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Short communication

Surgical preparation of mice for recording cardiorespiratory parameters *in vivo*

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HIGHLIGHTS

- Mice are maintained under anaesthesia and ventilated for more than 6 h.
- This method enables multiple nerve recordings.
- Our approach enables complex physiological studies to be conducted *in vivo* in mice.

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ABSTRACT

Background: The explosion in the use of genetically modified mouse strains to investigate function in biology has an enormous potential to expand on pharmacological studies traditionally conducted in rats. A key limitation to date is the inability to record from multiple nerves in an anaesthetised mouse for long periods.
New method: Here we describe an *in vivo* preparation that maintains mice in a suitable physiological state, under anaesthesia, for at least 6hr and also enables multiple cardiorespiratory recordings over that time.
Results: Using the method described, blood pressure, heart rate, phrenic nerve activity, splanchnic nerve activity and heart rate were able to be recorded for hours in an anaesthetised, paralysed and mechanically ventilated mouse.
Comparison with existing method: Existing anaesthetised mouse preparations are limited by difficulties in maintaining mice under anaesthesia for long periods. This time constraint therefore limits the surgical time and number of cardiorespiratory variables recorded. It also limits the type of stimuli that can be administered and the length of recorded responses. The method described here optimises these variables to overcome these challenges.
Conclusions: In summary, we report an approach that enables physiological and pharmacological studies previously undertaken in larger animals or 'reduced' preparations, to be conducted *in vivo* in mice. We anticipate that the use of this preparation will enable a deeper understanding of genetic variation, and allow a much greater level of phenotypic characterisation in genetically modified mice.

1. Introduction

Mouse models are becoming increasingly popular for investigating the physiology of the nervous system, primarily because of the availability of genetically altered strains. Acute pharmacological studies investigating changes in autonomic control over many hours are commonly performed in rats (Farnham et al., 2008; Inglott et al., 2011; Shahid et al., 2012). The size, and relative robustness, of rats permits simultaneous recording of multiple nerves and repeated testing of agents and reflexes over many hours, whilst under anaesthesia (Shahid et al., 2012). Mice on the other hand, are 10 times smaller (~20–40 g compared with ~250–500 g rats), have a very small blood volume (1.1–2.3 ml) and are exquisitely metabolically fragile. Achieving an *in vivo* anaesthetised surgical preparation similar to the ones previously used in rat, rabbit and

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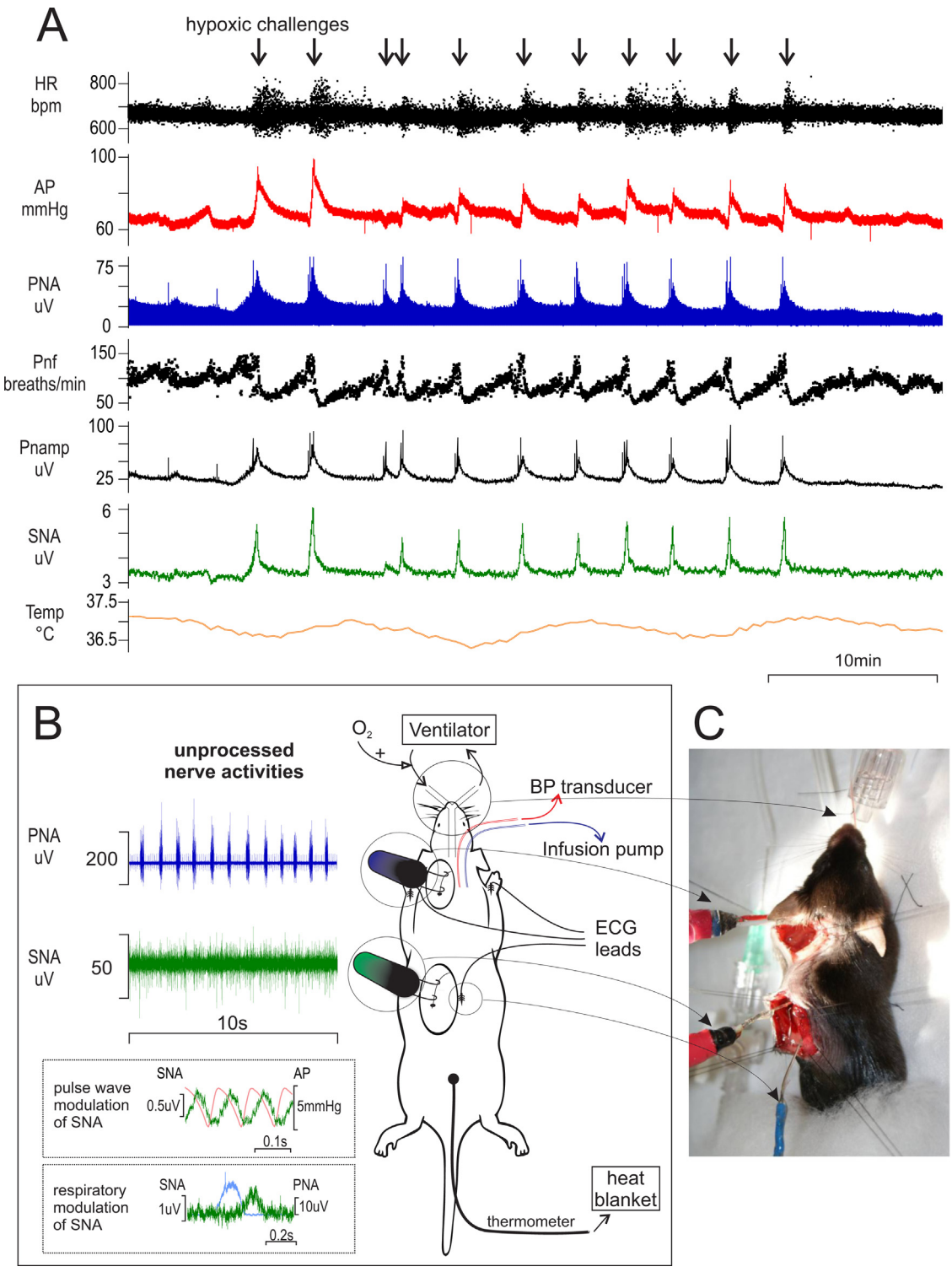

Fig. 1. (A) A trace from an anaesthetised, paralysed, vagotomised and artificially ventilated mouse, 7hr after induction of anaesthesia. Hypoxic challenges are indicated by arrows. The top trace shows heart rate (HR), the next trace shows arterial pressure (AP), followed by the rectified trace of phrenic nerve activity (PNA). The 4th trace show phrenic nerve frequency (PNf), which is an event channel triggered from PNA. The phrenic nerve amplitude (PNamp) is a realmark channel that records the peaks from the PNA trace. The second trace from the bottom is the rectified and smoothed sympathetic nerve activity (SNA) recorded from the splanchnic nerve. The bottom trace is the core temperature (Temp). (B) A cartoon of the surgical preparation with unprocessed recordings from the phrenic (blue) and splanchnic (green) nerves and ensemble averages (dashed boxes) showing pulse modulation and respiratory modulation of SNA. (C) A picture of the complete anaesthetised mouse preparation.

cat, while making observations over a period of hours is therefore extremely challenging, and has not been described previously. Here we describe an approach that enables, at a minimum, ventilation, paralysis, temperature regulation and measurement, and venous access, as well as recording of blood pressure, heart rate, ECG, phrenic nerve activity and splanchnic nerve activity (Fig. 1) in C57Bl6 and CD1 background mice maintained under anaesthesia for over 6 h. To develop this technique, 14, female, TRPV1$^{-/-}$ and TRPV1$^{+/+}$ (wild-type littermates) mice, on a C57Bl6 background were used. This technique has subsequently been used for studies