METHODICAL APPROACH TO ASSESSMENT OF THE MORPHOLOGICAL CHANGES IN ACUPUNCTURE OF TONGUE

N. Pirovski¹*, N. Dimitrov¹, D. Atanasova¹,²

¹Department of Anatomy, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria
²Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

ABSTRACT
Changes occurring in acupuncture and sublingually administration of medicines also have a morphological basis, which can be objectively assessed. Objective: Refine of the methodology for evaluating morphological changes associated with tongue acupuncture. Material and methods: Selection of experimental animals and appropriate protocol for material collection, fixation, staining and counting of selected morphological structures and elements. Results: The samples fixed with formalin 10% were fixed well, the consistency of the tissues remained hard and the cuts were obtained well in all thicknesses (from 5 to 15 μm). Cuts from 5 to 7 μm are suitable for visualizing the structure of the tongue. The sagittal cuts provide the largest area for single-cut exploration, as well as are more suitable than others to track the acupuncture channel in the chosen for our study point. Conclusions: The study can be carried out in rats, but if possible, it is preferable to take the material from pigs. The use of formalin 10% gives a better texture to the material and facilitates the cutting with the microtome. It is necessary to use a variety of colorings due to the wide range of structures that are involved with the acupuncture needle tract.

Key words: tongue, morphology, methodology, acupuncture

INTRODUCTION
The long-term planning of our scientific study on the morphology of the tongue is related to changes in acupuncture and sublingual drug use. In order to realize these changes and effects it is necessary to take into account the morphological prerequisites for this. The stepwise study is related to work on experimental animals prior to the administration of healthy volunteers. The selection for experimental animals has led to the conclusion that the most suitable animals for testing are pig (1). Practical accessibility required the use of rats, are already used for acupuncture tests (2). The tongue is a solid muscular organ with a multilayered flat non-keratinizing epithelium in which the receptors for taste are located. Our main interest is focused on structures that are more affected and fast to react to irritation and drugs, such as nervous structures, the immune system, the blood vessels and the connective and muscle tissue (3). Lymph system could also be involved (4).

PURPOSE
To clarify the morphological prerequisites of acupuncture on tongue. Establishing a suitable protocol for fixing, cutting and coloring of samples from a tongue in order to maximize the visualization of the structures and by minimizing the number of experimental animals and samples (5).

MATERIAL AND METHODS
Morphological description of the tongue from the point of view of the structures that could be influenced by acupuncture. The subject of study were 12 male Wistar rats aged between 20 days and 2 years. Animals were anesthetized with xylazine and ketamine, 6 of the rats were perfused with 4% paraformaldehyde in and the other 6 with
formalin in 0.1 M phosphate buffer (pH 7.36). Samples were embedded in paraffin and cut from 5 to 15 µm and stained with the following histological stains: haematoxylin and eosin (HE), Bismarck brown (BB) and toluidine blue (TB) (6).

RESULTS
Tissues fixed with paraformaldehyde did not fix well, so they remained soft and the cutting on the microtome proved impossible. The formalin-fixed samples were well fixed, the consistency of the tissues remained stiff and the cuts were obtained well at all thicknesses (from 5 to 15 µm). Sizes of 5 to 7 µm are suitable for visualizing the tongue structure, with slices of 12 µm giving imprecise results by overlapping cells and groups of cells in the cut thickness. Slices were made in the frontal, the horizontal and the sagittal plane around the center of the tongue body (Figure 1). Sagittal slices provide the largest area for single-sided study, and are more suitable than others for tracking the acupuncture needle tract when needling the tongue. The stains used have well visualized mast cells, blood vessels and muscles, and not so well nerve fibers and lymphatic pathways.

![Figure 1](image)

**Figure 1.** (A, D, G) Light photomicrographs of hematoxylin and eosin stained sections showing the epithelium (Eph) in frontal (A) and sagittal (G) sections of rat tongue, striated muscle (MT) in a horizontal section (D). (B, E, H) Bismarck Brown stained sections demonstrating mast cells (MC) located in the musculature. (C, F, I) Toluidine Blue stained sections visualizing a salivary gland (GL) in (B) and mast cells (I). Scale bars = 200 µm (A-C; G-I), 500 µm (D-F).

DISCUSSION
The main morphological differences of tongue acupuncture from corporal acupuncture are: different epithelial layer; a high concentration of muscle fibers in a different direction; speed and tone of muscles with high speed and rapid reactivity; very intense inervation from various areas of the brain; high concentration of blood, nerve and glandular elements and this corresponds with the normal anatomy features of the tongue (7). The number of mast cells in the lamina propria and the musculature in horizontal, sagittal and frontal sections is similar, so it is not necessary to follow them in all three directions. Sagittal slices provide the best visualization of elements of the immune system, the epithelium, the musculature in a proper plane for tracking the acupuncture changes confirmed from our previous studies (8). This is an advantage when studying the acupuncture needle tract due to the fact that it is small in size and limited slices could be made from each sample. To visualize nerve structures, lymphatic pathways and connective tissue fibers, cytoskeleton, it is necessary to increase the range of stains and techniques.
used. A fibroblast cytoskeletal response to mechanical stimuli has been reported for researchers (9).

CONCLUSIONS
For good tissue fixation of the tongue it is necessary to use strong fixatives such as formalin 10%. Sagittal slices provide the best visualization of structures that are involved with the acupuncture needle tract. Acupuncture affects all layers of the tongue and a variety of tissues and structures, both locally and by reflexogenic mechanism.

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