

This is an interest paper about the objective determination of fatigue in rodents. The authors have done a nice job describing the methods to perform an automatic analysis of time to fatigue in running rats. This study is an important contribution to experiments using animals in exercise physiology science. I have included some specific comments that the authors should consider.

Introduction

The authors can improve the introduction of the paper by describing previous studies that measured the total exercise time in rodents. It is important for the reader to understand how this measure has been made in order to understand the novelty of the proposed method.

The authors should also mention in the introduction the theoretical basis of the effects of heat on fatigue.

It is important to explain that the rats run through an electrical stimulus and the magnitude of this can drastically influence the total exercise time.

Line 72: please include *in rodents* ... and measure time to fatigue and exhaustion *in rodents*.

Methods

Is the sample size adequate? The total exercise time in running rats is a variable that presents a high coefficient of variation. Generally, a sample size greater than 6 is required.

Experimental protocol

Line 112. How many milliamps and how many millivolts were applied to the electric stimulus?

Line 114. Please change "treadmill running" to "constant-speed exercise"

Authors should provide further details of manual determination of total exercise time. For example, some authors establish as a criterion for the interruption of effort when the rat remains in the electric stimulus grid for more than 10 seconds.

Results

It is important for the authors to describe in graphs or tables the correlation coefficient between the time to fatigue values reached by the manual method and by the automatic method.

Is the automatic analysis specific for the Columbus treadmill? On the other hand, the Excel spreadsheet can be modified for use with other apparatus. There is variation in the size of the belt between different treadmills.

The authors should present a graph with the internal temperature values of the rats during running at 24°C and 32°C. This will contribute to the discussion of the effects of heat on fatigue.

Line 286. Delete "...results in faster fatigue and exhaustion" and maintain Exercise in a hot environment

Discussion

Paragraph 1 – If the automatic analysis does not differ from the manual, how important is the use of automatic analysis? The authors should explain this in the first paragraph of discussion.