

# METHOD OF RAT IMMOBILIZATION DURING EXPERIMENTAL ACUPUNCTURE

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## ABSTRACT

There is consistency in the location of biologically active points (BAP) in humans and animals, so it is assumed that the morphological changes after acupuncture in experimental animals are identical to changes in humans. Most often the experiments are made on rats, using various methods of fixing to ensure immobility during the impact in biologically active points. The aim of this study is to develop a method for immobilization of experimental animals, meeting the requirements of our experiments, in order to demonstrate the morphological changes that occur after acupuncture and to define the optimal conditions for its application.

The fixing of the experimental rats can be made with available materials or a special clamp. The used spacers for the experimental rats are basically two types - using a transparent tube and not using a transparent tube. On some animals prior anesthesia is made. After repeated application of a fixative developed by us we reached the following conclusions: 1. The fixing with a transparent tube without the use of anesthesia creates optimal conditions for experiments with needle-pricking of a rat. 2. For immobilization of experimental animals without the use of a tube, prior general anesthetic is required in order to avoid straining of the skeletal muscles and trauma on the psyche of the animal.

Today's trend is to create minimum traumatic conditions for animal fixation without the use of pre-anesthesia. The study complies with the ESA European Directive on animal welfare and is consistent with the Commission for the Ethical Treatment of humans and animals.

**Key words:** *acupuncture, biologically active points (BAP), rat, ST 36, acupuncture needle*

## INTRODUCTION

In our study of morphological changes after acupuncture an unexpected problem was fixing the experimental animals. Some of the used clamps show good immobilizing effect, but traumatize animals. Other devices are kind of friendly, but they do not fix well the test material. In the world practice

different fixatives are offered, which cover to some extent the necessary conditions to achieve results of high scientific value.

Choi et al (2010) used a transparent tube to fix the rat in a horizontal position (Fig. 1). Fixing is tight, so no further immobilization of limbs was necessary.

Tu et al (2012) also provided horizontal restraint, but without using a transparent tube (Fig. 2).

Horizontal fixing of experimental animals is preferred by other authors, with some additional limb immobilization in a horizontal or vertical position (Huang et al, 2007).

Chuansen et al (2011) placed the rat in a vertical position, immobilizing it with the help of a transparent tube (Fig. 3).

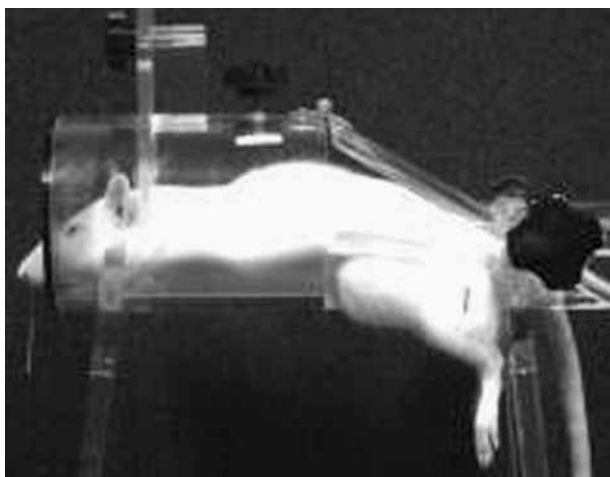
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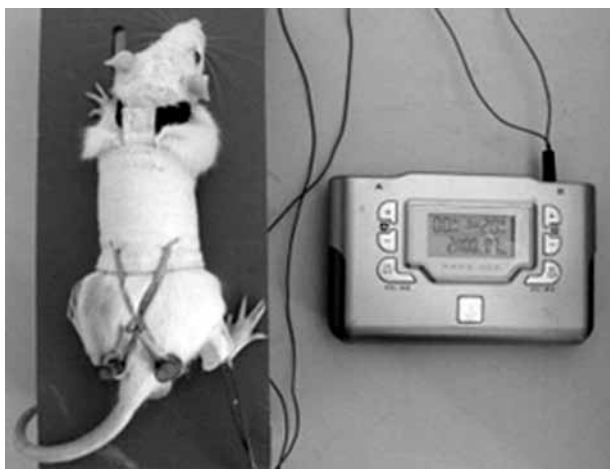
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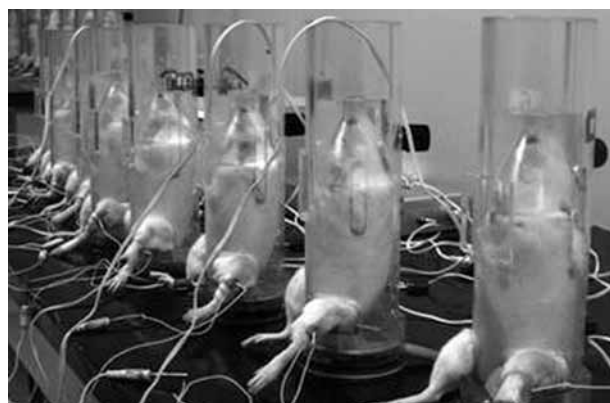
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*Fig. 1. Fixing the rat horizontally using a transparent tube (by Choi et al)*



*Fig. 2. Fixing a rat in a horizontal position without the use of a transparent tube and without anesthesia (by Tu et al)*



*Fig. 3. Fixation of experimental animals in a vertical position using a transparent tube (by Chuansen et al)*

According to Huanmin (2006) fixing devices with or without a transparent tube allows immobilization of the test animals without anesthesia.

The existence of numerous attempts to create a restraint apparatus shows that this problem is still not resolved and is a subject of future research.

### AIM AND OBJECTIVES

The aim of this study is, based on the available data for the most gentle fixation of experimental animals, to develop and implement a method to immobilize, meeting the requirements of our experiments so that we can demonstrate the morphological changes that occur after acupuncture and determine the optimal conditions for its implementation.

### MATERIALS AND METHODS

Usually in our research as experimental animals we use rat race „Wistar“, therefore in this study we examine different options for fixing that can be applied in experiments on these animals.

Fixing the experimental rats can be made with available materials or special fixings.

Fixing with a towel. In the temporary fixation with a thick cotton towel the animal is pressed against the underlying solid substrate while applying other permanent method of immobilization (Fig. 4). During a prolonged fixation the left hand of the experimenter's assistant, using a cloth, covers the chest of the experimental rat and the thumb and index finger of his right hand immobilize the limb which will be treated with acupuncture needle (Fig. 5).

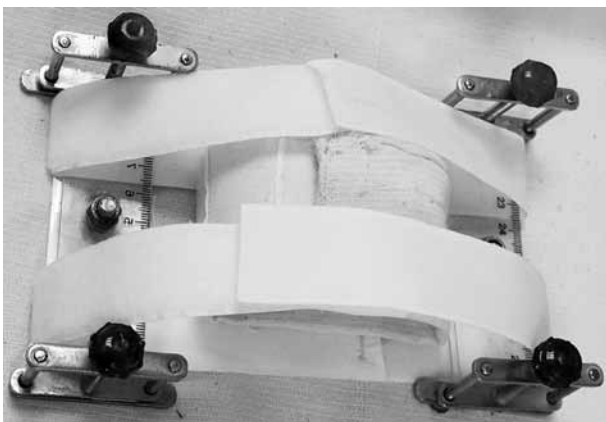


*Fig. 4. Temporary immobilization of a rat under non-anesthesia with a cloth without a clamp (own material)*

Immobilization with a clamp. We checked the effectiveness of our clamp designed for horizontal fixing of rats, which consists of H-shaped base, two metal linear graduated drivers, two textile straps to immobilize the animal and four metal retainers with rubber pads on their legs (Fig. 6).



*Fig. 5. Prolonged fixation of a rat under non-anesthesia with a cloth (own material)*



*Fig. 6. Device for fixing horizontally rats under anesthesia without the use of a transparent tube, developed by the authors (own material)*

To a part of the used animals anesthesia is previously applied: Ketamine (60-80mg/kg, ip) or Chloralhydrate (150 mg/kg, ip).

We measure the level of immobility of the experimental animals with different versions of fixing, their minimum trauma from immobilization devices and difficulties related to mobility of the animal that arise in the course of the experiment.

## DISCUSSION

Fixation using a cloth without using anesthesia does not give good results, as it does not allow a stable and long-term fixation. Another disadvantage is the need to involve an assistant to hold the animal during the experiment. Therefore, we use this method only as a temporary means until the animal is anesthetized or until applying other methods of fixing. Our view coincides with the findings of Tabosa et al (2002), who describes the use of a special sock to acupuncture limbs of the trained animals.

In immobilization with fixative without anesthesia, despite the stationary position of the



*Fig. 7. Fixing of a rat under anesthesia for horizontal immobilization (own material)*



*Fig. 8. Preparing for acupuncture of a back limb of a fixed and anesthetized rat (ST36 point is marked) (own material)*



Fig. 9. Acupuncture needle inserted in point ST36 of a fixed and anesthetized rat. (ST36 point is marked) (own material)

experimental animal, experiment can fail because of its attempts to release, in which it strains its skeletal muscles and makes putting an acupuncture needle in a designated point difficult (Gim, 2011). Therefore, the fixative we constructed is the most suitable for immobilization of pre-anesthetized animals in which muscle relaxes and allows needle-pricking without soft tissue trauma.

The type of fixtures used in other laboratories depends on the objectives of the study and used acupuncture points. Minimum traumatic for the experimental animal are the transparent tube retainers without prior anaesthesia (Almeida et al, 2008), but it demands experience of the operator, previously trained animals and short fixation. The most commonly studied acupuncture points are located on the limbs, which require access to them. When working with experimental station without using a transparent tube, as in our study, it is necessary to use general anesthesia in order the animal to be less mobile in acupuncture point ST 36. This is according to Koo et al (2002), Tae (2007) and other authors.

## CONCLUSIONS

1. Fixing with a transparent tube without the use of anesthesia creates optimal conditions for experiments with needle-pricking of a rat.
2. For immobilization of the experimental animals without the use of a tube is required prior general anesthetic to avoid straining of its skeletal muscles and trauma on the psyche of the animal.

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