



## MORPHOMETRIC ALTERATION IN THE ADRENALS OF THE WISTAR RATS SUBMITTED TO SUBACUTE SWIMMING STRESS.

### Morphology

**Luciana Hahmann** Faculty of Medicine, ITPAC, Porto Nacional, Tocantins, Brazil

**Tales Alexandre Aversi-Ferreira** Laboratory of Biomathematics, Department of Anatomy, Federal University of Alfenas, Alfenas, Brazil. System Emotional Science, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan.

**Wataro Nelson Ogawa\*** UnirG, Gurupi, Tocantins, Brazil. \*Corresponding Author

### ABSTRACT

The reaction to stress can generate the Cannon reaction whether the animal is submitted to stressing stimulus. A chronic stressing stimulus generates an increase of the adrenals in morphological terms, since the stress affect the hormonal and others systems via neural influence, however, no one study was performed to verify the effects of the subacute stress on the adrenal size. Therefore, the main this study was to verify the alterations of the adrenal size comparing two groups of Wistar rats submitted and not submitted to swimming stress. The results demonstrate that occurred an increase of the adrenals in the animals submitted to the pool with water in relation to animals submitted to the pool without water with significant statistical difference. In conclusion, the subacute stress is enough to generate a morphological alteration in the adrenals in Wistar rats.

### KEYWORDS

subacute stress, adrenals, morphometry.

### INTRODUCTION

The presence of stressor agent in the environment is signaled by sensorial system and follow to encephalon where will be translated in perceptions with different meanings idiosyncratically and it will to mobilize physiological responses that are dependent of ancient experience via learn-memorization, sensitization, habituation<sup>1</sup>. However, the previous experience of the stressor situation and the possibility to control it could attenuate the anxiety component or the stimulus could to assume a menacing character<sup>2</sup>.

The reaction to stress can generate the Cannon reaction (Rodrigues, 1989). Indeed, whether the animal is submitted to stressing stimulus as fear, anger, immobilization<sup>3</sup>, pain, hemorrhage, hypoglycemia<sup>4,5</sup>, sound waves<sup>6</sup>, stressing physical exercises as swimming<sup>7</sup>, it responses with behavior alterations of normal states and will entry in alert to fight or scape.

It is knowledge that a chronic stressing stimulus generates an increase of the adrenals<sup>4</sup> in morphological terms, since the stress affect the hormonal and other systems via neural influence<sup>8</sup> increasing the number of the cells in this organ.

However, according our knowledge, no one study was performed to verify the effects of the subacute stress on the adrenal size. Therefore, the main this study was to verify the alterations of the adrenal size comparing two groups of Wistar rats submitted and not submitted to swimming stress.

### MATERIAL AND METHODS

To purpose this research were used 32 Wistar rats (males and females) allocated in the Vivarium of the University of the Gurupi (UNIRG), all of them were clinically normal, at the same age, weighting from 200 to 250g, fed with feed and water *ad libitum*. Groups of four animals were allocated in the plastic box in a room with controlled temperature (25°C) and moisture, circadian cycle of 12h day/night. The noise was also controlled and kept around 54,7 dBA (IMPAC model IP-130). Clinic normality was observed in terms of behavior analysis in considering aspects as mobility, weight, ingestion of water and feed, eyes aspects, absence of wounds. The weights were measured in a scale CE, model SF-400 with precision of the 1 g.

All proceedings associated to animals were performed in according the resolutions of the Brazilians' law of the Animal Use in Research, number 11794 (October 8, 2008).

### Experimental groups

The rats were divided into 8 groups named S0, S5, S10, S15, A0, A5, A10 e A15 submitted to following experiments:

**S0:** 4 rats, placed once during 15 minutes in a pool without water then sacrificed (acute condition);

**S5:** 4 rats, placed once during 15 minutes per day during 5 days, in a pool without water, then sacrificed at 5<sup>th</sup> day (chronic condition);

**S10:** 4 rats, placed once during 15 minutes per day during 10 days, in a pool without water, then sacrificed at 10<sup>th</sup> day (chronic condition);

**S15:** 4 rats, placed once during 15 minutes per day during 15 days, in a pool without water, then sacrificed at 15<sup>th</sup> day (chronic condition);

**A0:** 4 rats, placed once during 15 minutes in a pool with water without submersion, then sacrificed (acute condition);

**A5:** 4 rats, placed once during 15 minutes per day during 5 days, in a pool with water, then sacrificed at 5<sup>th</sup> day (chronic condition);

**A10:** 4 rats, placed once during 15 minutes per day during 10 days, in a pool with water, then sacrificed at 10<sup>th</sup> day (chronic condition);

**A15:** 4 rats, placed once during 15 minutes per day during 15 days, in a pool without water, then sacrificed at 15<sup>th</sup> day (chronic condition).

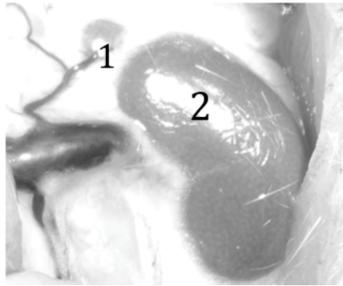
### Adaptation – Chronic protocol

The period of adaptation in environment conditions 'without water' and 'with water' for groups was performed in 15 days, 10 days and 5 days, respectively for the groups A [A0, A5, A10] and S [S0, S5 and S10]. The adaptation was performed in a plastic tank with diameter of 118 cm, 57 cm of height and volume of 50 L. The deep of the water was 2,5 cm. These procedures were performed for avoid or minimize the physical stress via swimming.

### Surgery procedures, adrenal removal and euthanasia

All rats were weighed immediately before the surgical procedures. The removal of the adrenals was performed forthwith after the anesthesia by ether inhalation via median laparotomy with incision in the medial abdominal wall using the iliac crest as a reference. The abdominal organs were away to access the kidneys where the adrenals with fatty tissue were visualized (figure 1), carefully cut and separated from the kidney using a no serrated clamp within 3 minutes, to minimize the ether effects.

After removed, the adrenals were placed in a filter paper soaked in physiological solution, dissected and immediately weighted with an analytical scale (Bioprecisa model FA2104N). The rats were sacrificed via an ether overdose.



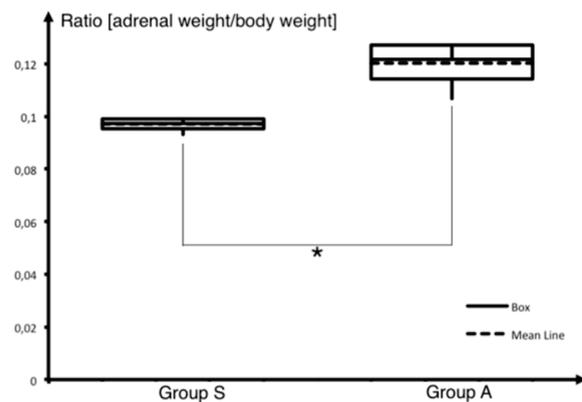
**Figure 1. Photograph of the retroperitoneal region of an Wistar rat evidencing the [2] left kidney and [1] adrenal.**

**Analysis of data and statistic**

For to verify the alterations in adrenals under effect of subacute stress, were calculate the relation of the average of both adrenal's weight/body's weight, called here of the R. The data were submitted to Q test for minimize the experimental errors. The average, standard deviation for each group and the T test for average comparison [p<0,10] was performed using the StatPlus:mac AnalystSoft Inc./2009 software.

**RESULTS AND DISCUSSION**

The results of the R demonstrate that occurred an increase of the adrenals in the animals submitted to the pool with water in relation to animals submitted to the pool without water [table 1, figure 2], with significant difference (T test p<0,10).



**Figure 2. Box graph showing significant difference of the average of the relation R [adrenal's weight/body's weight], between the group S [without water] and the group A [with water].**

According the results seems that the water or a wet environment is to Wistar rats an adverse stimulus that could be associate to many factors, internal and external, as well as phylogenetic concerning to transition from aquatic to terrestrial environment involving a genetic statement, others uncontrolled stressor factors in studied environment, the presence of females and males in the same environment.

From the moment that individual realize a stressor agent the organism presents a fast and a slow response, producing respectively, adrenaline and cortisol<sup>9</sup> and according these results is possible to verify that adrenal glands response fast to stress stimulus since the 5 day (table 1) with increasing its size.

**Table 1. Average and standard deviation of the R to the experimental groups.**

Groups	Without water	With water
15 minutes	0,082±0,003	0,077±0,004
5 days	0,093±0,005	0,107±0,045
10 days	0,097±0,003	0,122±0,026
15 days	0,100±0,013	0,133±0,028

On the other hand, Sanzovo & Coelho<sup>10</sup>, stated that the stress is a reaction to physical and psychosocial elements, and in physical terms the swimming induces stress and stimulates the adrenals response and 15 days were not sufficient to generate an adaptation to animal to return for homeostasis, according the data in table 1.

However, the physiological stress was not measure this work and this could address a new work to verify it, because the emotional stress presents complex etiology with internal and external sources<sup>11</sup>.

As animal's models are used to study indirectly human anxiety, it is plausible to conclude that subacute stress not is enough to generate a fitness of the organism to anxiety control, as well. However, it is important to state that a subacute stress is enough to generate a morphological alteration in the adrenals.

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