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Tracheotomy improves experiment success rate in mice during urethane anesthesia and stereotaxic surgery

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ABSTRACT

Urethane anesthesia is frequently used for acute experiments on small rodents in physiology and neuroscience. Severe respiratory distress is a common side-effect of urethane anesthesia in many strains of mice. Associated complications interfere with completion of experiments, and as a consequence more animals must be sacrificed. During experiments with stereotaxic brain surgery, we found that intubation by means of tracheotomy is an efficient way to maintain patent airways in these animals. Artificial ventilation of the animals is not required. In this paper we describe a simple, fast and reliable method for intubation of mice in experiments that involve a stereotaxic instrument. The method proved considerably easier to learn and apply than conventional intubation through the oral route. The incidence of breathing problems decreased from 77% in untreated mice to 9% in those that underwent tracheotomy. In addition, the success rate for our acute electrophysiological experiments increased from 24 to 77%. We conclude that tracheotomy reduces the number of sacrificed animals, and saves time and labor.

1. Introduction

The house mouse (*Mus musculus*) has emerged as the dominant mammalian model organism in molecular biology, and increasingly neuroscientists turn to mutant mouse models for the study of genes involved in central nervous system function. In neuroscience, the injection anesthetic urethane (ethyl carbamate, $NH_2COOCH_2CH_3$) has been widely used to induce surgical anesthesia in rats for acute *in vivo* electrophysiological recordings. However, our own experience, and conversations with colleagues, suggests that many laboratories experience low success rates during acute *in vivo* electrophysiology experiments with urethane anesthesia in mice.

Urethane, a carcinogenic substance that has fallen out of favor in many other research areas (Field and Lang, 1988), is favored for such experiments because it induces long-lasting steady level of surgical anesthesia with muscle relaxation (Maggi and Meli, 1986a) but has minimal effects on autonomic and cardiovascular systems (Maggi and Meli, 1986b; Hara and Harris, 2002; Janssen et al., 2004). Furthermore, surgical anesthesia is achieved without markedly enhancing GABAergic synaptic transmission (Maggi and Meli, 1986a; Hara and Harris, 2002). Instead, urethane has a spectrum of actions on ion channels that is distinct from other anesthetics and include minor to modest effects on GABA, glycine, ACh, NMDA and AMPA receptors (Hara and Harris, 2002). Thus, urethane is preferred in studies that require maintenance of network oscillations in the brain, such as theta and gamma rhythms, which are critically dependent on GABAergic neurotransmission (Buzsaki, 2002). Urethane can be used alone or together with the more rapidly metabolized drug ketamine (Klausberger et al., 2003), which primarily act on NMDA receptors (Franks, 2008).

Breathing problems are common during long-term general anesthesia in mice (Stoenica et al., 2006), and occur frequently during urethane anesthesia. First the breathing becomes noisy or wheezing, usually starting 30–60 min after sufficient anesthetic depth for surgery has been achieved. The initial respiration problems are followed by more labored, wheezing, and irregular breathing. In the final stage, the animals make gulping breaths that can include large movements of the torso. This progression of symptoms can be relatively swift or extend across 1–2 h. These breathing problems seem to arise from salivation and airway secretion that accumulate in the respiratory tract (Olson et al., 1994). Breathing problems may also occur in rats under urethane anesthesia, but mice seem to be more affected by secretion than rats. In the absence of reliable pharmacological interventions (see Section 4), ensuring patent airways by intubation seems to be necessary for overcoming

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the breathing problems and low experiment success rate in mice during urethane anesthesia.

Intubation of the trachea can be achieved either through the conventional oral route or by tracheotomy, but is considerably complicated when a stereotaxic instrument is required for the experiment. For reasons discussed below, we abandoned oral intubation in favor of tracheotomy. Tracheotomy is commonplace in research on small laboratory animals involving inhalation anesthesia, open thorax surgery and lung physiology. Tracheotomy has also been used to ensure patent airways and artificial ventilation in rats during acute *in vivo* electrophysiological experiments under urethane anesthesia (e.g. Bland and Whishaw, 1976) but is not essential (e.g. Kocsis and Vertes, 1997; Marshall et al., 2002; Klausberger et al., 2003; Li et al., 2007). Tracheotomy has also been used to ensure patent airways in mice during stereotaxic surgery (Ellrich and Wesselak, 2003) and urethane anesthesia (Stoenica et al., 2006). Although implicit, these papers neither addressed the challenges associated with urethane anesthesia and stereotaxic surgery in mice, nor documented the efficacy of tracheotomy. Here we show that tracheotomy reduces the occurrence of breathing problems and improves experiment success rate in mice during urethane anesthesia and stereotaxic surgery from 24 to 77%. This reduces the number of animals needed to complete projects, and saves time, labor, and cost.

2. Materials and methods

The mice used for this study were also part of *in vivo* electrophysiology studies, the results of which will be reported elsewhere (Moldestad et al., in preparation). Thirty-nine adult male mice were used (C57bl6, $n = 25$; mice expressing dominant negative KCNQ2 channel subunits (Peters et al., 2005), $n = 14$. Genotype and anesthesia were not balanced across groups but reflected availability and ongoing refinement of protocols, respectively. Six mice were excluded because of procedural errors. The mice were housed in transparent polycarbonate cages in a temperature and humidity controlled vivarium with free access to food and water. Lights were on between 0600 and 1800 h. The animals were tested during the light period. All experimental procedures were approved by the section for comparative medicine at the medical faculty, University of Oslo, and by the Norwegian animal research authority. The procedures complied with both national laws and European Communities Council Directive of 24 November 1986 (86/609/EEC) governing the use of animals in research.

2.1. Equipment

The tracheal tube was fashioned from the distal end of a commercial intravenous catheter (Venflon Pro BD, 18–22 GA, BD, NJ, USA) cut down to 20–25 mm length and passed through a roughly hexagonal-shaped thin sheet of silicone rubber according to a design from Manna et al. (2003) (Fig. 1B). The distal end of the catheter tapered to facilitate insertion into the trachea, and the gauge of the catheter used varied between individuals (18–22 GA) according to animal size.

2.2. Chemicals and reagents

Urethane, ethyl carbamate crystals (Fluka, Sigma–Aldrich, MO, USA), was dissolved in 0.9% saline to prepare an injection solution of 0.15 g/ml. Ketamine (Ketalar, Pfizer, NY, USA), bupivacain with epinephrine (Marcain–Adrenaline 5 mg/ml and 5 μg/ml, AstraZeneca, UK), and Simplex eye ointment (Optha, Denmark), were also used.

2.3. Tracheotomy procedure

The mice were brought to the laboratory 30 min before induction of urethane anesthesia and allowed to settle down in a dark enclosure. An intra-peritoneal (i.p.) injection of 1.2 or 1.5 g/kg urethane was administered. For animals receiving 1.2 g/kg this was followed 10 min later by an i.p. injection of 20 mg/kg ketamine to ensure a more rapid induction of surgical anesthesia (Klausberger et al., 2003). The scalp (in preparation for stereotaxic surgery) and the throat were shaved with an electrical razor and small injections of bupivacain/epinephrine were administered along the line of incision in the neck for long lasting local analgesia. The depth of anesthesia was tested by pinching the hind-paw or the ear, and surgery was initiated only after the pinching consistently failed to elicit a reflex.

For the intubation surgery we adapted methods described for young rats by Manna et al. (2003) and for mice (Schwarte et al., 2000; Ellrich and Wesselak, 2003). The animal was placed in a supine position and fixed in place with plaster strips. The head, tethered with a suture around the upper incision teeth to the operating surface, pointed in the direction of the experimenter. The fore limbs were extended to the side and fixed with plaster strips (Fig. 1A). Five to 10 min after giving bupivacain/epinephrine for local analgesia, a 1.5 cm long median cervical skin incision was made between the upper thorax aperture and the lower jaw. The lobes of the thyroid gland were then separated bluntly at their isthmus (Fig. 1C; see Iwaki et al., 2001 for anatomical details). The incised skin and the thyroid lobes were kept retracted by hemostats, but often such retraction was unnecessary. To expose the larynx and the trachea, the sternohyoid muscles were spread bluntly and pulled aside (Fig. 1D; alternatively these pretracheal muscles could be pulled gently to one side). The next steps often caused respiratory problems and needed to be performed quickly and gently. A forceps was used to bluntly separate the connective tissue under the length of the exposed trachea and a thin tube was inserted underneath the trachea in such a way that it straddled the incision and kept the trachea exposed (Fig. 1E). The tracheostomy was made between two tracheal rings with the sharp tip of a catheter needle (Fig. 1F). Next, the distal end of the tracheal catheter (Fig. 1B) was inserted into the trachea trough the resulting hole. Once this was accomplished, and the correct positioning of the tracheal tube was confirmed by judging the symmetrical chest expansion, the critical phase was completed and the animal was usually allowed 5 min to recover from any respiration problems. When the respiration was judged stable, the tube was secured in place by one or two ligatures around the trachea (Fig. 1G). The thin tube underneath the trachea was then removed and the incision sutured. The exteriorized end of the tracheal tube was cut to approximately 5 mm length (Fig. 1H). Finally, the animal was moved to the recording setup and fixed in the stereotaxic frame (Fig. 1I). Care was taken not to accidentally move or block the tracheal tube while fixing the animal. It was not necessary to artificially ventilate the animals. The eyes were protected with Simplex eye ointment to keep the cornea from drying, and stable body core temperature was maintained throughout the experiment with a DC temperature regulation system (FHC, ME, USA). All surgical procedures for the tracheotomy were done without magnification.

2.4. Analysis

To assess the effectiveness of tracheotomy in alleviating respiratory distress, we analyzed the frequency of breathing problems during the experiments. We also analyzed whether or not the acute *in vivo* electrophysiological experiments were successfully completed and the duration of these experiments. The frequency of