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Full Length Article

Sevoflurane exposure has minimal effect on cognitive function and does not alter microglial activation in adult monkeys

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ABSTRACT

Postoperative Cognitive Dysfunction (POCD) is a complication that has been observed in a subset of adult and elderly individuals after general anesthesia and surgery. Although the pathogenesis of POCD is largely unknown, a growing body of preclinical research suggests that POCD may be caused by general anesthesia. A significant amount of research has examined the effects of general anesthesia on neurocognitive function in rodents, yet no studies have assessed the adverse effects of general anesthesia on brain function in adult nonhuman primates. Thus, this study sought to determine the effects of an extended exposure to sevoflurane anesthesia on cognitive function and neural inflammation in adult rhesus macaques. Five adult rhesus macaques (16–17 years of age) were exposed to sevoflurane anesthesia for 8 h and, and micro-positron emission tomography (PET)/computed tomography (CT) imaging and a battery of operant tasks were used to assess the effects of anesthesia exposure on ^{18}F-labeled fluoroethoxybenzyl-*N*-(4-phenoxypyridin-3-yl) acetamide ([^{18}F]-FEPPA) uptake, a biomarker of microglia activation, and aspects of complex cognitive function. Exposure to sevoflurane anesthesia for 8 h did not increase [^{18}F]-FEPPA uptake in the adult monkey brain. Sevoflurane anesthesia significantly decreased accuracy (mean difference = 22.79) on a learning acquisition task 6 days after exposure [$t(3) = 6.92$, $p = 0.006$], but this effect did not persist when measured 1 week and 2 weeks after additional exposures. Further, sevoflurane anesthesia had no impact on performance in 4 additional cognitive tasks. These data suggest that exposure to anesthesia alone may not be sufficient to cause persistent POCD in adult populations.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a phenomenon that can occur in patients after surgical procedures conducted under general anesthesia. POCD is defined by deficits in one or more cognitive domains, determined through preoperative and postoperative neuropsychometric testing, that persist for weeks or even months after surgery Rasmussen (2006). Although more common in elderly populations, transient POCD can also occur in middle-aged adults (Johnson et al., 2002; Monk et al., 2008). Currently, the pathogenesis of POCD remains largely unknown; however, many preclinical studies suggest that the onset of POCD may be caused by general anesthetic agents (Jevtovic-Todorovic et al., 2013; Wang and Orser, 2011).

Due to its rapid induction, minimal airway irritation, and minor cardiovascular and respiratory side effects, sevoflurane is a frequently used volatile anesthetic for surgical procedures (Brioni et al., 2017). Sevoflurane acts as a positive modulator of γ-aminobutyric acid subtype A ($GABA_A$) and glycine receptors (Forman and Chin, 2008). In adult rodents, sevoflurane and isoflurane anesthesia have been shown to induce neural inflammation and apoptosis, while producing corresponding impairments in learning and memory (Alkire and Gorski, 2004; Culley et al., 2004; Karaman et al., 2017; Lin and Zuo, 2011; Liu et al., 2010).

Although several studies have examined the neurotoxic effects of exposure to general anesthesia on the central nervous system in neonatal nonhuman primates (Coleman et al., 2017; Creeley et al., 2014; Paule et al., 2011; Zhang et al., 2016) (Alvarado et al., 2017; Raper et al., 2015, 2018), no studies have assessed these effects in adult nonhuman primates. Using nonhuman primates as models for POCD is advantageous since their anatomy, neurochemistry, physiology, and behavior are more closely related to humans than that of rodents (Weerts et al., 2007). Further, their larger size allows for adequate monitoring and control of physiological parameters during anesthesia as is the case in the human situation. Therefore, the adult rhesus macaque was used in this study that incorporated microPET/CT imaging and a battery of operant tasks to assess the effects of an extended

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exposure to sevoflurane anesthesia on a biomarker of neural inflammation and aspects of complex cognitive function.

To measure aspects of neuroinflammation we used ^{18}F-labeled fluoroethoxybenzyl-*N*-(4-phenoxypyridin-3-yl) acetamide ([^{18}F]-FEPPA), a ligand for the translocator protein (TSPO). In the central nervous system, TSPOs are expressed primarily on activated microglia and can be used as biomarkers of neural inflammation (Imaizumi et al., 2008; Rupprecht et al., 2010; Schweitzer et al., 2010), and have been used extensively in the study of multiple sclerosis (Airas et al., 2018). Recent studies have demonstrated [^{18}F]-FEPPA to be a reliable radiolabeled ligand for detecting increased TSPO expression after general anesthesia exposure in neonatal rhesus macaques (Zhang et al., 2016) and in adult patients with Alzheimer's Disease (Suridjan et al., 2015). Here, we examined [^{18}F]-FEPPA binding as a measure of neuro-inflammation after exposure to sevoflurane anesthesia in the adult rhesus monkey.

In addition, cognitive function after sevoflurane anesthesia was assessed in these same monkeys using the National Center for Toxicological Research's Operant Test Battery (OTB). The OTB has served as a reliable tool for assessing the effects of drugs and other neurotoxicants on the behavior of nonhuman primates (Buffalo et al., 1994; Ferguson and Paule, 1993; Frederick et al., 1995; Paule, 2001; Paule et al., 2012) and for studying cognitive function in humans (Baldwin et al., 2004; Chelonis et al., 2011; Gleich et al., 2015; Walters et al., 2016). Furthermore, these operant tasks are currently being used to evaluate the neurocognitive effects of general anesthesia in pediatric patients (Gleich et al., 2015). Thus, by using translational assessment techniques in a nonhuman primate model of general anesthesia, this study aimed to elucidate the role anesthesia might play in the development of POCD in adult populations.

2. Methods

2.1. Animals and anesthesia exposure

Five adult rhesus monkeys (3 males, 16–17 years of age (7.3–8.5 kg) and 2 females, 17 years of age (7.2–7.3 kg) served as subjects. All monkeys were drug naïve for over 7 years and had been performing the operant test battery since they were ˜ 6 months of age. Two animals had previously been treated with ketamine for experimental purposes, and another animal had been treated with atomoxetine and nicotine. All exposures occurred after animals reached sexual maturity. Standard nonhuman primate chow (Purina Mills, Richmond, IN) was provided once a day following behavioral sessions (M-F) and on weekends at approximately the same time of day. The amount of daily chow given was monitored weekly to ensure that animals maintained approximately the same weight throughout the present experiment. Water was available ad libitum and vitamins, fresh fruit, and nut supplementation were provided 5 days per week. All animal procedures were approved by the National Center for Toxicological Research's (NCTR) Institutional Animal Care and Use Committee and conducted in full compliance with the National Research Council's Policy on Humane Care and Use of Laboratory Animals (Council, 2011).

2.2. Anesthesia exposure

Monkeys were anesthetized with the inhaled anesthetic, sevoflurane (2.5%; Patterson Veterinary Supply, Saint Paul, MN), for 8 h. Exposures occurred on Mondays with a maximum of one monkey being exposed on any given Monday. In addition to this 8 h experimental exposure, subjects were also exposed to sevoflurane for ˜2 h during 4 separate microPET sessions (see microPET section for details). Subjects were fasted overnight prior to all sevoflurane exposures. Animals were given an intramuscular injection (i.m.) of glycopyrrolate (0.04 mg/kg) to reduce saliva production and an i.m. injection of ketamine (10 mg/kg, Patterson Veterinary Supply, Saint Paul, MN) to immobilize the animal for insertion of the endotracheal tube. Upon tracheal intubation, sevoflurane (5%) was delivered in 75% oxygen at a flow rate of 800 ml - 1 l per min. Once the animal was fully anesthetized, an IV catheter was inserted (for administration of IV fluid), and the sevoflurane concentration was reduced to 2.5% for the remaining duration of the exposure.

Soft cushions and warm water heating pads were placed underneath and around the animal to reduce pressure and maintain body temperature at 37 °C. IV fluid (0.9% Normal saline) was delivered at a rate of 1.7 ml · kg^{-1} · hr^{-1} (to maintain the IV for emergency intervention) and lubricant was applied to the eyes. Two investigators and 1 veterinarian remained in the room during the entire exposure to monitor sevoflurane administration. Respiratory rates, blood oxygen saturation (SpO_2), rectal temperatures, and systolic, diastolic, and mean arterial blood pressure were continuously monitored (DRE Waveline Monitor, Louisville, KY, USA). Heart rate and expired CO_2 concentrations were also continuously monitored via capnography (Tidal Wave Hand-held Capnograph, Respironics, Murrysville, PA, USA), and venous blood (0.25 ml) was collected at 2 h intervals for the measurement of plasma glucose concentrations, venous blood gases, pH values, and hematocrits (GEM©Premier™ 4000, Instrumentation Laboratory, Lexington, MA). Physiological parameters remained stable for all monkeys during the

Table 1
Physiological parameters during 8 h anesthesia exposure.

Time (h)	Heart Rate (bpm)	Resp. Rate (bpm)	SpO_2 (%)	Expired CO_2 (mmHg)	Temp °C	Systolic bp (mmHg)*	Diastolic bp (mmHg)*	Mean bp (mmHg)*	Glucose (mg/dL)*	HCO_3 (mmol/L)*	pH (venous)*
0.5	112 ± 14	23 ± 10	94 ± 3.2	32 ± 4	36 ± 1.7	48 ± 6	23 ± 6	34 ± 6	–	–	–
1	106 ± 13	27 ± 10	95 ± 2.5	27 ± 7	36 ± 2.3	58 ± 23	23 ± 6	36 ± 11	–	–	–
1.5	107 ± 14	26 ± 12	97 ± 2.7	27 ± 7	36 ± 1.7	61 ± 19	28 ± 7	41 ± 11	–	–	–
2	104 ± 15	24 ± 9	97 ± 3.5	27 ± 6	36 ± 1.3	61 ± 22	31 ± 10	45 ± 17	61 ± 2	29 ± 4	7.3 ± 0.04
2.5	99 ± 18	26 ± 11	97 ± 4	27 ± 6	36 ± 1.4	55 ± 13	29 ± 7	39 ± 11	–	–	–
3	98 ± 18	25 ± 5	99 ± 1.6	26 ± 5	36 ± 1.7	60 ± 14	31 ± 6	44 ± 11	–	–	–
3.5	98 ± 19	25 ± 8	100 ± 0.5	25 ± 5	36 ± 2.1	62 ± 19	35 ± 8	48 ± 16	–	–	–
4	98 ± 18	26 ± 6	100 ± 0.5	25 ± 5	36 ± 1.7	66 ± 13	33 ± 9	48 ± 12	61 ± 1	28 ± 3	7.4 ± 0.05
4.5	101 ± 15	24 ± 5	99 ± 1	23 ± 5	36 ± 0.6	63 ± 15	34 ± 9	47 ± 12	–	–	–
5	103 ± 13	27 ± 5	99 ± 1	24 ± 4	37 ± 0.4	68 ± 13	36 ± 10	51 ± 11	–	–	–
5.5	103 ± 12	27 ± 6	99 ± 0.9	22 ± 4	37 ± 0.4	67 ± 10	37 ± 9	50 ± 8	–	–	–
6	106 ± 12	25 ± 6	100 ± 0.9	24 ± 3	37 ± 0.7	64 ± 8	33 ± 6	46 ± 6	60 ± 1	27 ± 3	7.4 ± 0.04
6.5	105 ± 11	27 ± 5	100 ± 0.6	22 ± 4	37 ± 0.8	66 ± 8	34 ± 6	47 ± 7	–	–	–
7	105 ± 10	28 ± 4	100 ± 0.4	21 ± 5	37 ± 0.6	67 ± 7	35 ± 2	48 ± 3	–	–	–
7.5	106 ± 10	25 ± 7	100 ± 0.5	23 ± 3	37 ± 0.8	69 ± 7	36 ± 4	50 ± 5	–	–	–
8	107 ± 10	31 ± 13	100 ± 0.5	22 ± 3	37 ± 0.9	71 ± 10	36 ± 5	52 ± 9	60 ± 1	26 ± 3	7.4 ± 0.03

Note. SpO_2 = blood oxygen saturation. Data are expressed as means and standard deviations for each physiological parameter during 30 min recording intervals. In some instances sample size is smaller (* n = 4) owing to an equipment failure at the start of an exposure.

entire duration of each sevoflurane exposure, and the animals did not display any signs of respiratory depression or hypoxia (See Table 1). In no instance was additional intervention (inotropes, pressors, bolus fluid) necessary.

2.3. MicroPET/CT imaging

MicroPET/CT imaging was used to assess the effects of sevoflurane exposure on neural inflammation. To determine baseline levels of microglial activation, microPET/CT imaging was conducted at least 1 week prior to the first sevoflurane exposure. Then, to compare the effects of sevoflurane exposure to baseline measures, MicroPET/CT imaging was conducted 1 day, 1 week, and 2 weeks after the 8 h experimental sevoflurane exposure.

Prior to microPET/CT imaging, monkeys were immobilized with an intramuscular injection of ketamine (10 mg/kg). Then, during the microPET and CT imaging sessions (140 min), anesthesia was maintained with 2.5–3.5% sevoflurane gas delivered in 75% oxygen, 25% nitrogen via tracheal intubation. An electronic heating pad was used to maintain body temperature at approximately 37 °C, and physiological parameters were monitored as described during the 8 h experimental exposure to sevoflurane (blood gas analyses were not analyzed during microPET/CT scans). A transmission scan (10 min) using a rotating ^{57}Co point source was performed prior to injection of the radiolabeled FEPPA. An attenuation correction sinogram file was created using a blank dataset and the acquired transmission dataset and the software supplied by the scanner manufacturer. This histogram file was specified when reconstructing the emission sinogram data and was multiplied with the emission data thereby performing attenuation correction.

For each imaging session, [^{18}F]-FEPPA (mean = 357.05, SD = 10.99 MBq) was injected into the lateral saphenous vein of each anesthetized animal. Immediately following the injection, a set of serial microPET images were recorded using a Focus 220, high resolution small animal PET scanner (Siemens Preclinical Solution, Knoxville, USA) to assess the influx and accumulation of the radiotracer (24 frames, 5 min each). Immediately after microPET imaging, a CereTom CT scanner (Neurologica Corps Danvers, MA, USA) was used to obtain brain images of the animal for co-registering anatomical (CT) with molecular (PET) data. Once scanning was complete, monkeys were monitored in a shielded isolation area until complete recovery (˜ 4 h or 2 ^{18}F half-lives) and then returned to their home cage.

Medical image analysis software, ASIPro™ (Concorde Microsystems, Inc., Knoxville, TN, USA) was used for the quantitative analyses of the imaging data. The frontal cortex, temporal cortex, and basal ganglia were selected as the regions of interest (ROIs) and the cerebellum was used as a reference ROI. Three-dimensional ROIs (5–10 pixels in diameter) were drawn in the coronal plane with reference to transverse and sagittal planes displayed simultaneously. A brain atlas (Saleem and Logothetis, 2012) that contains the delineation of the brain was used as guidance to manually draw reduced spatially-standardized ROIs of prefrontal cortex, temporal lobe, and basal ganglia (striatum), which were used to quantify the average SUV value of these regions in the images. Additionally, the ROIs were placed on each microPET image with the aid of the corresponding CT image for individual monkeys. [^{18}F]-FEPPA accumulation in the ROIs was converted into standard uptake values (SUVs) using the following formula: SUV = average concentration of radioactivity in the ROI (MBq/ml) x body weight (g)/ injected dose (MBq). SUV Ratios (SURs) were then calculated as: SUV of ROI (e.g. frontal cortex, temporal cortex, or striatum)/SUV of cerebellum.

2.4. Behavioral testing

Behavioral tasks were conducted in primate operant test chambers using behavior panels that included press-plates and response levers (See Fig. 1). Animals were transferred from their home cages to operant test chambers in transport chairs and banana flavored food pellets were used as reinforcers for all tasks. Testing occurred daily (M-F) at the same time of day (+/- 1 h). Subjects performed five operant tasks; Progressive Ratio (PR; 10 min), Incremental Repeated Acquisition (IRA; 35 min), and Conditioned Positioned Responding (CPR; 5 min) tasks occurred on one test day and Delayed Matching to Sample (DMTS; 30 min) and Temporal Response Differentiation (TRD; 20 min) tasks occurred on alternate test days. A brief description of each task is provided below. For a more detailed description of these tasks refer to Paule et al. (Paule et al., 2011). During the imaging portion of the study animals were behaviorally tested only 3 or 4 days per week.

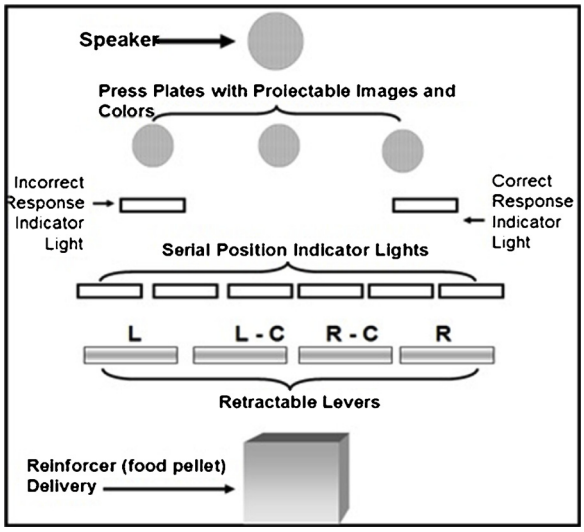

Fig. 1. Depiction of operant test chamber response panel.

2.4.1. Incremental repeated acquisition (IRA)

The IRA task required subjects to learn a specific sequence of responses that differed each test session. Briefly, the IRA task started with a one lever 'sequence' (IRA1) for which one of four horizontally aligned response levers was designated as the correct lever and a response on that lever resulted in the illumination of a "correct" indicator light and the delivery of a reinforcer. A response on any of the other three levers resulted in the illumination of the "incorrect" indicator light and a 2 s timeout. After twenty one-lever response sequences were completed subjects advanced to IRA level 2. This level required the monkey to find the correct lever for this level, as indicated by the illumination of the correct indicator light, and then respond on the correct lever for IRA1 to get a reinforcer. After 20 errorless two-lever response sequences, the task was incremented to a three-lever response sequence and so on, up to a maximal six-lever response sequence. The IRA task continued until the subject completed 20 errorless six-lever response sequences or until the total task time of 35 min had expired. The correct sequence of lever pressing varied each session, thus allowing a measure of response acquisition (learning) each session. The dependent variables for the IRA task were percent task completed, response rate, and accuracy.

2.4.2. Conditioned positioned responding (CPR)

The CPR task measured stimulus discrimination. Subjects were required to make a right or left choice response depending upon which color was presented to them at the initiation of a given trial. At the start of a trial, the center one of three horizontally aligned press plates was randomly illuminated red, yellow, blue, or green and after a response to it, it immediately extinguished and the two side press-plates illuminated white. If the center press-plate was previously illuminated red or yellow then a left choice response was correct; if the center press-plate was previously illuminated green or blue, a right choice response was correct. If the monkey made a correct choice the next trial started

Table 2
Experimental schedule.

Week	Day	Event
1-2	Mon-Fri	Baseline OTB
3	Mon	MicroPET baseline scan (2 h)
3	Tues-Fri	OTB (E1)
4	Mon	OTB (E1)
4	Tues-Fri	OTB
5-6	Mon-Fri	OTB
7	Mon	Sevoflurane (8 h)
7	Tues	MicroPET (2 h)
7	Wed-Fri	OTB (E2)
8	Mon	OTB (E2)
8	Tues	MicroPET (2 h)
8	Wed-Fri	OTB (E3)
9	Mon	OTB (E3)
9	Tues	MicroPET (2 h)
9	Wed-Fri	OTB (E4)
10	Mon	OTB (E4)
10	Tues-Fri	OTB (Post study)
11	Mon-Fri	OTB (Post study)

Testing and scanning of the 5 animals used for this study occurred over about 1 year. Animals were consistently exposed to sevoflurane on Mondays, with microPET scans taking place on Tuesdays. Baseline OTB testing was performed for 10 days prior to the Baseline microPET scan which occurred on week 3. E1 consisted of all data collected over 6 days (typically 4 test sessions) following the baseline scan (week 3 and 4). Only 6 days were included in E1 to make this point identical to E2, E3, and E4. Data for E2, E3, and E4 were typically collected over 6 days of OTB data, typically consisting of 4 test sessions. The post study data point consisted of 14 days (7–21 days after the last microPET scan), typically 10 test sessions, after E4. Animals were never tested in the same chamber on consecutive days.

immediately whereas an incorrect choice resulted in a 10-sec timeout (all plates darkened) before the random presentation of a new problem. The CPR task ended once 60 pellets were obtained or 5 min had elapsed. The dependent variables for the CPR tasks were percent task completed, response rate, and accuracy (See Fig. 3). Observing response latency and choice response latency were also measured but not included in the figure.

2.4.3. Temporal response differentiation (TRD)

The temporal response differentiation (TRD) task measured time perception and response inhibition. For the TRD task, only the left retractable lever was utilized. Subjects were required to hold the lever in the depressed position for a minimum of 10 s but no longer than 14 s in order to receive a reinforcer. Releasing the lever too early or too late had no programmed consequences and the monkey could immediately start a new trial. The TRD task ended once 120 pellets were obtained or 20 min had elapsed. The dependent variables for the TRD task were percent task completed, accuracy, and mean duration lever press (MDLP) (Table 2).

2.4.4. Delayed matching to sample (DMTS)

The delayed matching-to-sample (DMTS) task measured short-term memory. Only the three press-plate manipulanda were used for this task. For each trial, the center press plate was randomly illuminated with one of seven geometric symbols (sample stimulus). A press to the center press-plate extinguished the sample stimulus and initiated a randomly selected delay interval (0.01–80 s). Subsequently, all three press-plates were illuminated, one plate with a matching symbol and the other two with non-matching symbols. A response to the correct (matching) symbol was reinforced, while a response to an incorrect symbol was followed by a 10 s time-out, during which all press-plates darkened, and responses had no programmed consequences. The DMTS task terminated after 120 pellets were obtained or 30 min elapsed. The dependent variables for the DMTS task were percent task completed and accuracy (shown in Fig. 4). Additional measures recorded but not displayed in the figure include observing response latency and choice response latency.

2.4.5. Progressive ratio task (PR)

The PR task assessed appetitive motivation. Only the far right of the four, horizontally aligned retractable response levers was extended during the PR task. Subjects were placed under a PR 1 + 1 schedule such that within each session they were required to increase the number of lever presses required for each subsequent reinforcer by one response. Initially, one lever press resulted in reinforcer delivery. Thereafter, the number of responses required for subsequent food pellets was progressively increased by one. The PR task ended once 100 reinforcers were earned or after 10 min elapsed. The dependent variables for the PR task were percent task completed, response rate, and total responses.

2.5. Statistical analyses

Statistical analyses for microPET data were conducted using GraphPad Prism 6. One-way Repeated Measures ANOVAs were conducted to compare differences in SURs in each ROI ($\alpha_{critical} = 0.05$). In contrast to our previous work, scan "time" was not included as a factor, as we have previously shown that[^{18}F]-FEPPA provides a consistent signal across a 2-hour scan time. This was also done to improve the relative signal to noise ratio of the signal from the [^{18}F]-FEPPA. Statistical analyses for behavioral data were conducted using Statistica 13 (64 bit) and graphical presentations of data (means and standard deviations) were created using Graphpad Prism 6. A power calculation was not performed for this work. Sample size was based on previous PET and behavioral studies designed to evaluate the impact of perinatal anesthesia in the rhesus monkey (Paule et al., 2011; Zhang et al., 2016). Four different exposures were defined for the analysis of behavioral data. Exposure 1 consisted of the first 2 h exposure to sevoflurane required to obtain the baseline microPET, Exposure 2 consisted of the 8 h experimental exposure to sevoflurane plus the 2 h sevoflurane exposure required for the microPET scan on the day following the experimental 8 h exposure, Exposure 3 consisted of the 2 h sevoflurane exposure required for the microPET scan 1 week after the 8 h experimental exposure, and Exposure 4 consisted of the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the experimental exposure. If a subject did not make at least 6 responses for any given task, all data except percent task complete and response rate were excluded from the analysis. The data were examined for outliers, but none were observed. Animals were tested on 2 or 3 of the 5 OTB tests on any given day. In several instances, single data points are missing owing to interruptions to the test schedule and in once instance an apparatus failure (PR E2 and E4, IRA E2 and E4, CPR E2 and E4, TRD E3). Dependent samples *t*-test (two-tailed) were conducted for each behavioral endpoint for each task. Means for the first 10 days (4–5 sessions per task) prior to Exposure 1 (baseline on graphs) were compared to the first 6 days (2–3 sessions per task) after each exposure. Accordingly, 4 comparisons (Baseline versus Exposure 1, Exposure 2, Exposure 3, and Exposure 4) were made for each measure on each task. Moreover, a comparison was made between baseline performance and performance 2–3 weeks after the final exposure (post study on graphs, 7–21 days after exposure 4, 5–6 sessions per task). A Bonferroni correction was applied to all *t*-tests used on behavioral studies to control for multiple comparisons ($\alpha_{critical} = 0.01$). We corrected for five comparisons (Baseline vs. E1, E2, E3, E4, and post-study).

3. Results

There were no differences in general appearance, health, or body weights before and after each sevoflurane exposure for any of the 5 monkeys. Results from the physiological recordings are reported in Table 1 [see (Bertrand et al., 2017; Choi et al., 2016) for reference

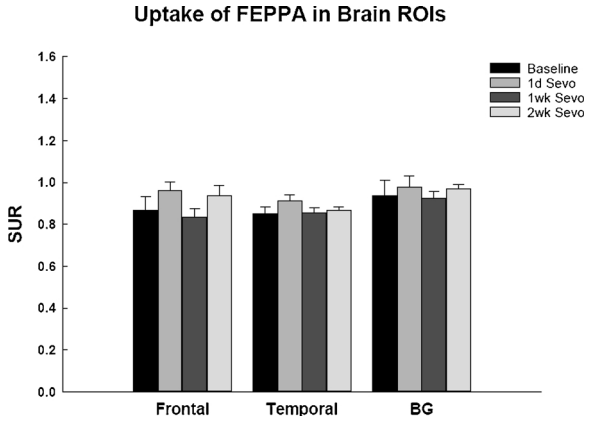

Fig. 2. Uptake of ^{18}F-labeled fluoroethoxybenzyl-*N*-(4-phenoxypyridin-3-yl) acetamide ([^{18}F]-FEPPA) expressed as standard uptake value ratios (SUR) in the frontal cortex, temporal cortex, and basal ganglia (striatum) during baseline and 1 day, 1 week, and 2 weeks post sevoflurane exposure (8 h). Data are shown as the means ± SDs.

values]. No monkey suffered from hypoxia as indicated by ≥ 90% blood oxygen saturation. Results from microPET imaging revealed that the 8 h sevoflurane exposure did not cause significant neural inflammatory responses. There were no significant differences in FEPPA uptake (SURs) in the frontal cortex, temporal cortex, and basal ganglia 1 day, 1 week, and 2 weeks post-exposure (See Fig. 2). Similarly, sevoflurane exposures did not significantly impact performance in the CPR, TRD, DMTS, and PR tasks at any of the time points observed (See Figs. 3 and 4; observing and choice response latency data for CPR and DMTS not shown). However, the 8 h experimental exposure to sevoflurane (Exposure 2) significantly decreased accuracy [Mean difference = 22.79, $t(3) = 6.92$, $p = 0.006$] in the IRA task (See Fig. 5).

4. Discussion

This study employed microPET/CT technology and a battery of operant tasks to examine the effects of a prolonged exposure to sevoflurane anesthesia on neural markers of inflammation (increased TSPO expression) and various aspects of cognitive function in 5 adult rhesus macaques. Results revealed that an 8 h exposure to sevoflurane anesthesia produced a mild, transient effect on learning task performance, while having no effects on neural inflammatory responses. Further,

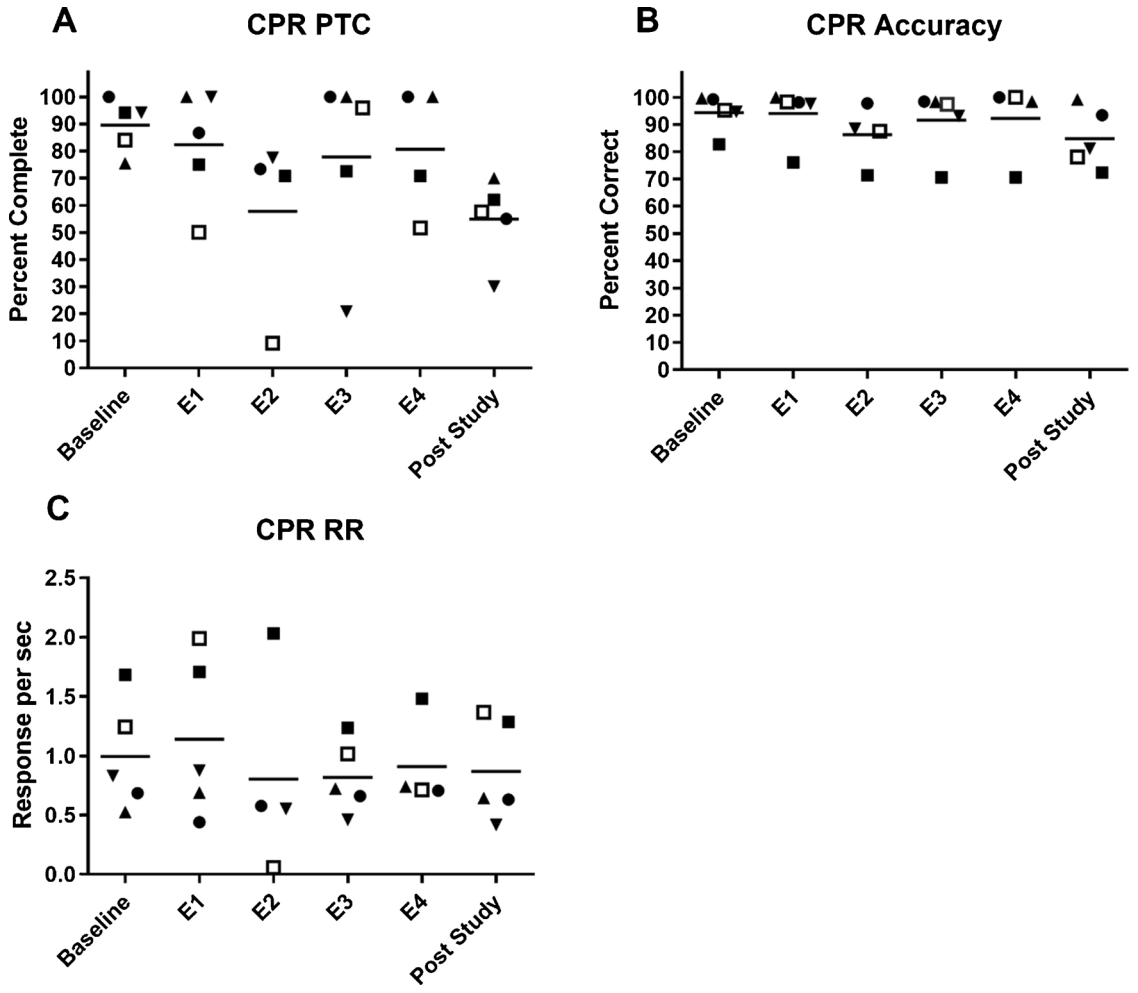

Fig. 3. Scatterplot depicting: (A) percent task completed (PTC), (B) accuracy, and (C) response rate (RR) in the conditioned positioned responding (CPR) task. Baseline = average performance for the 10 test days prior to the baseline microPET; Exposure 1 (E1) = average performance for the first 6 days post the 2 h sevoflurane exposure required to obtain the baseline microPET; Exposure 2 (E2) = average performance for the first 6 days post 8 h experimental sevoflurane exposure plus the 2 h sevoflurane exposure required for the microPET scan on the day following the experimental 8 h exposure; Exposure 3 (E3) = average performance for the first 6 days following the 2 h sevoflurane exposure required for the microPET scan 1 week after the 8 h experimental exposure; Exposure 4 (E4) = average performance for the first 6 days following the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the experimental exposure; Post Study = average performance for days 7–21 following the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the 8 h experimental exposure.

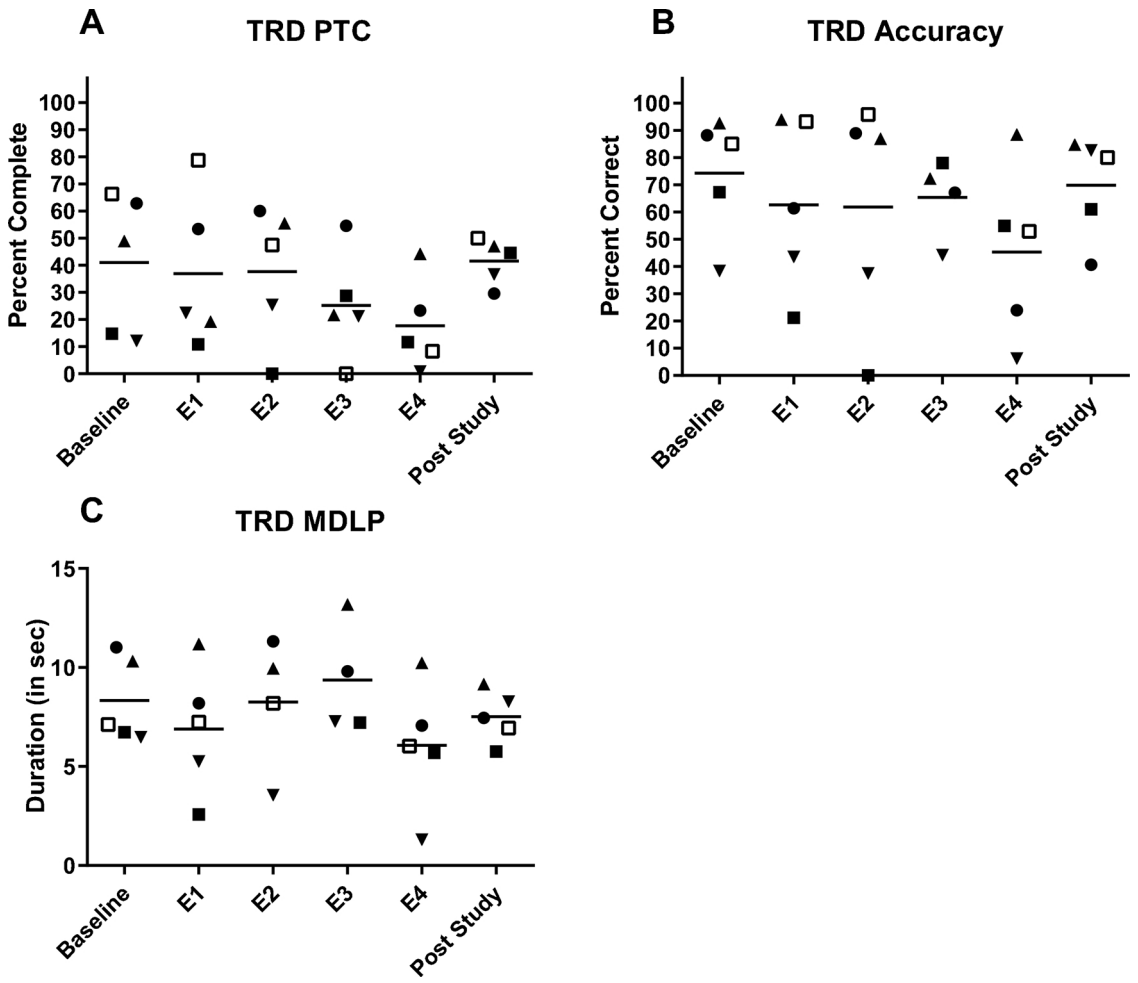

Fig. 4. Scatterplot depicting: (A) PTC, (B) accuracy, and (C) mean duration lever press (MDLP) in the temporal response differentiation (TRD) task. Baseline = average performance for the 10 test days prior to the baseline microPET; Exposure 1 (E1) = average performance for the first 6 days post the 2 h sevoflurane exposure required to obtain the baseline microPET; Exposure 2 (E2) = average performance for the first 6 days post 8 h experimental sevoflurane exposure plus the 2 h sevoflurane exposure required for the microPET scan on the day following the experimental 8 h exposure; Exposure 3 (E3) = average performance for the first 6 days following the 2 h sevoflurane exposure required for the microPET scan 1 week after the 8 h experimental exposure; Exposure 4 (E4) = average performance for the first 6 days following the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the experimental exposure; Post Study = average performance for days 7–21 following the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the 8 h experimental exposure.

sevoflurane anesthesia did not impact performance of cognitive tasks designed to specifically measure short term-memory, time perception, visual discrimination, and motivation. These findings suggest that general anesthesia alone may cause minor POCD in adult populations; however, this effect is transient and unlikely related to neural inflammation. Importantly, because general anesthesia can cause nausea, and the OTB requires an animal to work for food, it is also possible that the observed effects are related to feeling unwell after the extended exposure as opposed to any true cognitive defect.

The only measure impacted by sevoflurane exposure was performance in the IRA task. This task required monkeys to learn a novel sequence of lever presses each session, a behavior believed to be highly dependent upon the integrity of the frontal cortex and hippocampus (Kesner and Churchwell, 2011; Ketchum et al., 2016). Nonetheless, the impairment in sequential learning observed here was transient and only persisted for approximately 1-week post exposure. These findings contrast with a previous study in rhesus monkeys that revealed a 24 h exposure to ketamine anesthesia during the first week of life produced deficits in IRA performance (Paule et al., 2011) lasting years. Seeing as performance in all other operant tasks here remained intact and there was no effect on neural inflammatory responses, these findings suggest that the adult monkey brain is very resistant to the neurotoxic effects of general anesthesia in comparison to the developing monkey brain. While it is possible that cognitive impairments could have been observed if a different behavioral battery was used, we believe that in this experimental cohort extended sevoflurane had little or no effect on cognitive function.

The finding that sevoflurane anesthesia had minimal impact on cognitive function in adult monkeys is consistent with previous studies in adult rodents that examined the long-term effects of general anesthesia on cognitive function. For example, Callaway et al., (2012, 2015)showed that a 4 h exposure to either sevoflurane or desflurane anesthesia had no impact on spatial learning and memory retention in the Morris Water Maze (MWM) in adult rats when assessed for up to 3 months post exposure (2012, 2015). Likewise, a 2 h exposure to isoflurane anesthesia had no impact on radial arm maze performance in adult rats 2 weeks after exposure (Crosby et al., 2005).

The finding that sevoflurane anesthesia had no effect on microPET markers of microglia activation is also in agreement with previous research in rodents. Wang et al. (2015) demonstrated that although isoflurane anesthesia increased Iba-1 expression and levels of the proinflammatory cytokines, IL-1β and TNF-α, in the hippocampus of aged mice, it had no impact on these measures in adult mice. Similarly, Kawano et al. (Kawano et al., 2018) revealed that isoflurane anesthesia did not impair trace fear conditioning nor did it alter levels of IL-1β and TNF-α in adult rats; nonetheless, impairments in these measures were

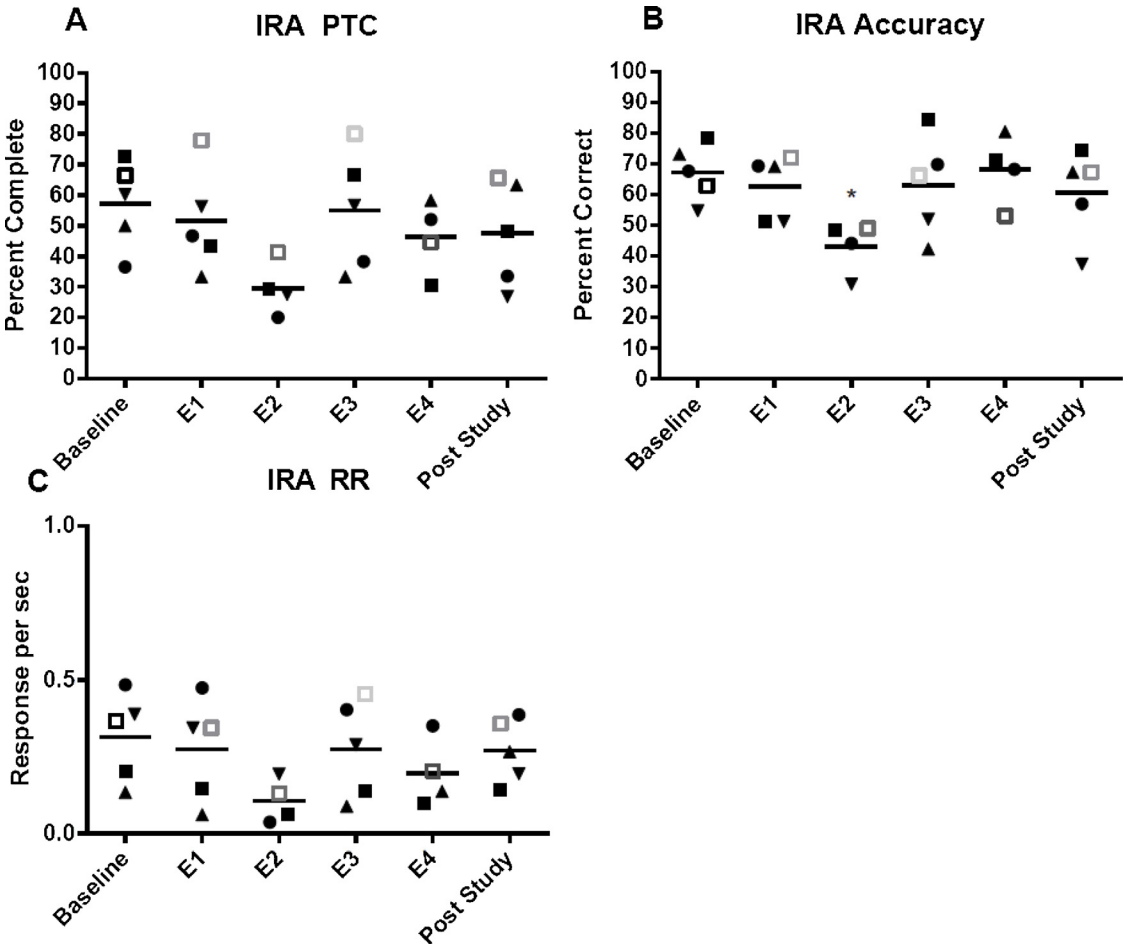

Fig. 5. Scatterplot depicting: for (A) percent task completed (PTC), (B) accuracy, and (C) response rate (RR) in the incremental repeated acquisition task (IRA). Baseline = average performance for the 10 test days prior to the baseline microPET; Exposure 1 (E1) = average performance for the first 6 days post the 2 h sevoflurane exposure required to obtain the baseline microPET; Exposure 2 (E2) = average performance for the first 6 days post 8 h experimental sevoflurane exposure plus the 2 h sevoflurane exposure required for the microPET scan on the day following the experimental 8 h exposure; Exposure 3 (E3) = average performance for the first 6 days following the 2 h sevoflurane exposure required for the microPET scan 1 week after the 8 h experimental exposure; Exposure 4 (E4) = average performance for the first 6 days following the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the experimental exposure; Post Study = average performance for days 7–21 following the 2 h sevoflurane exposure required for the microPET scan 2 weeks after the 8 h experimental exposure. * = $p < 0.01$ (Bonferroni correction applied).

observed in rats that underwent anesthesia + abdominal surgery. While our results do generally replicate what has previously been reported, our investigation was limited by the lack of non-TSPO based microPET markers for neuroinflammation.

The monkeys exposed to sevoflurane anesthesia in the present study were 16–17 years of age. The average lifespan of rhesus macaques living in captivity is 26 years, and they are typically considered old after approximately 20 years where they begin to show significant signs of physical decline (Colman, 2017). Thus, the monkeys in the current study were considered to be a “mature adult” rather than a “geriatric” model of general anesthesia. This again suggests that the middle-aged brain may be more resistant to the neurotoxic effects of general anesthesia in comparison to the more vulnerable aged brain, which is known to have an elevated baseline proinflammatory profile and a decreased ability to recover from CNS insults (McLinden et al., 2012; Sparkman and Johnson, 2008; Ye and Johnson, 1999).

It is important to note that the data presented here were collected from 5 adult monkeys utilizing a within-subjects design. In humans, POCD has been reported to occur in 30.4% of middle-aged adults at hospital discharge and in 19.2% of middle-aged 7 days after surgery (Monk et al., 2008). If anesthesia causes a sporadic “all or nothing” impairment, rather than a reliable graded decrement in performance, a substantially larger sample size would be necessary to detect impairments in cognition. Furthermore, not all OTB endpoints are equally sensitive. However, we used a liberal statistical approach to investigate the effects of sevoflurane anesthesia on OTB performance and little was seen. The inclusion of the Bonferroni correction made little difference on the number of significant endpoints, and there was no lasting trend in response to sevoflurane.

In addition, the neurotoxic effects of sevoflurane anesthesia were assessed using a surrogate marker of neuroinflammation. Thus, it is possible that sevoflurane exposure could have impacted other indices of neurotoxicity not measured here. Further, the monkeys used here did not undergo surgery, and they were relatively healthy. They did not have any underlying medical conditions and, therefore, were not taking any medications to treat chronic ailments. Thus, this study did not evaluate the ability of general anesthesia to interact with other variables shown to be associated with the development of POCD, such as inflammation induced by surgery (Kapila et al., 2014; Skvarc et al., 2018), preexisting medical conditions (Gvozdenovic and Antanaskovic, 2015; Hudetz et al., 2007; Messerotti Benvenuti et al., 2014; Silbert et al., 2015), and concurrent drug exposures (Shoair et al., 2015). While we did not find evidence for POCD, these data will help to define the conditions under which POCD is not likely to occur. Indeed, these data indicate that POCD is not likely to occur either in the absence of a medical procedure, or in a middle-aged population. However, it is

possible that an effect of sevoflurane might have been observed if different aspects of cognition had been evaluated, or if different markers of inflammation were used.

In conclusion, the present study found that an 8 h exposure to sevoflurane anesthesia did not increase microPET measures of neural inflammatory responses, nor did it have long lasting impairments on cognitive function in 5 healthy adult rhesus macaques. To our knowledge, this was the first study that assessed the neurotoxic effects of exposure to general anesthesia in adult monkeys. While the sample size is limited, these data suggest that anesthesia alone is not sufficient to cause POCD in a "mature" adult population. As such, future research should focus on either true geriatric populations or investigate factors which could contribute to the development of POCD like the co-occurrence of surgery, pathology related to Alzheimer's disease, or the use of other medications in conjunction with anesthesia.

Disclaimer

This document has been reviewed in accordance with United States Food and Drug Administration (FDA) policy and approved for publication. Approval does not signify that the contents necessarily reflect the position or opinions of the FDA nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the FDA.

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Conflict of interest

Authors John Chelonis, Xuan Zhang, Charles Fogle, Mi Li, and John Chelonis have worked for the Food and Drug Administration for the last 3 years and declare no conflict of interest. Jennifer Walters is currently at Washington State University, and prior to that was also at the Food and Drug Administration and has no conflict of interests. Merle Paule retired 9 months ago from the Food and Drug Administration and has no conflict of interest. John Talpos has worked for the Food and Drug Administration for the last 32 months. Prior to that John Talpos worked for Janssen Pharmacutica (Belgium) and has no conflict of interest with the publication of this material.

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