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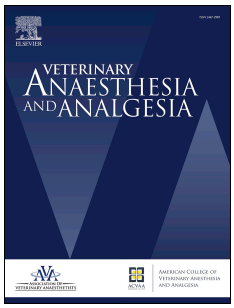

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FULL RESEARCH STUDY

The effect of atropine and propofol on the minimum anaesthetic concentration of isoflurane in the freshwater turtle *Trachemys scripta scripta (yellow-bellied slider).*

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Running head: Atropine reduces $MAC_{isoflurane}$ in terrapins.

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The authors declare no conflict of interest

CW, MFB, AKOA and TW conceived the study, all authors contributed to study design, LK, JQZ, SMH and CW carried out the experiments, LK and CW prepared the manuscript and all authors contributed to its editing and approved the final version.

Abstract

Objective To determine if the administration of atropine would reduce the measured minimum anaesthetic concentration of isoflurane ($MAC_{isoflurane}$) in freshwater turtles, the yellow-bellied slider, *(Trachemys scripta scripta).*

Study design Paired, blinded, randomised, prospective studies of i) the effect of atropine in isoflurane anaesthetized freshwater turtles (*Trachemys scripta scripta*) and ii) the effect of atropine in yellow-bellied sliders in which anaesthesia was induced with propofol and maintained with isoflurane.

Animals *Trachemys scripta scripta* (n = 8), female, adult.

Methods Atropine (2 mg kg^{-1}) or an isovolumetric control injection of saline were administered intraperitoneally 15 minutes prior to induction of anaesthesia with isoflurane. Individual $MAC_{isoflurane}$ was then determined by end tidal gas analysis in a bracketing design by an experimenter blinded to the administered drug, with a 2-week washout period. The experiment was repeated, with atropine (2 mg kg^{-1}) or saline administered intravascularly in combination with propofol for anaesthetic induction. Linear mixed modelling was used to determine the effects of atropine and propofol on the individual $MAC_{isoflurane}$. Data are presented as mean ± standard deviation.

Results Premedication with atropine significantly reduced $MAC_{isoflurane}$ (p = 0.0039). In isoflurane induced *Trachemys scripta scripta,* $MAC_{isoflurane}$ decreased from 4.2 ± 0.4% to 3.3 ± 0.8% when atropine had been administered. Propofol as an induction agent had a MAC-sparing effect ($p < 0.001$) such that $MAC_{isoflurane}$ following propofol and a control injection of

saline was 2.3 ± 1.0%, which decreased further to 1.5 ± 0.8% when propofol was combined with atropine.

Conclusions and clinical relevance Atropine, presumably by inhibiting parasympathetically mediated pulmonary artery constriction, decreases right-to-left cardiac shunting and the $MAC_{isoflurane}$ in yellow-bellied sliders, and thereby may facilitate control of inhalant anaesthesia. Propofol can be used for induction of anaesthesia and reduced the required concentration of inhaled anaesthesia assessed 1.5 hours following induction.

Keywords atropine, cardiac shunting, chelonians, MAC, propofol, reptile**s**

Introduction

Inhaled anaesthetics act upon the central nervous system (CNS) after diffusion across the alveolar surface of the lung, or faveolar surface in reptiles, and transport via the cardiovascular system. The efficacy of inhalation anaesthetics therefore depends in part on the ability of the cardiopulmonary system to convey the anaesthetic to the CNS. The potency of inhaled anaesthetics is typically described in mammals by the minimum alveolar concentration (MAC) required to prevent 50% of a population responding to a supramaximal nociceptive stimulus (Quasha et al. 1980). This stimulus is usually surgical incision in humans, or electrical or mechanical stimulation in animals (Quasha et al. 1980). MAC is determined as the alveolar anaesthetic concentration expressed in volumes percent. This volume percent represents an alveolar partial pressure of that anaesthetic, which at equilibrium equals that of the arterial blood and highly perfused tissues such as the CNS. MAC can be reduced by co-administration of other anaesthetic or analgesic drugs and premedication with sedatives (Quasha et al. 1980; Larouche et al. 2019).

It has proven difficult to determine MAC in reptiles, (defined as the mean anaesthetic concentration, since reptiles have faveoli rather than alveoli (Glass & Wood 1983, Bertelsen 2019) particularly in turtles. We recently provided theoretical evidence that reptiles' low minute ventilation and potential for large cardiac right-to-left (R-L) shunts profoundly slow equilibration and uptake of volatile anaesthetics (Williams et al. 2020). This makes the determination of reptile MAC more challenging. Reptiles have a high capacity for cardiac shunting owing to incomplete anatomical division of the ventricle (Hicks 1998). Recirculation of systemic venous blood into systemic arteries (R-L shunt) lowers arterial oxygen partial pressure and slows the uptake of inhaled anaesthesia (Eger & Severinghaus 1964; Tanner et al. 1985; Williams et al. 2020). In reptiles, the magnitude of R-L shunting is under parasympathetic regulation, where vagal innervation of the pulmonary artery induces

constriction of pulmonary vasculature, which reduces pulmonary blood flow and directs blood flow towards the systemic circulation (Garcia-Parraga et al. 2018; Filogonio et al. 2020). Consistent with these considerations, pharmacological inhibition of the vagal innervation of pulmonary vasculature by an infusion of atropine, an antagonist of the muscarinic receptors, reduces intracardiac R-L shunt and reduces $MAC_{isoflurane}$ in tortoises (Greunz et al. 2018).

Freshwater turtles, such as *Trachemys spp.,* exhibit large cardiac shunts that change markedly with changes in ventilation and have been extensively studied (Burggren 1975; Wang & Hicks 1996). Given their large capacity for R-L shunting, freshwater turtles are ideal subjects for investigating the effect of anti-cholinergic drugs such as atropine on the $MAC_{isoflurane}$ in this species. Since they are commonly kept as pets (Pendelbury 2010), this information would help to optimise the anaesthesia protocols used in this species. Furthermore, propofol is often used to induce anaesthesia prior to intubation, and it is therefore clinically relevant to investigate whether propofol, at appropriate doses [10-20 mg kg^{-1}; (Ziolo & Bertelsen 2009)], leads to a transient reduction in MAC.

Therefore, we hypothesized that the administration of atropine would reduce the MAC value of isoflurane in this species. We also hypothesized that induction of anaesthesia with propofol would lead to a further reduction in MAC.

Methods and materials

This blinded and randomised study used eight female individually-identified *Trachemys scripta scripta.* This sample size was calculated based on comparison with previous literature (Bertelsen et al. 2005; Greunz et al. 2018), and follows calculations presented in (Larouche et al. 2019) with significant effect size set as 0.75% difference in F_E'Iso constituting MAC between two treatments, and estimated standard deviation of MAC in reptiles with known

cardiac shunts of 0.48% F_E'Iso, and using values of α and β errors of 0.05 and 0.2 respectively.

Freshwater turtles were housed in 1000 L tanks containing 800 L water (26 °C) with free access to a basking spot (30-35 °C) for behavioural thermoregulation with basking ultraviolet light A & B and heat provided by 160 W EXOTerra lamps (Hagen Deutschland GmbH & Co. KG, Holm, Germany). Animals were fed twice weekly with commercial catfish pellets (EFICO Alpha 838F, BioMar SAS, France), fresh greens, fruits and freshwater plants and Biorept Sticks (Tropical, Poland). The photoperiod was maintained at 12:12 hour light:dark cycles. Individual age was unknown as all freshwater turtles had been in private collections before donation and arrival at the facility. Animals were judged healthy based on clinical examination and behaviour and had been acclimatised at the facility for several months prior to the period of experimentation. Freshwater turtles were fasted for at least 24 hours prior to experiments to prevent digestion-related heart rate (HR) changes (Wang et al. 2001) and alleviate any consequences of atropine on gastric motility.

The study consisted of two parts: the first experiment investigated the effect of atropine on $MAC_{isoflurane}$, while the second experiment determined whether atropine affected $MAC_{isoflurane}$ following the induction of anaesthesia with propofol, since this is a commonly used drug in clinical practice. The study was performed under ethical and legal permit from the Danish Licensing Authority no. 2015−15−0201−00684.

A crossover design was used, where each animal was anaesthetized twice in each experiment, once with a saline control and once with atropine. Animal order was randomized by lottery (drawn from a bag of numbered lots, each one representing a specific animal) and first treatment randomisation was performed using www.random.org. Every animal had a recovery period of at least 14 days between treatments. End-experimental body weight of the freshwater turtles was 824 ± 155 g [mean ± standard deviation (SD)] and 957 ± 162 g for

111 experiments 1 and 2, respectively. In experiment 2, each study started at the same time of day
112 (± 1 hour), and MAC was obtained no earlier than 55 minutes after propofol injection.
113 Atropine sulphate salt (Sigma, Germany) was dissolved in sterile saline (0.9 % NaCl,
114 Fresenius Kabi, Germany), to give an atropine concentration of 1 mg mL^{-1} in experiment 1
115 for wide intracoelomic dispersal and uptake, and 2 mg mL^{-1} in experiment 2 for practical
116 intravascular administration). Atropine was administered in both experiments at a dose of 2
117 mg kg^{-1} to ensure efficacy during the long procedure (Cruz et al. 2014; Greunz et al. 2018).
118 Aliquots were stored at -18 °C. All drugs: atropine, lidocaine hydrochloride (Lidocaine 20 mg
119 mL^{-1}; Mylan, UK), and propofol (Propofol-Lipuro 10 mg mL^{-1}; B. Braun, Germany) were
120 administered at room temperature (~21°C). The animal selected on each study day was
121 weighed and placed on a heat mat (Melissa, Denmark) set at 28-30 °C (Espindola et al. 2019)
122 prior to drug injection.
123
124 Experiment 1 induction
125 Atropine (2 mg kg^{-1}using a 1 mg mL^{-1} solution in saline) or a control isovolumetric saline
126 injection was administered via the intracoelomic route in the left pre-femoral area and the
127 freshwater turtle then visually monitored for spontaneous movement, head and leg tone in a
128 plastic box (40 cm x 30 cm x 20 cm) for 15 minutes. The intracoelomic route was used in this
129 group to minimize the stress of injection in unsedated animals. Sedation was not used as
130 single agent anaesthesia was required for the initial MAC study. The freshwater turtle was
131 then moved to an induction box with 0.25 mL isoflurane L^{-1} of induction chamber volume on
132 paper towel (Tork, Essity, Denmark) out of direct animal contact within a fume hood (Greunz
133 et al. 2018; Bertelsen 2019). Sedation level was assessed visually every 5 minutes, tracheal
134 intubation was attempted when reactive movement subsided and head and pectoral limbs

were relaxed. A maximum time of 90 minutes was allowed before attempted intubation.

Experiment 2 induction

Both atropine (2mg kg^{-1}, or an isovolumetric control injection of saline) and propofol (15 mg kg^{-1}) were co-injected intravascularly into the subcarapacial sinus via a 23-gauge (0.64 x 30 mm, Henke Sass Wolf, Germany) needle at 100^{o} relative to the syringe (Ziolo & Bertelsen 2009). The needle bevel was directed dorsally, the needle angled toward the dorsum of the shell and inserted approximately 0.5 cm ventral to the carapace and dorsal to the neck on the dorsal midline. Negative pressure was used to confirm correct positioning via blood withdrawal before and mid-injection (Stegmann et al. 2017). If positive reflexes persisted after 3 minutes (Ziolo & Bertelsen 2009), the animal was re-injected with half the propofol dose. If induction of anaesthesia was unsuccessful after a maximum of three injections, the experiment was terminated, and the animal placed in recovery with a 14-day withdrawal before re-entering the protocol.

Anaesthetic maintenance and MAC assessment

After successful induction of anaesthesia, the animal was placed on the covered heating mat. Lidocaine (0.05 mL dose in all animals, of 20 mg mL^{-1} lidocaine hydrochoride) was applied topically to the glottis and an endotracheal tube (ETT) matching glottal diameter was inserted via the glottis and taped to the mandible. ETT specifics: 17-24 gauge (0.6-1.5 mm) modified to 5-6 cm length from intravascular catheters (Venflon Pro and Nexiva; BD, NJ, USA) or orogastic tubing (Fuchigami, Japan). The ETT was connected to an anaesthetic circle breathing system (Anesthesia Workstation; Hallowell EMC, MA, USA) with an agent-specific vaporiser (Northern, UK). Capnography was used to confirm adequacy of ventilation from the capnograph waveform (Cardell Touch Veterinary Monitor 8013-001, Midmark

Animal Health, OH, USA) and an AX+ pre-calibrated mainstream gas-analyser (Masimo, CA, USA). If a leak was suspected, the animal was reintubated with an ETT a size larger, and this was defined as the point of successful intubation. Minute volume was set at 150 mL $minute^{-1}$ kg^{-1} consisting of 36 mL kg^{-1}, 4.1 breaths $minute^{-1}$, 6-10 cmH_2O maximum airway pressure using 0.65 L $minute^{-1}$ oxygen flow (Mans et al. 2019; Doneley et al. 2018). The ventilation system was initially flushed with isoflurane at 2.5% or 3.5% (Greunz et al. 2018), and the initial setting was adjusted to the MAC of the last animal from that treatment (Experiment 1). Cloacal temperature was measured using the temperature probe of the Cardell Monitor and maintained using the covered heating pad and hot water filled gloves dorsally if required (Espindola et al. 2019).

The following variables were recorded every 5 minutes: cloacal temperature, airway pressure (via the Hallowell EMC), end-tidal isoflurane (F_E'Iso), inspired Isoflurane (F_I'Iso), end-tidal CO_2 (P_E'CO_2) (via the AX+ analyzer) and heart rate (HR) using a doppler probe positioned over the brachial artery (Nicolet Vascular Elite no. 100, Natus Medical, Denmark) with aqueous non-irritant gel (Aquasonic 100, Parker Laboratories Inc, NJ, USA) which was also applied as lubrication to the temperature probe. The eyes were lubricated if open, once following induction, also with non-irritant gel (Neutral, Optha, Denmark). Palpebral or, when lost, corneal reflexes and assessment of head tone and limb muscle tone were recorded. Palpebral and corneal reflexes were assessed using a light digital touch, and coded present or absent. Assessment of head, jaw and limb tone was by manually raising the head, opening the jaw and withdrawing the limbs, and coded present or absent. Reflexes present/lost for more than 5 minutes (equal to two consecutive measurements) were considered regained/lost in analysis.

Steady F_E'Iso and F_I'Iso were attained, defined by a maximum difference of 0.1% in the preceding 20 minutes. Then, the supramaximal stimulus was delivered as a standardized

pelvic limb interdigital pinch administered and assessed by an experimenter blinded to treatment. The stimulus was applied until a positive response was obtained or for a maximum duration of 1 minute, with a Mayo-Hegar needle holder (18 cm tips blunted with tape, set to its first auto-static clamp, Aesculap, PA, USA). The same instrument was used on all animals. Between each pinch, limb and digit were switched, and response was recorded with a camera (TV-IP 572-WI, Trendnet. Inc, CA, USA). The response was evaluated positively if the animal retracted the stimulated limb and purposefully moved other parts of the body. If the response was positive, inspired percent of isoflurane was increased by 10-20% of its previous value. In case of a negative response, the isoflurane level was set to a 1% lower setting on the vaporiser. In both instances, the response was reassessed after 20 minutes of stable readings. When a positive response was followed by a negative response, MAC was recorded as the average of the F_E'Iso values at the two points (Quasha et al. 1980), and isoflurane flow was ended. MAC determination within 2.5 hours of successful intubation was used given that MAC in reptiles has been reported to decline with time of anaesthesia as initial equilibration is slow (Barter et al. 2006, Williams et al 2020), and to limit the variability of time following propofol induction in experiment 2. If a positive then a negative response was not achieved within 2.5 hours, isoflurane administration ceased, and MAC was recorded as the mean of the F_E'Iso at a negative response followed by a positive response, or the last measured value following repeated positive or negative responses. The effect of including or excluding the data from animals where a positive then a negative response was not possible, was determined in the MAC analysis.

Recovery

After isoflurane delivery ended, mechanical ventilation was maintained, and the recurrence of spontaneous ventilation, limb withdrawal in response to limb extension, palpebral reflexes

and temperature were checked every 5 minutes. The animal was extubated when multiple limb withdrawal responses were present, or it attempted self-extubation. Recovery from anaesthesia was defined as positive limb withdrawal and palpebral reflexes, spontaneous breathing and spontaneous movements as well as strong head tone. The animal was then placed in a box (40 x 30 x 19 cm, SmartStore, Denmark) with air circulation and water provided in a thermostatically controlled chamber (Gram, Jumo Dtron 08.1 Tempatron TT32 programmable Digital Timer, Denmark) at 30 °C and 12:12 hour light:dark cycle. Each animal was checked and recovered for at least 20 hours before it was returned to the original tank.

Statistical analysis

Data were analysed using the statistical program R Studio (R Studio Team 2015). Visual assessment of $MAC_{isoflurane}$ data with standard residual *versus* fitted values graph showed homogenous variance and a Shapiro-Wilk test confirmed normal distribution of data ($p > 0.05$). A linear mixed model with the individual as a random effect was chosen to analyse MAC and recovery time data using the nlme package. Setting treatment order as a nested random effect did not change the significance of the model, but better captured the experimental design, and was therefore used. Model selection confirmed that additional variables did not increase the validity of the model. The significance of treatment (e.g., propofol or atropine) was assessed via likelihood ratio tests (Zuur et al. 2009; Winter 2019).

HR data for both positive and negative responses to supramaximal stimuli were analysed. HR were not normally distributed, and a linear mixed model with the individual as a random effect was chosen to analyse these data, given the low dependence of the data analysis on normality of residuals (Winter 2019). Individual temperatures were included in HR data analysis. The linear models used, with package citations, for HR data, data from the

order of reflex loss and resumption and times to extubation and recovery are presented in the supplemental material with discussion of the results. Actual power and effect size of the experiments were analysed using package (pwr). All data in the text, figures and supplementary material are presented as mean ± standard deviation (SD) unless stated otherwise, statistical significance was defined at $p < 0.05$.

Results

Minimum anaesthetic concentration (MAC) assessment

$MAC_{isoflurane}$ was significantly reduced in both induction protocols by the administration of atropine (df = 19, F = 10.7792, $p = 0.0039$) and by propofol induction (df = 19, F = 48.9, $p < 0.001$) with both atropine and control treatments. Atropine decreased $MAC_{isoflurane}$ in seven of the eight individuals (Fig.1), with an overall $MAC_{isoflurane}$ of 4.2 ± 0.4% in the control group and a mean of 3.3 ± 0.8% when atropine was given prior to isoflurane induction. Following the administration of propofol $MAC_{isoflurane}$ was 2.3 ± 1.0% with the control treatment and 1.5 ± 0.8% after the administration of atropine). Fig.1 graphically represents these data, while Supplementary Table S1 reports these data in the context of $MAC_{isoflurane}$ in other reptile species. Effect sizes of atropine administration were large for both isoflurane and propofol inductions (Cohen's D = 0.91 and 0.61 for experiments 1 and 2 respectively). From a total of 32 trials (eight animals x four MAC determinations), in 24 trials a positive then a negative MAC bracket was successfully completed. The significant effect of atropine was robust to removal of datapoints where no second MAC bracket was possible in experiment 2 (four trials). In another four trials (experiment 2) it was not possible to determine a positive then a negative MAC bracket within the 2.5-hour period from anaesthetic induction, and MAC was determined from a negative then a positive MAC bracket. Induction of anaesthesia with propofol decreased $MAC_{isoflurane}$ in all individuals (Fig. 1). Repeat dosing of propofol was required in five out of

16 trails in experiment 2, and the average propofol dose required to enable intubation was 17.8 mg kg^{-1}. From the animals where repeated propofol injections at induction were required to enable intubation, there was no effect of repeat propofol injection on $MAC_{isoflurane}$. All animals completed the study. However, one turtle was anaesthetised on two separate occasions with a 2- week withdrawal because induction of anaesthesia with propofol was unsuccessful when first randomly allocated. Temperature at the time of MAC measurement was 29.0 ± 0.3 °C in experiment 1 and 29.6 ± 0.8 °C in experiment 2. Further details of reflex responses, HR and recovery are presented in Supplementary Figs 1-4 and Tables S2a and b.

Discussion

In this study we showed that atropine decreased the $MAC_{isoflurane}$, both with and without the use of propofol for induction of anaesthesia in *T. scripta scripta*. This finding confirms our hypothesis that, based on a presumed mechanism of a decrease in R-L shunting following atropine injection, MAC of isoflurane was reduced. This mechanism was present and active during the period of MAC reduction following atropine administration in a related species - *Chelonoidis carbonaria*. The mechanism of atropine's effect, preventing pulmonary artery constriction, and thus reducing R-L shunt has been studied in *Trachemys scripta* (Greunz et al. 2018; Wang & Hicks, 1996). As shunts vary between individuals, the administration of atropine could be predicted to reduce inter-individual variability in $MAC_{isoflurane.}$ However, the variability of $MAC_{isoflurane}$ differed between the induction treatments (SD of 0.8 $\%_{iso}$ in atropine alone, 0.8 in atropine after propofol, 0.4 in control alone and 1.0 in control with propofol). Thus, atropine alone does not decrease the intra-individual variability in $MAC_{isoflurane}$ in *T. scripta*. This may result from a limitation of the isoflurane vaporiser since they have a maximum output of 5%, - so variability in the control group may be artificially constrained by the limit of 5% on isoflurane delivery.

Reported reptile $MAC_{isoflurane}$ range between 1.11 - 3.3%, a considerably higher interspecific variation than for mammalian species, where $MAC_{isoflurane}$ ranges from 1.15-1.63% (Quasha et al. 1980; Larouche 2019). The concept of MAC assumes equal anaesthetic partial pressure in alveoli (faveoli in reptiles), the arterial blood, CNS, and venous blood draining the CNS (Quasha et al. 1980). These assumptions are not necessarily upheld when measured in reptiles, because of the possibility of R-L shunted blood and much lower minute ventilation which slow CNS equilibration (Williams et al. 2020). Therefore, MAC in the same individual may vary with or without atropine, as decreased R-L shunting in atropine-treated chelonians (Greunz et al. 2018) results in isoflurane partial pressure in the arterial blood and brain and spinal cord being closer to that in end-tidal gas. Inter-specific variation in reptilians may also be partially dependent on R-L shunt fraction, with pythons exhibiting minimal shunting and the lowest reported MAC, and chelonians at the upper end for both (Greunz et al. 2018; Larouche 2019; Williams et al. 2020). However, interspecific variation is not solely due to shunting, as the highest values of 2.2% and 3.3% were observed in chelonians, where R-L shunting was minimized by atropine treatment (Greunz et al. 2018). High inter-individual variation is also noted in this and other reptilian studies (Barter et al. 2006; Greunz et al. 2018), and may be affected by variation in tissue composition, any covariance with age and sex, and circadian rhythm (Quasha et al. 1980). Although sex and time of day were controlled in the study design, the freshwater turtles in this study had been in different environments prior to their housing at the facility. Their age and historical body condition and tissue composition may have varied greatly, and be part of this unexplained intra-individual variation, as previously reported in mammals (Lemmens et al. 2008; Boveri et al. 2013). The animals were subjectively more active when handled during their second treatment, however treatment order (atropine *versus* saline) did not affect the MAC.

Measuring MAC in chelonians is subject to some anatomical and methodological difficulties. Given large vital capacity of reptiles in general, and specifically the complex ventral lung anatomy of chelonians (Cieri & Farmer 2016), complete mixing of gases within the lung during mechanical ventilation may be less likely, which may complicate the relationship between end-tidal and faveolar gases. Also, the complete tracheal rings of chelonians present difficulties in the accurate measurement of end-tidal gases owing to the potential for leaks with uncuffed endo-tracheal tubes. Hence, differing $P_E'CO_2$ might reflect the potential for leaks as well as differences in the metabolism of the individual animals, and result in differing accuracy in the measurements of F_E'Iso. Together with R-L shunting, these intra and inter-individual variations have previously rendered MAC determination in this species impossible. In this study, despite all efforts to eliminate leaks and provide adequate mechanical ventilation, it was not possible to quantify positive to negative responses in all animals, within a practical time period (2.5 hours from intubation). This time limit was imposed to reduce variation in MAC resulting from duration of anaesthesia, as reported in other reptilian species (Barter et al. 2006). This phenomenon probably reflects the slow equilibration of inhaled anaesthetics in these species (Williams et al. 2020).

The use of propofol reduced the required concentration of volatile anaesthetics in other species (Dzikiti et al. 2011). In the present study, propofol reduced the MAC of isoflurane in *T. scripta* significantly both with and without atropine administration (from 3.3 ± 0.8% to 1.5 ± 0.8% with atropine and 4.2 ± 0.4% to 2.3 ± 1.0% without atropine). The decrease was obtained in all animals. This study used an initial dose of 15 mg kg^{-1} propofol (17.8 mg kg^{-1} mean final administered dose), which should result in at least 60 minutes of anaesthesia in this species, and possibly no longer than 90 minutes at this temperature (Ziolo & Bertelsen 2009). The $MAC_{isoflurane}$ following propofol injection probably varies depending on time from

propofol administration as the propofol is metabolised. The final brackets of $MAC_{isoflurane}$ were completed 91± 26 minutes after first propofol injection, with MAC determined no earlier than 55 minutes after propofol. This suggests that the $MAC_{isoflurane}$ is still significantly affected by propofol at this time. Lidocaine was used topically on the glottis at a set volume that corresponded to a dose of 0.8-1.68 mg kg^{-1}; while any plasma concentrations may theoretically also affect MAC (Quasha et al. 1980), lidocaine plasma concentrations from glottal surface administration were expected to be minimal. The sub-carapacial sinus was used here for propofol administration, as it provided vascular access in this species in unsedated animals (as required to determine MAC) with minimal manual manipulation (Hernandez-Divers et al. 2002). However, it should be noted that there are clinical reports of accidental submeningeal injection and clinical abnormalities following its use for venipuncture and injection in chelonians (Innis et al. 2010; Quesada et al. 2010), and the use of the sub-carapacial/supra-vertebral sinus is therefore not currently recommended in clinical practice across chelonian species. Access to the jugular vein or brachial plexi may be more appropriate, especially in sedated individuals and particularly in terrestrial species with a greater doming of the dorsal carapace (Bertelsen 2019; Mans et al. 2019).

The determination of an effective dose of an anaesthetic is important in all species, as noxious stimuli impair welfare if consciously perceived. Nociception also results in a cascade of physiological changes e.g., increased HR produced by nociceptive stimulation potentially extending to inducing catabolic states and limiting rates of tissue healing (Williams et al. 2019). Hence, use of atropine to decrease R-L shunting during isoflurane anaesthesia may be of clinical value to allow a constant and more controllable anaesthetic level. Surgical anaesthesia usually requires 1.3 x MAC to account for individual variation (Bertelsen 2019), while anaesthetic plane is best judged from local nociception response such as limb

withdrawal to pinch, rather than corneal reflexes.While heart rate and pulmonary flow data collected following atropine injection will be influenced by its physiological effects, the use of propofol for induction, and use of atropine as a premedication where data are collected from animals after an adequate period for atropine's elimination (Cruz et al. 2014; Joyce et al. 2018), can be recommended.

This study suggests that 15 mg kg^{-1} propofol given intravenously for rapid anaesthetic induction and 2 mg kg^{-1} atropine reduce isoflurane MAC when anaesthetizing *T. scripta*, the latter by potentially decreasing R-L shunting. However, the site of propofol injection is subject to clinical judgement based on the species and situation, as discussed above. Future studies can elucidate whether lower doses of atropine, expected to have a shorter active period, yield similar effects and clinical applicability.

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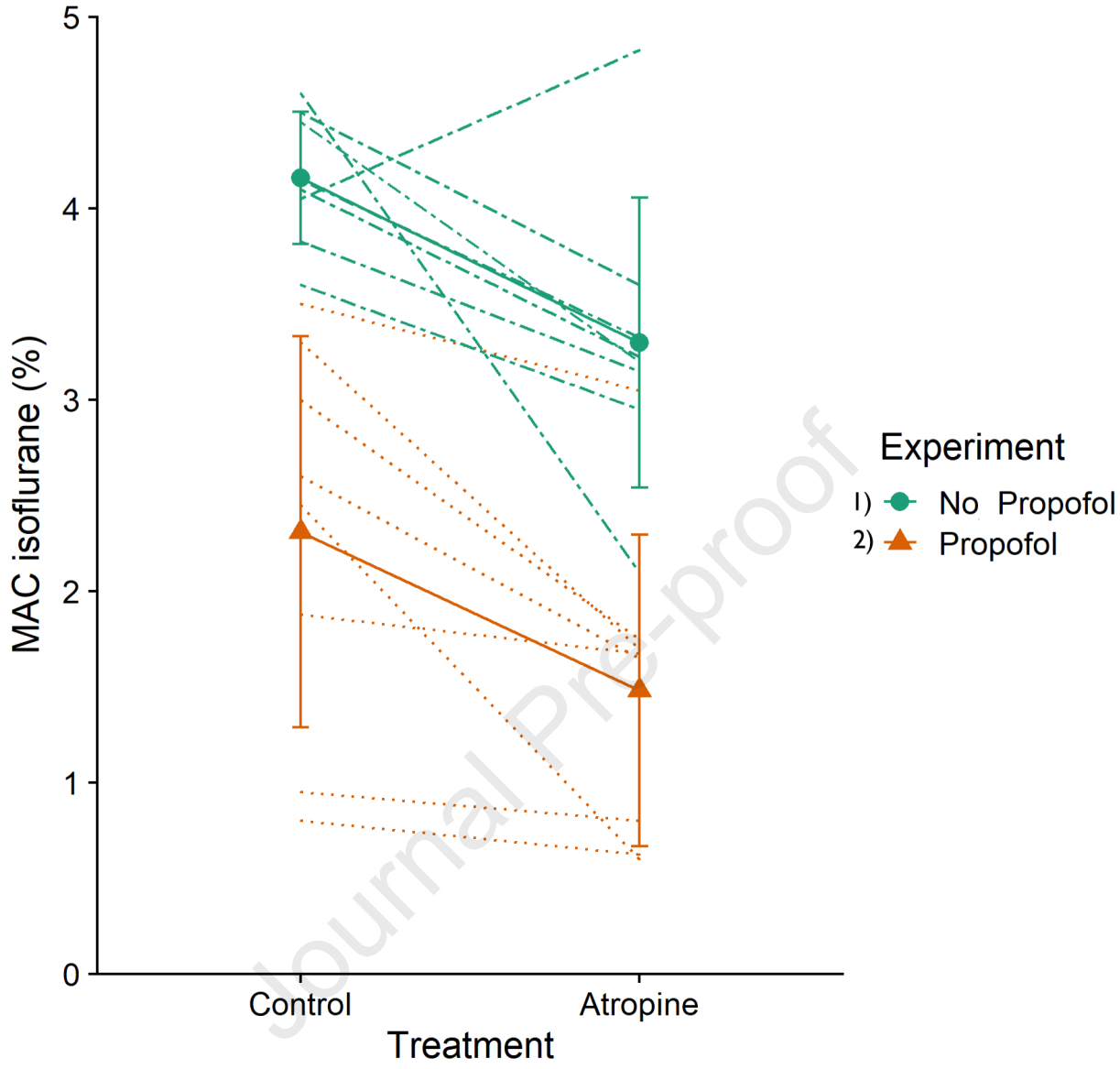

Figure Legends

Figure 1. Minimum anaesthetic concentration (MAC) in *Trachemys scripta scripta* (paired study $n = 8$) as mean ± standard deviation for end-tidal isoflurane (%) with atropine (2 mg kg^{-1}) or isovolumetric control (saline) treatments. Measurements were completed after induction of anaesthesia with isoflurane and intracoelomic injection of atropine or saline (turquoise circles, Experiment 1) and after induction of anaesthesia with propofol (orange triangles, Experiment 2) with intravenous administration of atropine or saline. Dashed turquoise lines indicate the change in MAC with atropine for an individual animal after isoflurane induction (Experiment 1), and dotted orange lines show the effect of atropine in individual animals after propofol induction (Experiment 2). Both atropine treatment ($p = 0.0039$) and propofol treatment ($p < 0.001$) significantly reduced MAC, and this decrease in MAC after atropine administration was present in all but one animal.