing a significant role in the development of POCD.

Anesthesia alone has also been reported to result in cognitive deficits in experimental animals when administered for longer durations [15–19]. This raises the question of how anesthetics alone induce POCD when there is no surgical intervention. The studies of Maze and Ma and co-workers employed only the shortest duration of anesthesia required to perform the surgery (15–20 min). No effects of these short durations of anesthesia on cognitive outcome or cytokine levels were found leading to the conclusion that anesthesia does not play a role in POCD [4,5,11–13]. Interestingly, duration of anesthesia was identified as a risk factor for development of POCD by The International Study on Post-Operative Dysfunction (ISPOCD) [2].

Anesthetics have well documented immune-modulating effects [20] and could potentially contribute to the inflammatory reaction during surgery. In clinical studies differential effects of inhalational and intravenous anesthetics on surgery-induced inflammation and alteration in cell-mediated immunity have been reported with inhalation anesthesia having a more pronounced inflammatory effect [21]. With respect to POCD, a recent study in experimental animals has reported increased IL-1β but not TNFα in the hippocampus 6 h after isoflurane inhalation (1.2%, 2 h) in adult Fischer rats [22] and subsequent cognitive deficits two weeks after exposure suggesting isoflurane-induced neuroinflammation could indeed contribute to subsequent cognitive dysfunction.

Therefore, in the present study we sought to investigate the effects of a moderate dose and duration of anesthesia (4 h, 1.4% MAC isoflurane) on systemic and hippocampal cytokine levels at early time points after exposure and at the time of assessment of cognitive outcome of rats. We also examined the addition of surgical trauma through laparotomy on these parameters and hypothesised that anesthesia alone would increase pro-inflammatory cytokines and impair cognition and that surgical trauma would have an additive effect.

2. Materials and methods

2.1. Rats

This study was approved by the University of Melbourne Animal Ethics Committee on the use of animals in a research project. Male Sprague Dawley rats used in the study (8–10 weeks of age, weighing 327.57 ± 4.05 g) were obtained from the Biological Research Facility, University of Melbourne, for use in these experiments. Rats were housed 3–4 per cage, with free access to food and water, in a climate and humidity controlled room on a 12 h light–dark cycle.

2.2. Anesthesia and surgery

Rats were randomly assigned to anesthesia alone, anesthesia plus surgery or no anesthesia control group ($n = 17$ isoflurane; $n = 17$ isoflurane + surgery; $n = 26$ no anesthesia controls). Rats in the anesthesia groups were placed 3 at a time in an anesthetic induction chamber filled with 5% isoflurane for approximately 2–3 min or until unconscious as previously described [19]. Rats were then removed and attached to one of the nose cones of our custom designed anesthetizing apparatus into which the anesthetic was delivered at a flow rate of approximately 1.5 L/min, and adjusted to maintain isoflurane concentration at 1.8% and oxygen and carbon dioxide at constant levels within the chamber. This concentration of isoflurane was chosen to be higher than 1 MAC in rats (previously determined in our laboratory as 1.4% in young adult Sprague Dawley rats) and enough to conduct surgery in all rats without eliciting a response. Gases within the anesthetic chamber were continuously monitored (Ohmeda Excel 210 SE anesthetic machine, Datex Instrumentarium Corp., Helsinki, Finland). No anesthesia control animals were placed into the induction chamber and received 100% oxygen at an identical flow rate for 10 min to simulate anesthetic induction before being returned to their home cages.

Typically in each experiment a total of 6 rats were treated at the same time with equal numbers assigned to isoflurane alone and isoflurane plus surgery groups. A number of no anesthesia control rats were also included on the same day. Rats were exposed to 1.8% isoflurane in 100% oxygen for 4 h. After approximately 1 h of anesthesia, rats in the isoflurane plus surgery group were shaved along the abdomen and swabbed with 70% ethanol before an incision to the length of 3 cm was made longitudinally along the abdominal median line. The incision was covered with gauze and left open for a period of 10 min to model surgical environmental conditions before both the abdominal and skin layers were sutured separately (Prolene 4-0 polypropylene suture, Ethicon Inc.).

Blood pressure was monitored non-invasively at hourly intervals in anesthetised rats using a tail cuff (Coda, Kent scientific, USA). Temperature was measured at 30 min intervals and normothermia (37 ± 1 °C) was maintained using warming mats. Saline solution (0.9%; 0.25 mL/100 g body weight/h) was administered subcutaneously to maintain hydration during anesthesia. All rats, including all anesthesia alone, anesthesia plus surgery and no anesthesia controls received analgesia (buprenorphine, 10 μg/kg subcutaneously) 10 min before ceasing anesthesia and 24 h after treatment to manage post-operative pain.

Groups of rats ($n = 4$ in treatment groups and $n = 6$ no anesthesia controls) were sacrificed at 6 or 48 h post-isoflurane to analyse serum and hippocampal cytokines. The remaining rats ($n = 9$ in treatment groups and $n = 14$ no anesthesia controls) were assigned to cognitive testing. These rats were allowed to recover for 1 week before cognitive testing on day 8.

2.3. Behavioral testing

Behavioral testing was performed in a closed, quiet, light-controlled room in the Biological Research Facility at the Howard Florey Institute. Rats were acclimatized to the conditions of the facility for at least 4 days prior to testing. On day 8 after exposure to anesthesia, rats were subjected to context fear conditioning. The fear conditioning chambers (Coulbourn Instruments, Whitehall, PA, USA) were soundproofed and illuminated with a small light bulb on the rear wall. Chambers were wiped with 70% ethanol before and between each rat to remove odor cues. Each rat was placed into a chamber and allowed to explore for 3 min before receiving a single shock (1 mA, 1 s duration), delivered through a stainless steel rod floor. Rats remained in the chamber for a further 30 s before being returned to their home cages. After an inter-trial interval of 2 h,

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