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## DMP ID: https://doi.org/10.48321/D1VS45

Title: Impacts of immune system activation on locomotor behavior of male and female anurans of the species Xenopus laevis.

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Funder: São Paulo Research Foundation (fapesp.br)

Funding opportunity number: 2020/14174-4

Grant: 2020/14174-4

Template: Digital Curation Centre (português)

#### Project abstract:

Locomotion is essential for the survival and reproduction of animals, but requires energy and metabolic resources. Thus, locomotion can conflict with other aspects of physiology, such as the activation of the immune system in the face of an infection. Consequently, the allocation of energy and metabolites can impose limits on immune responses or activity patterns. When an animal is sick, immune responses can include behavioral changes such as behavioral depression resulting in reduced activity and locomotion. This reduction may be associated with energy saving which may be allocated to the activation of the immune system to fight an infection. This study investigated the effect of a simulated infection on locomotor performance and voluntary movement of male and female anurans of the species *X. laevis.* The results show that a simulated infection resulted in a reduction in the locomotor endurance of individuals of both sexes when compared to the same individuals before injection. No differences between sexes in the treatment response were observed, however. Jump force also differed when compared to control animals, but also differed between sexes with females being more affected by the immune challenge. Voluntary movement, contrary to what was expected, was only

marginally affected by the simulated infection. Our data suggest that a simulated infection leads to reduced locomotor performance in *X. laevis*, corroborating the data observed in the literature. However, there seem to be only few differences between sexes suggesting that similar mechanisms are likely responsible irrespective of differences in energy allocation strategies between the sexes.

Start date: 03-01-2021

End date: 08-31-2021

Last modified: 01-18-2024

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# Impacts of immune system activation on locomotor behavior of male and female anurans of the species Xenopus laevis.

The data generated will be of two types: a) Numerical measurements of variables of locomotor performance and voluntary movement of each animal of each sex before and after treatment with LPS. These data will be inserted into spreadsheets in .xlsx format. b) Graphic images obtained after statistical analysis of the data in TIFF format and 1000 dpi quality c) all the data commented above will be compressed in a .zip format file.

## Experimental Groups

Six animals were used for each test (locomotor endurance; jump force; voluntary movement), totaling 18 animals of each sex. Animals were testes first without treatment (control group) and subsequently after LPS injection (treatment group). Therefore, each individual was a control of itself. Each animal was tested separately.

#### Locomotor endurance

Animals were tested three times with a rest interval of 48 hours between trials. We recorded the total time spent moving, the total distance covered, and the number of laps on the track.

#### Jump force

This test was repeated on three days, with 48-hour intervals between trials. Forces were recorded at 500 Hz during 60 second recording sessions and frogs were induced to jump multiple times per session. The three best jumps per session were analyzed using the Kistler Bioware software. To do so the peak X-, Y- and Z-forces were extracted and the overall resultant force was calculated.

#### Voluntary movement

Were analyzed with a stopwatch and the total time spent moving, total time spent in the shelter and the number of breaths.

## Tables and graphs.

The project was developed with approval and permission from the ethics committee for the use of animals in experiments by the Cuvier Committee at the MNHN.

#### Does not apply to this project.

The .zip file created will be made available in the scientific data repository of the University of São Paulo (http://dadoscientificos.usp.br) and can be accessed by searching the project title, authors' names, or searching related topics to the research topic, such as "anurans, locomotion, infection, LPS, sexual dimorphism, example .

Only the authors will have access to the data to modify them if necessary. The data will be open for public consultation and may be used as long as they are referenced.

All data contained in the tables.

#### Question not answered.

The data are available in the repository of the University of São Paulo (http://dadoscientificos.usp.br) with open

access.

No.

The principal researcher Thaysa Gomes de Oliveira.

# Planned Research Outputs

## Text - "Results"

## Locomotor Endurance

<u>Body mass and test days</u>: Body mass showed no significant difference after treatment (male: p=0.587; female: p=0.910, respectively). Body mass was also not different between sexes (p=0.144) (Table 1 and 2). No difference between test days was observed either (Table 3).

<u>Total time spent moving</u>: The ANOVA demonstrated significant differences between control males *vs.* males injected with LPS (n=6; f= 5.3; p=0.036), with a 17% reduction of time on the track after treatment with LPS. Control females were also different from females injected with LPS (n=6; f= 16; p=0.001), with a reduction of 21% after treatment with LPS (Table 3; fig 1). No differences between sexes were detected either in control or treatment animals (n=12; p=0.435) (Table 4; Fig. 1).

<u>Number of laps until exhaustion</u>: The results showed a significant difference between control males *vs.* males injected with LPS (n=6; f=21; p<0.001) and between control females *vs.* females injected with LPS (n=6; f=12.7; p=0.003). The decrease in performance was 31% in males and 30% in females after treatment with LPS (Table 3; Fig. 1). There was no difference between sexes, irrespective of treatment (n=12; p=0.052) (Table 4; Fig. 1).

<u>Total distance covered:</u> The results showed a significant difference between control males *vs.* males injected with LPS (n=6; f= 8.9; p=0.009) and between control females *vs.* females injected with LPS (n=6; f= 13; p= 0.002), with a decrease of 58% in males and 63% in females after treatment with LPS (Table 3; Fig. 1). There was no difference between the sexes irrespective of treatment (n=12; p=0.068) (Table 4; Fig. 1).

#### Jump Force

Body mass and test days: No differences in body mass were observed after treatment (males: p=0.520; females p=0.057, respectively). Body mass did show a difference between the sexes (p=0.002) with males being heavier than females (Table 1 and 2). There was no difference between test days (Table 3).

<u>Maximum jump force</u>: The results showed a significant difference between control females *vs.* females injected with LPS (n=6; *f*= 6; *p*=0.025), with a 26% reduction in jump force after LPS treatment. For males the result was also significant (n=6; *f*= 6; *p*=0.036), with a reduction of 10% after treatment with LPS. There was a difference between the sexes (n= 12; *p*< 0.001), with jump forces being 27% lower in females compared to males. After LPS injections the difference was exacerbated, with jump force being 45% lower in females compared to males (Table 4; Fig. 2).

## Voluntary movement:

Body mass and test days: Body mass did not change after LPS injection in either males or females (males: p=0.356; females: p=0.810). Body mass was different between the sexes (p=0.002), with females being 41 % heavier than males (Table 1 and 2). There was no difference between test days (Table 3).

<u>Total time spent moving</u>: The results showed that there was no difference between control males *vs.* males injected with LPS (n=6; f=1.16; p=0.111). Similarly, no difference was observed for females LPS (n=6; f=2.15; p=0.163) (Table 3; fig.3). There was also no difference between the sexes (n=12; p=0.564) (Table 4; Fig. 3).

<u>Total time in the shelter</u>: The results showed that there was no treatment effect for either males (n=6; f= 1.56; p=0.230) or females (n=6; f= 0.16; p=0.688) (Table 3; fig.3). There was also no difference between the sexes

(n=12; *p*=0.712) (Table 4; Fig. 3).

<u>Number of breaths</u>: There was a significant difference between control males *vs.* LPS-injected males (n=6; f=10.5; p=0.005) with a 44% increase in breaths after LPS treatment. Similarly, there was also a difference between control females *vs.* LPS females (n=6; f=2.46; p=0.038) with an increase of 11% after treatment with LPS (Table 3; fig.3). There was a significant difference between the sexes (n=12; p<0.001). Control females took 40% more breaths than males. After LPS injection the number of breaths became 47% higher in females compared to males (Table 4; Fig. 3).

## Planned research output details

Title	Type	Anticipated release date	access	Intended repository(ies)	Anticipated file size	License	Metadata standard(s)	May contain sensitive data?	May contain PII?
Results	Text	2022-08-07	Open	None specified		Creative Commons Attribution 4.0 International	None specified	No	No