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Combined implanted central venous access and cortical recording electrode array in freely behaving mice

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A B S T R A C T

Establishing a long-lasting, functioning venous access in a non-anesthetized mouse is very challenging at least. Since we needed a reliable venous access to titrate intravenous anesthetics, we refined and combined previously described methods. The tunneling of the catheter from the cranial to the pectoral wound, the fixation of the catheter in the external jugular vein with two sutures, and a tissue adhesive allowed us to combine this method with the implantation of intracranial recording electrodes. With this approach we neither have to restrain the animal causing excessive stress nor do we need an additional anesthetic, interfering with the effects of the intravenous anesthetic. This approach can help to establish a greater understanding of the concept of consciousness by identifying the neural circuits which mediate the effect of intravenous anesthetics. In addition - due to the flexible design of the recording electrode array - our approach can also be applied to investigate further neuroscientific hypotheses.

- Establishment of a reliable chronical venous access for the application in freely behaving mice.
- The jugular venous access can be combined with all kinds of neurobiological recording and application designs.
- The design of the venous access allows chronic combinations with telemetric and tether-bound systems.

A R T I C L E I N F O
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Resource availability:	*N.A.*

Method details

Chronic venous cannulation has already been established in mice in the 1960s and was first described for the jugular vein in 1979 [1,2]. Over the years these publications were followed by various studies refining the previously described techniques [3,4]. We extensively studied the literature, evaluated the (dis-) advantages of each proposed technique and then combined this method with the implantation of a recording electrode array. This novel approach allows the investigation of a broad field of complex neurophysiological processes.

Animals

All experimental procedures were approved by the Com. on Animal Health and Care of the State of Upper Bavaria, Germany (ROB-55.2–2532.Vet_02–19–121). An overview of the surgical implantations is presented in Fig. 1 (that uses a mouse sketch from wiki commons [5]). To establish this model, we used ten adult male mice (C57BL/6 N; Charles River Laboratories GmbH, Germany) which were housed individually under a 12/12-hour light/dark cycle with free access to food and water. The body weight (BW) ranged from 26 to 38 g. In the present study we only included four animals as three animals died (one during the surgery due to hemorrhagic shock and two during establishment of the anesthetic protocol). Another three animals had to be excluded as the catheter dislocated during the recovery period. The dislocation issue was solved after adjusting our protocol. With the new protocol, the dislocation rate was less than 10%.

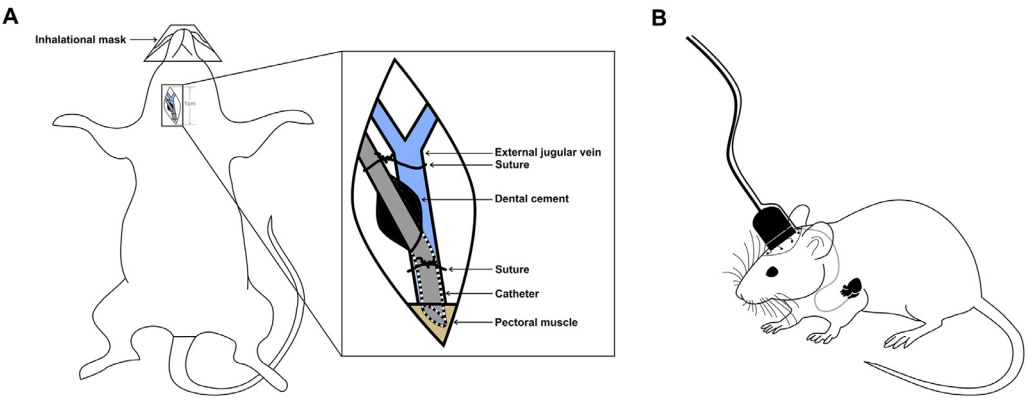

Fig. 1. (A) After cranial incision the animal is placed in a supine position. Details of the catheter implantation are displayed on the right side. The part of the catheter which is inside the vein is depicted as dashed line. (B) After the surgery the mouse is connected to the cable and can move freely due to a swivel system. The part of the catheter which is covered by skin is depicted as gray line.

Anesthesia and implantation of venous access and recording electrode array

For induction of anesthesia we placed the animals in a chamber with 4 Vol.-% isoflurane. After loss of righting reflex, the mice were transferred to a stereotactic device (prone position) and anesthesia was maintained with isoflurane 1.0–2.0 Vol.-% (CP-Pharma, Germany) throughout the procedure. For hair removal on the scalp, we pre-shaved the fur and carefully applied depilatory cream (Balea, dm-drogerie markt GmbH + Co. KG, Germany) for 30 s that was wiped off with warm water and a swab. Afterwards we performed a cranial incision of approx. 1.5 cm (Fig. 2A). We then injected carprofen (4 mg/kg, Zoetis Deutschland Gmbh, Germany) subcutaneously and applied lidocaine 1% (Aspen Germany GmbH, Germany) into the incision. For the next steps we removed the animal from the stereotactic device and put it in a supine position. After hair removal (as described above), we cut the skin for approx. 1 cm, 0.5 cm lateral of the sternum (Fig. 2B). We carefully dissected the tissue with two micro forceps to expose the *Vena jugularis externa* under visual control of a stereomicroscope. We then placed two 7–0 perma-hand silk sutures (#768 g, Johnson & Johnson Medical GmbH, Germany) at the caudal (next to the *Musculus pectoralis*) and rostral end (right after bifurcation) of the vein, but only closed the rostral one. If necessary, we ligated small branches (Fig. 2C). Then, we tunneled the catheter from the cranial to the pectoral incision with a blunt forceps – a technique adapted from Mokhtarian et al. [6]. A small incision was made between the ligatures using a micro-scissor (#OC947R, Aesculap AG, Germany). We inserted the catheter which was previously flushed with heparin solution (50 I.E./ml; ratiopharm GmbH, Germany). The catheter was a polyethylene tube (0.28 mm ID, 0.61 mm OD; Smiths Medical International Ltd, UK) whose tip was smoothed using gentle heat from a Bunsen burner (Fig. 2D). Before further processing, we flushed the catheter to ensure that the catheter was not sealed and evaluated the tip using a microscope. If the tip showed any sharp edges or fragments we would discard it. A small drip of dental cement

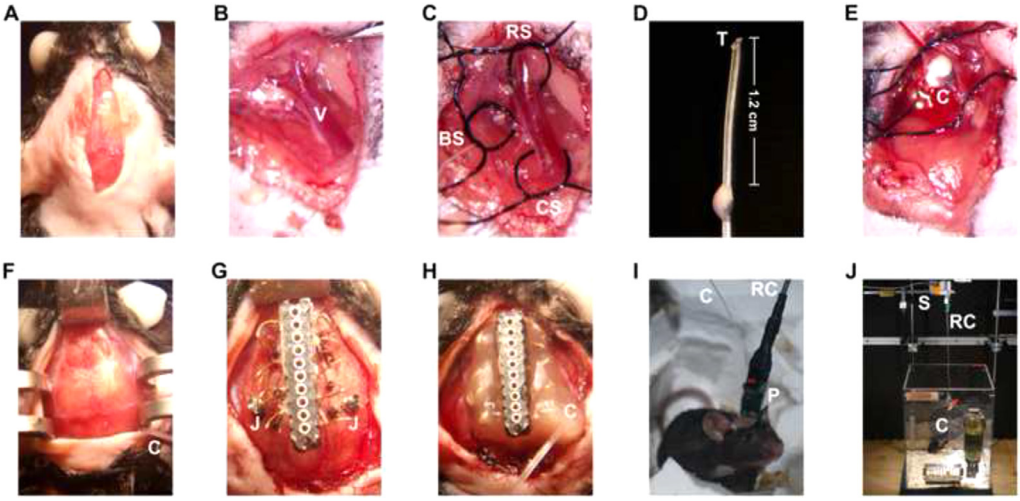

Fig. 2. (A) First step of the surgery: ~1.5 cm cranial incision (B) After transferring the animal into a supine position, a pectoral incision was performed and the Vena jugularis externa (V) was exposed. (C) Two sutures were placed around the vein: at the rostral (RS) and caudal (CS) end. In addition, a small branch (BS) was ligated. (D) Modified polyethylene tube with a smoothed tip (T) and drop of dental cement 0.5 cm from the tip (E) Inserted catheter (C) with sutures in front of and behind the drop of dental cement for fixation (F) After catheter placement, the animal was transferred to stereotactical device. The catheter (C) exited at the caudal end of the wound. (G) Implantation of nine gold wire electrodes and two jeweler's screws (J) on both hemispheres (H) The jeweler's screws and the electrodes were fully covered with dental cement. The catheter (C) was embedded into this structure. (I) Mouse during the recovery period with catheter (C), pre-amplifiers (P) and recording cable (RC) in its home cage. (J) Display of the whole setup with the catheter (C) bound to the recording cable (RC) which is connected to a commutator. The recording cable including the pre-amplifiers were mounted on a weight-neutral swivel system (S) allowing the animal to move freely in its home cage.

(PALADUR, Kulzer, Germany) on the catheter 1.2 cm from the tip ensured fixation (Fig. 2D). The total length of the catheter was 25 cm. After catheter insertion, the caudal ligature was closed and an additional knot was tied around the catheter at the rostral suture (Fig. 2E). Before skin closure, we applied two drops of a tissue adhesive (B. Braun Surgical S.A., Spain). We then fixated the animal in the stereotactic device (Fig. 2F) to implant the intracranial epidural electrodes as previously described [7,8]. In short, we removed the periosteum, drilled eleven 700 µm holes and inserted blunt-tipped self-tapping jeweler's screws (ø 600 µm) into two holes, one on each side. Afterwards we spread a layer of cyanoacetate adhesive as well as dental cement on the skull to fix a printed circuit board (PCB)-socket (PRECI-DIP SA, Switzerland). The PCB-socket was equipped with nine custom-made gold wire electrodes (Haefner & Krullmann Gmbh, Germany) soldered to it. The type of electrode can be adjusted to the experimental design, for example local field potential or electromyogram electrodes. Wire placement was according to the cortical EEG-target areas. The gold wires were 8 mm long and 150 µm in diameter with ball shaped ends to prevent cortical injuries and to increase the surface area. After socket fixation, we implanted the electrodes with their tips placed underneath the skull, but above the dura mater (Fig. 2G). To secure the electrodes, we applied an additional layer of dental cement after electrode insertion. The catheter located at the caudal end of the incision was also embedded into the dental cement structure. (Fig. 2H). After suturing the skin and applying lidocaine solution around the incision, we attached a recording cable to the PCB-socket (Fig. 2I), tied the catheter to the cable with an adhesive tape, and placed the animal into its home cage with the recording cable connected to a commutator (model SL-20, DRAGONFLY, Ridgeley, WV, USA) (Fig. 2J). The recording cable including the pre-amplifiers (1x amplification, npi electronic, Tamm, Germany) were mounted on a weight-neutral swivel system (custom made, Streicher M., Innsbruck, Austria) allowing the animal to move freely in its home cage. After surgery, the animals could recover for 10 days. During this period, we flushed the catheter every second day with 25 µl of heparin solution. In addition, we added carprofen to the drinking water from the first preoperative to the fourth postoperative day (0.067 mg/ml) for analgesia.

Method validation

We established this method to investigate the electrophysiological effects of intravenous anesthetics, e.g. propofol. To validate our method as well as correct catheter placement, we conducted experiments to determine the propofol dosage needed for observing loss of righting reflex (LORR). Our required dose was comparable to published experiments performing bolus injection [9]. As we were interested in the exact spectral electroencephalographic changes during the induction of general anesthesia, we slowly injected propofol in a second set of experiments. This approach allowed us to analyze the wake state as well as the various phases of anesthesia: slow wave anesthesia, burst suppression, and suppression (Fig. 3).

Before starting the anesthetic procedure, we measured the dead space of our system which was approximately 40 µl. For this reason, we flushed the catheter with 40 µl of propofol. The advantage of propofol is that it is a white (lipid) solution so one can observe the flushing of the catheter. After flushing the catheter with the anesthetic, we waited 30 min before starting anesthetic procedure. With the waiting period we made sure that any small amount of propofol which might have been injected during the flushing procedure has worn off. We then slowly injected propofol 2% (Fresenius Kabi Gmbh, Germany) using a micro-injection pump (CMA Microdialysis AB, Sweden). We started at a rate of 1 µl/min (≙10 µg/min) which we increased every two minutes by 1 µl. The median dosage at which the animals lost righting reflex was 5.4 ($n = 4$; minimum: 4.0; maximum: 6.3) mg/kg BW/min (Table 1). We increased the infusion rate until we observed 30 s of suppression. Throughout the entire procedure, we constantly recorded EEG. Each EEG channel was individually amplified (1000x) and band-pass filtered (0.1–100 Hz) online (DPA-2FL-Differential Amplifier and Filter, npi electronic, Tamm, Germany). The data were sampled at 250 Hz (Spike2, Cambridge Electronic Design Ltd., UK) and stored after analog-to-digital conversion (Power1401–3A, Cambridge Electronic Design Ltd., UK). For further analysis, we imported the data into MATLAB R2018b (The Mathworks, Natick, MA).

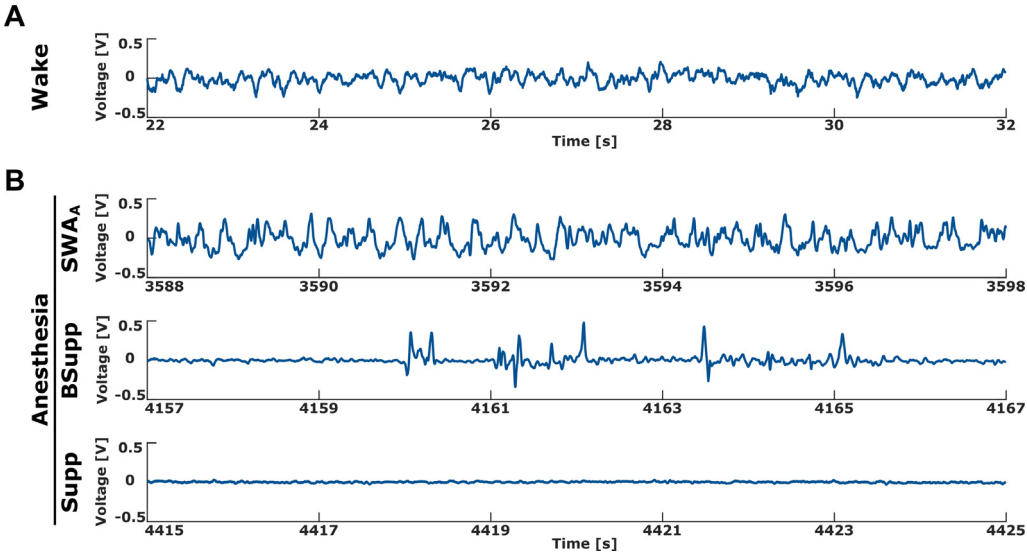

Fig. 3. Display of electrocorticogram (ECoG) during various phases of induction of general anesthesia with propofol (A) During the awake phase we observed an ECoG dominated by fast frequencies with small amplitudes (B) Slowly raising the infusion rate of propofol the animal lost its righting reflex at second 3510 and an infusion rate of 140 µg/min. Afterwards, the ECoG demonstrated a strong slow wave activity (SWAA). With increasing propofol concentration burst suppression (BSupp) – a pattern indicating extensively deep levels of anaesthesia with characteristics not comparable to SWA state – emerged (190 µg/min). This was followed by complete suppression (Supp) at a propofol infusion rate of 210 µg/min.

Table 1
Animal characteristics and infusion rate at which loss of righting reflex (LORR) was observed.

Animal Number	Body Weight [g]	Age [weeks	LORR [µl/min]	Dose [mg/kg BW/min]
1	26	19	12	4.6
2	35	27	14	4.0
3	36	32	22	6.1
4	38	68	24	6.3

Conclusion

Our method allows to selectively investigate the effect of intravenous anesthetics on the brain without causing excessive stress by restraining the animal or putting it under general anesthesia using an additional narcotic. This approach can help to establish a greater understanding of the concept of consciousness by identifying the neural circuits which mediate the effect of intravenous anesthetics.

Additional information

Thousands of people undergo general anesthesia each day [10]. This reversible state of immobility, amnesia, analgesia, and unconsciousness is induced by drugs whose molecular targets and cellular mechanisms have been well characterized over the last decade. However, the neural circuits which mediate the transient loss of consciousness are still unclear [11]. Because the investigation of these circuits requires recordings from intracranial electrodes, ethical concerns arise when trying to investigated this question in humans. Animal models can help to avoid these concerns and they can help analyze the influence of anesthetics on the brain. Mouse models became one of the most intensively studied ones in neuroscience due to low maintenance costs, ease of genetic modification, and practicability [12]. In anesthesia research, the mouse model is very suitable to

investigate the mechanisms of volatile drugs like sevoflurane. Because of the animal's size the model was almost exclusively used to study volatile anesthetics, since the establishment of a functioning venous access to titrate an intravenous anesthetic in a non-anesthetized animal is very challenging at least. Previous studies overcame this issue by either restraining the animal to place a catheter into a tail vein – in our experience not a highly reliable procedure – or putting the animal under inhalational anesthesia to establish a transient central venous access [9,13]. This approach does not allow to attribute the activation/inhibition of a neural circuit to one specific drug as an inhalational and intravenous anesthetic have been applied. To solve this problem, we established a murine model of a chronically implanted central venous access combined with intracranial electrodes for electrophysiological recordings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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