A novel approach to the application of electroacupuncture in awake rats

BACKGROUND

The primary focus of many animal studies of electroacupuncture (EA) is on the overall effects of the treatment, without due consideration of the impact of how the needles are applied in animal models. Most experimental studies of EA use anaesthesia before needle insertion and electrical stimulation, or alternatively use restraining devices while the animals remain awake, either of which may confound the effects of the needling per se. In a recent study, Wang *et al*[1] reported that rats remained in a polyethylene cylinder during the application of EA, without the need for either restraints or anaesthesia. Based on this observation, and given the potential difficulties that may be encountered during the application of EA, we set out to create a novel bottle-based device to help facilitate administration of EA without the use of anaesthesia or restraints.

CREATION OF DEVICE

A customised bottle device was fashioned out of a wooden board and six 500 mL polyethylene terephthalate (PET) water bottles. The necks of the bottles were cut off, fixed to the wooden surface, and used as burrows (figure 1). The cut edge of each bottleneck was covered with electrical tape to avoid any injury. Using this device it was possible to stimulate up to six conscious animals simultaneously without anaesthesia or restraints, thus increasing efficiency for the researcher (figure 1A).

EXPERIMENTAL ANIMALS

Eleven male Wistar rats were assigned to one of two experimental groups: animals in the Bottle group (n=6) used the new bottle device; while those in the Vest group (n=5) were restrained in a vest that maintained them in a suspended position. The vest comprised a hard waistcoat, which was placed around the trunk and secured using a system of Velcro straps that allowed the free movement of the front and hind paws. After experimental group allocation, all rats underwent EA treatment for 30 min/day, 5 days/week, for a total of 8 weeks, using the new bottle device or restraint vest, accordingly. Haemodynamic parameters were assessed as previously described.[2] All data are expressed as mean±SD. Student's t-test was used to compare variables between the two groups. A value of $p<0.05$ was considered to be statistically significant.

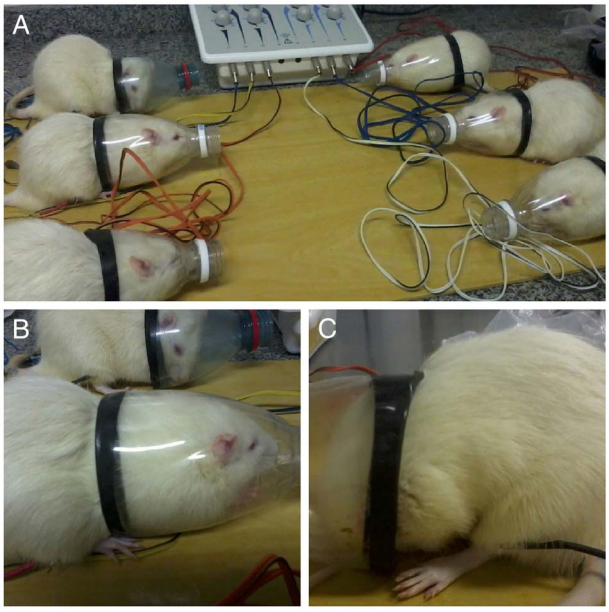

Figure 1 A custom-designed polyethylene terephthalate (PET) bottle device that enables the simultaneous stimulation of more than one animal using electroacupuncture (EA), without the need for either restraint or anaesthesia (A). Animals remained voluntarily in the bottle during electrostimulation at ST36 (B) and adopted a relaxed position within (C).

ETHICAL APPROVAL

The study protocol followed the standards set by the National Institutes for Health (NIH) *Guide for Care and Use of Laboratory Animals* (National Academies Press, Washington DC, USA). All procedures outlined in this study were approved by the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) Animal Ethics Committee (protocol no. 059/11).

FINDINGS

The rats remained inside the bottles voluntarily without restraint during the course of the EA treatment (figure 1B) and adopted a position of relaxation during the stimulation (figure 1C). No signs of stress (eg, piloerection) or pain were observed. As shown in figure 2A, values of heart rate (HR) were lower in animals using the bottle device compared to those that were restrained and suspended using the vest (338.2±14.3 vs 380.5 ±31.9 bpm, p=0.017). Furthermore, as illustrated in figure 2B–D, values of mean arterial pressure (MAP), systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) were significantly lower in the Bottle group compared to the Vest group (MAP: 101.6±2.6 vs 108.2±2.5 mm Hg, p=0.003; SAP: 125±2.1 vs 130.6 ±3.8 mm Hg, p=0.001; DAP: 82.2 ±2.9 vs 88.5±2.5 mm Hg, p=0.004).

COMMENT

We have created and successfully piloted a novel device that avoids

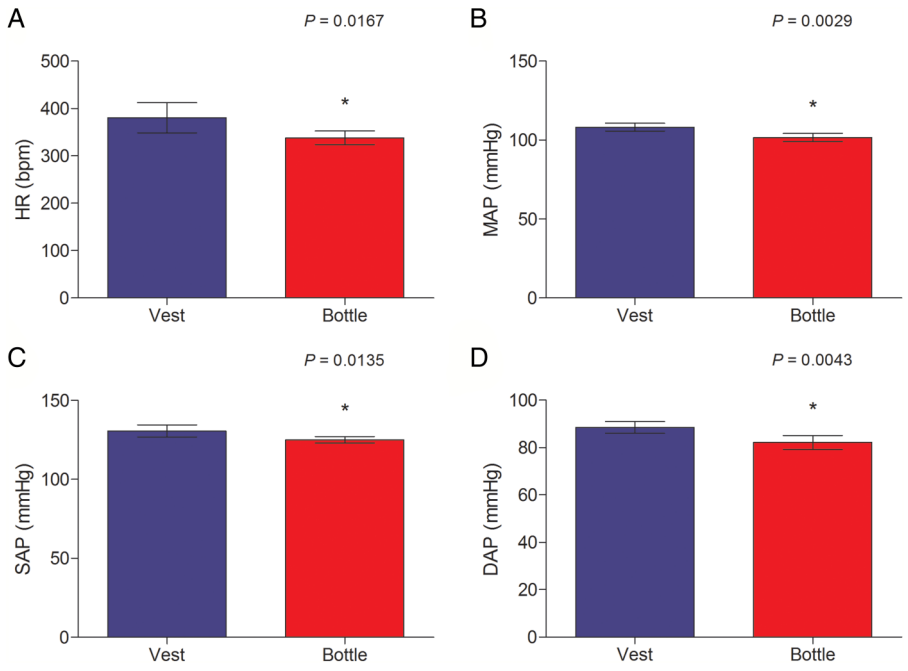

Figure 2 Haemodynamic measurements of: (A) heart rate (HR, bpm); (B) mean arterial pressure (MAP, mm Hg); (C) systolic arterial pressure (SAP, mm Hg); and (D) diastolic arterial pressure (DAP, mm Hg) in 11 rats voluntarily remaining in custom-designed burrows made out polyethylene terephthalate (PET) bottles (Bottle group, n=6) or more conventionally restrained in a waistcoat with Velcro straps (Vest group, n=5). Data are presented as mean±SD. *p<0.05 vs Vest group.

the need for restraints or anaesthesia in the application of EA. The vast majority of EA animal studies involve acute protocols that utilise general anaesthesia with the aim of reducing the stress caused by restraint of the animals for stimulation.[3] However, it is known that anaesthesia can have a cardiodepressant effect on haemodynamic parameters, decreasing blood pressure (BP) and HR.[4] By contrast, restraint may stress the animals and thereby elevate BP and HR. Moreover, we believe that subjecting animals to anaesthesia and restraint does not correlate with clinical acupuncture practice. A recent mouse study by Watanabe *et al*[5] demonstrated that acupuncture per se, when appropriately applied, does not have an impact on various markers of stress; however, their experiment also involved the use of restraint to model stress. Therefore, there is an ongoing need to establish a method whereby animals can receive EA without the use of anaesthesia or restraint.

In the present experiment, the animals remained relaxed during EA, as was the case in the study by Wang *et al*,[1] wherein animals were not anaesthetised or restrained and remained relaxed during the EA application in a PET cylinder. This was also the case in our previous experiment using the same device.[2]

CONCLUSIONS

This bottle device seems to be a feasible and highly effective alternative to anaesthesia and/or restraint when stimulating rats with EA. It is also an inexpensive tool, which is easy to use. Future research should include a larger validation dataset and compare this device against both other methods of EA application and versus untreated animals in their normal habitat. It would also be informative to evaluate its potential impact on more sensitive indices, such as HR variability.

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