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Original Research

Experimental evidence refuting the assumption of phosphorus-31 nuclear-spin entanglement-mediated consciousness

Rong Chen[1,†], Na Li[1,†], Hao Qian[1], Rui-Han Zhao[1] and Shi-Hai Zhang[1,*]

[1]*Department of Anesthesiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 430030, Wuhan, P. R. China*

*Correspondence: zhv@hust.edu.cn (Shi-Hai Zhang)
† These authors contributed equally.

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Phosphorus-31 nuclear-spin entanglements within $Ca_9(PO_4)_6$ molecules (Posner molecules) have been proposed to be central for neural processing. However, this has yet to be proven experimentally. Relatedly, increasing calcium ion concentration in the cerebrospinal fluid has been proposed to enhance consciousness by accelerating Posner molecules' creation. A dependence on calcium isotope is also expected. Here we test these predictions experimentally by measuring the loss of righting reflex ED_{50} for mice to sevoflurane - an increase in loss of righting reflex ED_{50} indicates a higher level of consciousness and *vice versa*. Our mice's findings demonstrate that intracerebroventricular injection of EGTA enhances the sevoflurane-induced loss of righting reflex ED_{50} while injecting calcium-40 chloride or calcium-43 chloride causes an opposite effect. Further, the identical effects of calcium-40 and calcium-43 indicate an absence of calcium isotope dependence. Here, our findings disprove conventional proposals that calcium ion concentration correlates with consciousness.

Keywords

Quantum consciousness; anesthesia; Posner molecules; entanglement

1. Introduction

Consciousness is an unsolved scientific enigma. Diverse quantum models attempt to explain consciousness mechanisms (Fisher, 2015; Hameroff and Penrose, 2014; Jibu and Yasue, 1995; Kumar et al., 2016; Poznanski et al., 2019).

Fisher (2015) first proposed the phosphorus-31 nuclear-spin entanglement model. According to Fisher's proposal, in bulk water, the survival time of nuclear-spin entanglements for phosphorus-31 is too short (within one second) in free phosphate ions due to proton attack. However, if the phosphorus-31 are the constituents of $Ca_9(PO_4)_6$, Posner molecules, its unique structure protects phosphorus-31 nuclear-spin entanglements from proton attack. As a result, the decoherent time lasts as long as 21 days or longer for the entanglements (Swift et al., 2018). Long-lived quantum entanglement is central in quantum consciousness processing (Swift et al., 2018). Thus, Posner molecules play a crucial role in processing consciousness (Fisher, 2015; Swift et al., 2018).

However, though attractive, Fisher's proposal is speculative (Fisher, 2015; Swift et al., 2018) and lacks experimental evidence to support or disprove it. Fisher's entire proposal cannot be tested experimentally, but a partial test is possible because some verifiable predictions can be derived from the proposal (Fisher, 2015). The testable predictions include varying calcium concentrations and the existence of calcium isotope dependence on consciousness (Fisher, 2015). Since Posner molecules are important in enabling phosphorus-31 nuclear-spin quantum entanglement to mediate consciousness in the brain, it is expected that varying calcium ion concentrations in the extracellular fluid would affect Posner molecules formation and accordingly it will influence the levels of consciousness (Fisher, 2015). So, an increase of $^{40}Ca^{2+}$ concentration in the cerebrospinal fluid is expected to increase the number of Posner molecules and increase the level of consciousness. Conversely, a decreased $^{40}Ca^{2+}$ concentration is expected to lower the level of consciousness.

Calcium isotope dependence is also expected in Fisher's proposal (Fisher, 2015). Specifically, $^{43}Ca^{2+}$ would disturb and decohere the phosphorus-31 nuclear-spin entanglements if $^{43}Ca^{2+}$ replaces the $^{40}Ca^{2+}$ within the Posner molecules (Fisher, 2015). Therefore, an increase of $^{43}Ca^{2+}$ concentration in the cerebrospinal fluid would lead to a decrease in the animal's consciousness level, opposite to the effect of $^{40}Ca^{2+}$.

We use the loss of righting reflex (LORR) ED_{50} for mice to sevoflurane to measure consciousness level (Franks, 2008; Franks and Lieb, 1994; Li et al., 2018) to test the predictions mentioned above. LORR ED_{50} closely correlates to the consciousness level; as described in our previous study, an increased LORR ED_{50} indicates increased consciousness level and *vice versa* (Li et al., 2018). By quantifying consciousness levels, we disprove Fisher's proposal because our findings oppose his predictions.

2. Materials and methods

2.1 Animals

C57BL/6 male mice (7 weeks old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (SCXK

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[jing] 2016-0006; P. R. China). The animals were kept in a 12:12 hour cycle of light/darkness with free access to water and food at room temperature (24 ± 1 °C) for a week before experiments. When experiments were performed, the animals were 8 weeks old. All animal operations and experimental protocols conformed to the US National Institutes of Health guide for laboratory animals' care and use (NIH Publications No. 8023, revised 1978). They were approved by the Institutional Animal Care and Use Committee (approval No: S164) at Tongji Medical College, Huazhong University of Science and Technology.

2.2 Reagents

Sevoflurane was obtained from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). Calcium-43 carbonate ($^{43}CaCO_3$) was purchased from ISOFLEX USA (San Francisco, USA) with an abundance of 90%. As the abundance of calcium-40 is 96.9% in nature (Wieser et al., 2004), natural $CaCl_2$ was regarded as $^{40}CaCl_2$ in this study. $CaCl_2$, NaCl, NaOH, and HCl were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, P. R. China). A calcium chelator ethylene glycol-bis (2-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, P. R. China). 0.9% sodium chloride solution was obtained from Baxter Healthcare Co., Ltd. (Shanghai, P. R. China).

2.3 Reagent preparations

The calcium-43 isotope we obtained was calcium carbonate, an insoluble powder. We had to transfer $^{43}CaCO_3$ powder into $^{43}CaCl_2$ solution before use. $^{43}CaCO_3$ powder 0.5 *g* was dissolved in 10 mL HCl solution (1 mol/L) to transfer $^{43}CaCO_3$ powder into $^{43}CaCl_2$ solution. The final concentration of $^{43}Ca^{2+}$ ion was 50 mmol/L, verified by ABL800 FLEX blood gas analyzer (Radiometer Medical ApS, Brønshøj, Denmark). EGTA 0.38 *g* was dissolved in NaOH 1 mol/L. The final concentration of EGTA was 7.5 mmol/L. All injection solutions were prepared in the 0.9% NaCl solution, adjusted to an osmolality of 300 ± 10 mOsm/L by NaCl or tri-distilled water and adjusted to pH values 7.35-7.45 by NaOH or HCl solution. The solutions were filtered through a 0.22-μm filter (Millipore, Bedford, MA, USA) before use.

2.4 Intracerebroventricular injections

Sixty C57BL/6 male mice (8 weeks old) were weighed and randomly divided into four groups, with 15 mice in each group. The mice in the four groups received intracerebroventricular (ICV) injections of 0.9% NaCl as control, EGTA 7.5 mmol/L, $^{40}CaCl_2$ 50 mmol/L, and $^{43}CaCl_2$ 50 mmol/L in a volume of 2 μL, respectively. The dosages of calcium chloride and EGTA injected into mice's lateral cerebral ventricle were determined as previously described (Erickson et al., 1978; Liang et al., 2004).

Basal LORR ED_{50} for each mouse to sevoflurane was determined. A sterile 25-gauge stainless steel guide cannula (RWD Life Science Co., Ltd., Shenzhen, P. R. China) was inserted into the lateral cerebral ventricle following a previously described method (Li et al., 2015; Marsh et al., 1999) with slight modifications. Briefly, under 2% sevoflurane anesthesia, the mouse was placed on a stereotaxic frame (RWD Life Science Co., Ltd., Shenzhen, P. R. China). After disinfecting the surgical area three times with 75% alcohol, a 1 cm midline incision was made behind the eyes toward the cranium's posterior. The skull was exposed, and the periosteum was cleaned by wiping the skull with a sterile cotton swab. A hole 0.5 mm in diameter was made, 0.3 mm posterior and 1.0 mm lateral to the bregma using a dental drill. The guide cannula was inserted 3.0 mm below the skull surface and fixed to the skull with dental cement. A matching stylet was then inserted into the guide cannula to prevent infection and obstruction. After 1 day of recovery, the mouse was sedated with 1% sevoflurane. The stylet was then removed, and a 30-gauge cannula was inserted into the guide cannula. Saline or a test agent was contained in a 5-μL Hamilton syringe. The syringe connected to the cannula was fixed in a syringe pump (RWD Life Science Co., Ltd., Shenzhen, P. R. China). All agents were warmed to 37 °C before infusion. The ICV injection was performed automatically by the pump at a rate of 1 μL/min. A total volume of 2 μL was injected. After injection, the 30-gauge cannula was kept in place for 5 min. The cannula was then slowly pulled out, and the stylet was inserted back into the guide cannula to prevent the backflow of the injected solution. Sevoflurane was then discontinued, and the mouse could recover. When the mouse could move freely, the LORR ED_{50} for the animal to sevoflurane was determined.

2.5 Loss of righting reflex testing

To determine the LORR ED_{50} for mice to sevoflurane, animals were individually placed in an isolated plastic mesh cage fixed in a 1-liter clear plastic chamber. One side of the chamber was connected to the oxygen source and a sevoflurane vaporizer (Aika, Ichikawa Shiseido, Tokyo, Japan). The other side was connected to an infrared gas monitor (BeneView T5; Shenzhen Mindray Bio-Medical Electronics, P. R. China) to measure sevoflurane, oxygen and CO_2 concentrations in real-time. The monitor was chosen because it can provide a precision of 0.01% when sevoflurane concentration is more than 1%, whereas most commercial monitors only provide a precision of 0.1% under the same condition. The chamber was warmed (Li et al., 2018), and the chamber temperature was monitored continuously and maintained at 36.0 ± 0.2 °C.

When the mouse was placed in the chamber, pure oxygen was supplied at a rate of 600 mL/min immediately. When the chamber's oxygen concentration increased to 99%, sevoflurane gas mixed in pure oxygen was provided by the vaporizer. The initial sevoflurane concentration was 1.00% in the chamber. After 15 minutes at that concentration to equilibrate the mouse with sevoflurane gas, the chamber was rotated 180° to place the mouse on its back in a V-shaped trough, and its righting reflex was observed. LORR was defined as the supine mouse unable to turn itself onto all 4 paws three times within 1 min (Kelz et al., 2008). According to the mouse's righting reflex, a stepwise increase or decrease of 0.10% sevoflurane in the chamber was given. Specifically, if the mouse's righting reflex disappeared, a decreased 0.10% sevoflurane concentration was given; otherwise, an increased 0.10% sevoflurane concentration was supplied. After 15 minutes of equilibration at each sevoflurane concentration, the mouse's righting reflex was observed again. LORR ED_{50} was the average value of the two critical sevoflurane concentrations at which the mouse either lost or returned its righting reflex (Fukagawa et al., 2014). During the measurements, the actual concentrations of sevoflurane in the chamber were continuously monitored and adjusted to guarantee that the difference between the actual

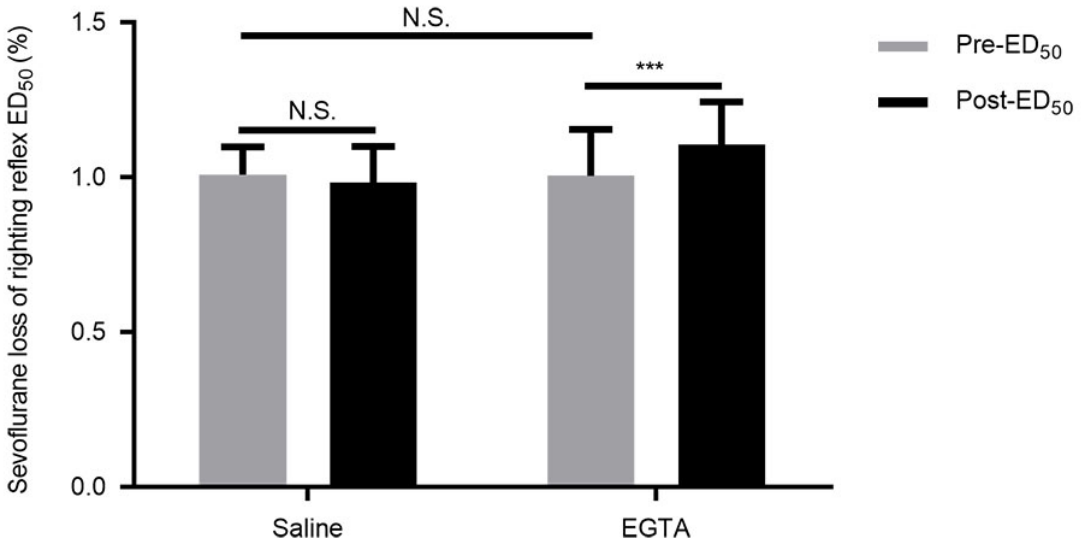

Fig. 1. Effects of normal saline and EGTA on righting reflex ED_{50} for mice to sevoflurane. Pre-ED_{50} denotes the basal LORR ED_{50}. Post-ED_{50} presents LORR ED_{50} after lateral ventricle administration of normal or EGTA. The difference in the values of the pre-ED_{50} between the two groups is not significant. The value of the pre-ED_{50} is comparable to the post-ED_{50} in the saline group. The post-ED_{50} value is significantly higher than the pre-ED_{50} in the EGTA group. Data are shown as mean (SD), n = 12 in the saline group and n = 13 in the EGTA group. ***$P < 0.001$; N.S. = not significantly different (two-way repeated-measures ANOVA followed by the Bonferroni correction as a *post hoc* test).

concentration of sevoflurane and the target concentration was at ± 0.01%. All righting reflexes were observed by a trained observer who was unaware of drug administration. All determinations were made between 8 and 18 o'clock.

After measurements, the mice could recover for 1 week. After recovery, the mice were weighed again. Since weight loss is an overall measure for possible neural pathologic damages that inevitably affect animal feeding, the mice that did not reach the weight a week before were excluded in this study as described previously (Perrin et al., 2004). To confirm successful ICV injection, the mice were then anesthetized with 3% sevoflurane, and 1 : 10 of Evans blue 2 μL was injected through the guide cannula. The mice were then decapitated, and the brain was removed to verify diffusion of the injected dye throughout the ventricular system (Davisson et al., 1998). Mice without dyed ventricles were regarded as unsuccessful ICV injections attempts and were excluded (Davisson et al., 1998).

2.6 Statistical analyses

The sample size was determined based on the literature (Hu et al., 2012). A larger number of mice were enrolled in this study than the literature (Hu et al., 2012) to allow for possible exclusion of mice that did not pass stated experiment requirements. Statistical analyses were performed using GraphPad Prism software version 6.07 (GraphPad Software Inc., USA). LORR ED_{50} data were presented as mean ± SD. Two-way repeated-measures analysis of variance (ANOVA) was used to analyze the data, followed by the Bonferroni correction as a *post hoc* test. $P < 0.05$ (two-tailed) was designated as statistically significant.

3. Results

To investigate the effects of calcium concentrations and calcium isotopes on sevoflurane-induced LORR ED_{50}, sixty C57BL/6 male mice were included. No mice were excluded due to weight loss. Three mice in the saline group and two in each of the other three groups were excluded due to unsuccessful ICV injection. Finally, data of the LORR ED_{50} of 51 mice, 12 in the saline group and 13 in each of the other three groups were taken into statistical analysis. Two-way repeated-measures ANOVA was used to analyze the data, with one main factor for time and the other for the group. Analyses revealed that regardless of the treatment condition, LORR ED_{50} values between the first and the second measurements had critical differences ($F_{1,47} = 35.47$, $P < 0.0001$). Not surprisingly, the effects of group type on LORR ED_{50} values were significant ($F_{3,47} = 7.77$, $P = 0.0003$) regardless of the measurement condition. The Bonferroni *post hoc* multiple-comparison test analyzed the effect of a group within each level of time and the effect of time within each group level. A strong time × group interaction was found ($F_{3,47} = 34.32$, $P < 0.0001$).

3.1 Effects of saline and EGTA on LORR ED_{50}

The basal value of LORR ED_{50} was determined for the 12 mice to sevoflurane in the saline group, before the ventricular cannulation, was at 1.01 ± 0.09% (95% CI, 0.95% to 1.07%). The value of LORR ED_{50} for the same mice to sevoflurane, determined after ICV injection of saline was at 2 μL, was 0.98 ± 0.12% (95% CI, 0.91% to 1.06%). The two values were highly comparable ($P > 0.9999$, Fig. 1). Since the mice were determined twice for their LORR ED_{50} to sevoflurane, there existed a possibility that the residual sevoflurane of the first determination might influence the second one. However, one day was allowed for the animals to recover between the two determinations. This interval is regarded as adequate for the animals to exhale out the sevoflurane completely. The lateral ventricular catheterization itself might also influence the second LORR ED_{50}. The highly comparable LORR ED_{50} values before and after saline injection of the mice in the control group ruled out these possibilities, indicating that the two determinations were independent. Therefore, LORR ED_{50} values of the mice af-

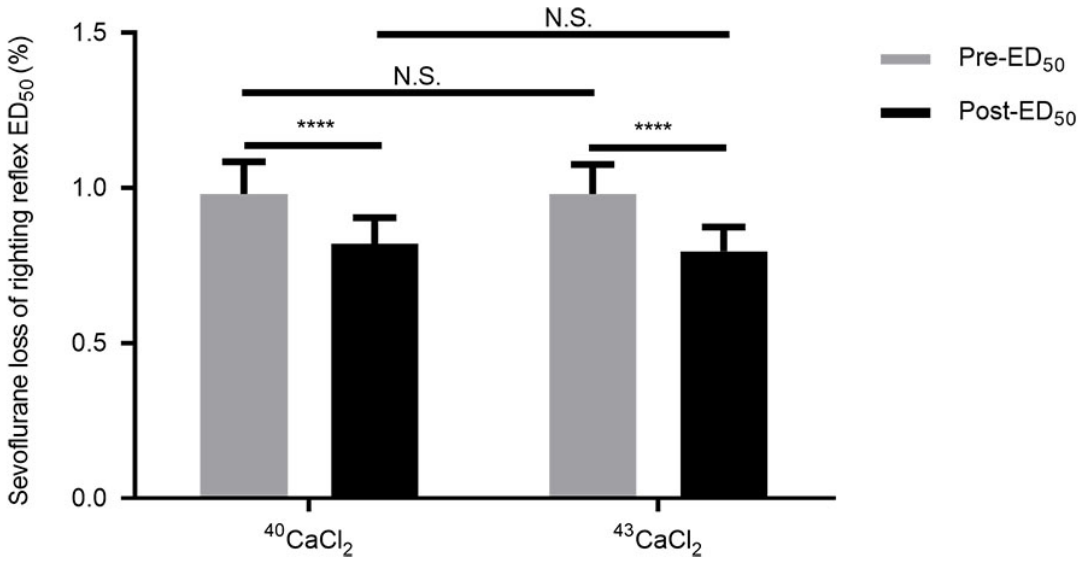

Fig. 2. Effects of $^{40}CaCl_2$ and $^{43}CaCl_2$ on righting reflex ED_{50} for mice to sevoflurane. Pre-ED_{50} denotes the basal LORR ED_{50}. Post-ED_{50} presents LORR ED_{50} after lateral ventricle administration of $^{40}CaCl_2$ or $^{43}CaCl_2$. The difference in the values of the pre-ED_{50} between the two groups is not significant. The post-ED_{50} value is significantly lower than the pre-ED_{50} in the $^{40}CaCl_2$ group or in the $^{43}CaCl_2$ group. The post-ED_{50} values of the two groups are not significantly different. Data are shown as mean (SD), n = 13 mice per group. ****$P < 0.0001$; N.S. = not significantly different (two-way repeated-measures ANOVA followed by the Bonferroni correction as a *post hoc* test).

ter ICV injections of EGTA, $^{40}CaCl_2$ and $^{43}CaCl_2$ in the other three groups were respectively independent on their first determinations.

To examine the effect of decreased calcium ion concentration in the cerebrospinal fluid on a consciousness level, EGTA, a calcium chelator, was directly injected into the mice's lateral ventricle. The basal LORR ED_{50} for the 13 mice to sevoflurane in the EGTA group was 1.00 ± 0.15% (95% CI, 0.91% to 1.09%). The LORR ED_{50} for the mice to sevoflurane after EGTA ICV injection was 1.10 ± 0.14% (95% CI, 1.02% to 1.19%), significantly higher than the basal value ($P < 0.001$, Fig. 1).

3.2 Effects of calcium-40 chloride and calcium-43 chloride on LORR ED_{50}

To examine the prediction of calcium isotope dependence on a consciousness level, the mice in the $^{40}CaCl_2$ and $^{43}CaCl_2$ groups received ICV injections of calcium-40 chloride and calcium-43 chloride, respectively. Calcium-40 injection resulted in a significant decrease in LORR ED_{50} for the mice to sevoflurane as compared with the basal value (0.82 ± 0.09%, 95% CI, 0.77% to 0.87% vs. 0.98 ± 0.10%, 95% CI, 0.92% to 1.04%, $P < 0.0001$, Fig. 2). Similar change was observed after calcium-43 chloride injection (0.80 ± 0.08%, 95% CI, 0.75% to 0.84% vs. 0.98 ± 0.09%, 95% CI, 0.92% to 1.04%, $P < 0.0001$, Fig. 2). Further analysis showed that equimolar ICV injections of calcium-40 and calcium-43 had no difference in LORR ED_{50} values ($P > 0.9999$, Fig. 2), indicating no calcium isotope dependence.

4. Discussion

Several quantum models based on quantum dynamics have been proposed with attempts to demystify the mechanisms of consciousness. For example, the electric spin entangled neural microtubules (the "Orch OR" theory) (Hameroff and Penrose, 2014), phosphorus-31 nuclear spin entanglements (Fisher, 2015), photon-spin interface (Kumar et al., 2016), and unpaired electron-spin activating nuclear spin-based mind-pixels (Hu and Wu, 2004), all set upon theoretical speculations or simulations. Hence, debates on consciousness models persist because there is no direct experimental evidence to confirm or reject any theory of consciousness (Hameroff and Penrose, 2014), primarily due to a lack of measures to probe consciousness. Anesthesia is a reversible unconscious state induced by general anesthetics. General anesthetics are relatively selective for consciousness while they spare many non-conscious brain activities (Craddock et al., 2015; Hameroff, 2006), anesthesia and consciousness may share the same mechanism, thus making general anesthetics natural probes for consciousness (Craddock et al., 2015; Hameroff, 2006).

In this study, LORR ED_{50} for mice to sevoflurane was used as a measure to quantify consciousness levels in mice. Thereby, we tested the predictions derived from the proposal that phosphorus-31 nuclear-spin entanglements protected by Posner molecules may mediate consciousness, first proposed by Fisher in 2015 (Fisher, 2015). It is necessary to give a brief introduction to the proposal first. Nine Ca^{2+} and six PO_4^{3-} ions form a Posner molecule, $Ca_9(PO_4)_6$. Two calcium-phosphate "structural clusters" with atomic constituents $Ca_9(PO_4)_6$, subsequently named "Posner clusters", were first found by Posner and Betts (Posner and Betts, 1975) while they were examining the X-ray crystal structure of bone mineral-hydroxyapatite. The term "Posner cluster" is expanded to "Posner molecule," which has been assumed to be central in consciousness due to its unique chemical structure (Fisher, 2015). Posner molecules are assumed to protect the phosphorus-31 nuclear-spin entanglements located within those molecules (Fisher, 2015). The phosphorus-31 nuclear-spin entanglements within the Posner molecules, for encoding information, remain coherent for times of a day, 21 days, or possibly much longer (Swift et al., 2018; Weingarten et al., 2016). This would allow Posner

molecules to function as "neural qubits" to transmit quantum information to the spatially separated neurons in the brain (Fisher, 2017; Weingarten et al., 2016). In addition to the phosphorus-31 nuclear-spin entanglements within the molecules, their nuclear spins are further entangled among Posner molecules once two bound Posner molecules start rotating about one another (Weingarten et al., 2016). Once at rest, Posner molecule pairs are susceptible to proton attack and therefore are melted and release their Ca^{2+} ions into the neuronal cytoplasm (Weingarten et al., 2016). Because the Posner molecules in the spatially separated neurons are entangled, when disentangled, all the molecules are melted simultaneously and subsequently release a swarm of Ca^{2+} ions (considering nine Ca^{2+} ions in each Posner molecule) in the neurons. This might modulate intraneuronal Ca^{2+} levels and thus make multiple neurons spike simultaneously. The simultaneous spiking of spatially separated neurons is considered a mechanism of consciousness (Weingarten et al., 2016).

If the Ca^{2+} ion concentration in the cerebrospinal fluid is reduced, in line with Fisher's proposal, a decrease in the number of Posner molecules in the cerebrospinal fluid may occur, and the consciousness level may be lowered (Fisher, 2015). For the same reason, an elevation in the Ca^{2+} concentration in the cerebrospinal fluid may increase consciousness level. However, our findings in mice contradict these predictions. EGTA reduces Ca^{2+} concentration in the cerebrospinal fluid after ICV injection (Liang et al., 2004). Contrary to the prediction that the consciousness level of the mice would be reduced after ICV injection of EGTA, instead, an increased consciousness level of the mice, as shown by an increased LORR ED_{50} value for the mice to sevoflurane in comparison to the basal value, was found after the animals received ICV injection of EGTA in this study. A significantly decreased consciousness level of the mice was found after ICV injection of $^{40}CaCl_2$ in this study, again in contradiction to Fisher's prediction. Unlike calcium-40 with zero nuclear spin, calcium-43 nuclei have 7/2 nuclear spins. In Posner molecules, if calcium-43 ions replace the calcium-40 ions, especially the central ones, the decoherent time for the phosphorus-31 nuclear-spin entanglements is expected to be shortened and thus, the consciousness level of the animals is expected to reduce - i.*e.,* calcium isotope dependence on consciousness is expected (Fisher, 2015). Indeed, we found the consciousness level of the mice was reduced after ICV injections of $^{43}CaCl_2$. However, further analysis showed no difference in LORR ED_{50} between ICV injections of $^{40}CaCl_2$ and $^{43}CaCl_2$ ($P > 0.9999$). The identical effects of $^{40}CaCl_2$ and $^{43}CaCl_2$ on the LORR ED_{50} indicate no calcium isotope dependence on consciousness.

Our findings are consistent with previous studies (Erickson et al., 1978; Harris, 1979). ICV injection of $CaCl_2$ increases ethanol-induced sleeping time, whereas EGTA decreases sleeping time in mice, as reported previously (Erickson et al., 1978; Harris, 1979). The inhibitory effect of calcium was also found in *in vitro* studies, as an increase in extracellular Ca^{2+} ion concentration suppressed neuronal excitability, while a decrease in extracellular Ca^{2+} concentration excited neurons (Lu et al., 2010; Ma et al., 2012; Wang et al., 2004). Although exploring the mechanisms of varying calcium concentrations on neural excitability is beyond the scope of this study, it is necessary to point out that varying calcium ion concentrations affect Posner molecules the most and, therefore, affect consciousness more than other possible protein- or ion-involved mechanisms, as Posner molecules are crucial for consciousness, superseding any other potential mechanisms according to the proposal (Fisher, 2015). Therefore, our study is vital to test the predictions derived from Fisher's proposal (Fisher, 2015) if it overshadows other possible mechanisms.

Consciousness is a challenging problem (Miller, 2005), both in philosophy and in science. Philosophically, consciousness belongs only to humans - consciousness is a feeling of oneness. Scientifically, however, it seems unjustified to claim that consciousness is uniquely specific to humans (Trewavas and Baluška, 2011). For living organisms, even a little bit of consciousness confers sentience and awareness of environment. Since consciousness is a vital function of living organisms, it merits scientific study. However, scientific study on consciousness has methodological problems - consciousness is difficult to define and therefore measure. Fortunately, we have inhaled anesthetics at hand. The potency of inhaled anesthetics can be quantified (Koblin et al., 1998). Therefore, consciousness can be quantified by using inhaled anesthetics in an alternative way.

Our previous study found that the nuclear spin property of ^{129}xenon partly antagonizing its anesthetic property is why the less anesthetic potency of ^{129}xenon than xenon isotopes with zero nuclear spin (Li et al., 2018). The existence of isotopic dependence of xenon anesthesia suggests a possible nuclear-spin mechanism of consciousness in the brain. Therefore, inhaled anesthetics can be applied to investigate nuclear-spin involving the mechanism of consciousness. In Fisher's theory (Fisher, 2015), Posner molecules can be considered the nuclear-spin property of ^{129}xenon. In our work, sevoflurane was the anesthetic property of ^{129}xenon. The use of sevoflurane to detect Posner molecules' effect on consciousness is reasonable since Posner molecules partially antagonizable effect is predicted to reduce the anesthetic potency of sevoflurane. Although we disprove Posner molecules in consciousness, it does not mean that nuclear spin is not crucial for consciousness. Nuclear spin may also be implied in sleep. The similarities between sleep and general anesthesia exist (Kelz et al., 2008), as sleep is a natural while general anesthesia is a drug-induced unconscious state.

5. Conclusions

In conclusion, our findings refute the predictions that phosphorus-31 nuclear-spin entanglements within Posner molecules play a central role in consciousness.

Author contributions

R. C, N. L., H. Q., and R. Z. performed the experiments. N. L. and R. C. analyzed the data and wrote the paper, which all authors read. S. Z. conceived the study, designed the experiments, supervised the overall project. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal operations and experimental protocols conformed to the US National Institutes of Health guide for laboratory animals' care and use (NIH Publications No. 8023, revised 1978). They

were approved by the Institutional Animal Care and Use Committee (approval No: S164) at Tongji Medical College, Huazhong University of Science and Technology.

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Conflict of Interest

The authors declare no conflict of interest.

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