

Comm2 regulation of synaptic arborization at the neuromuscular junction and its impact on the locomotor system of *Drosophila Melanogaster*

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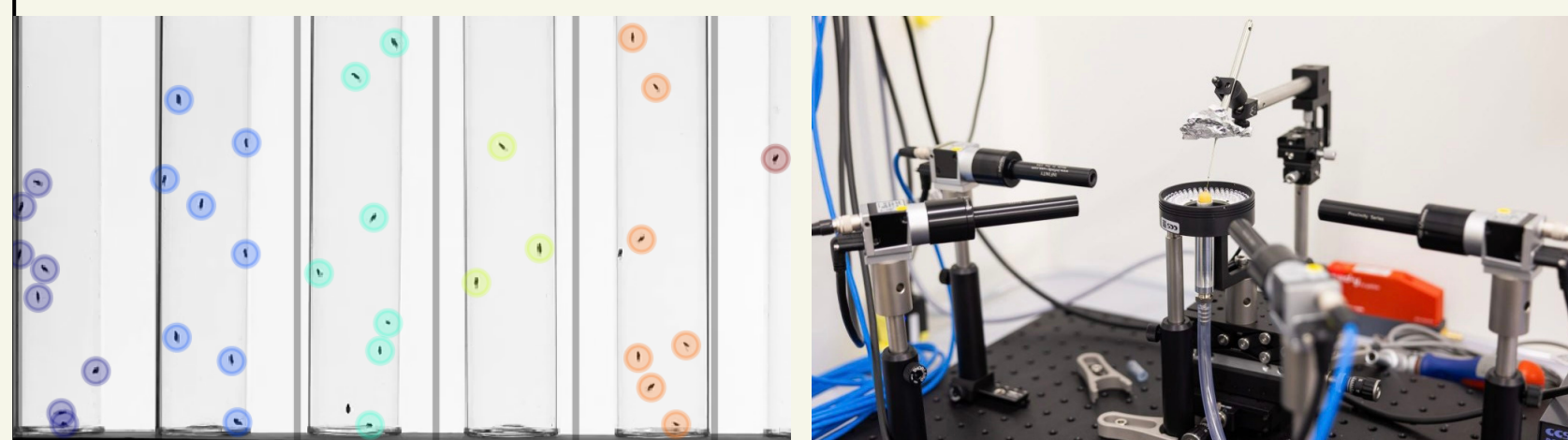


INTRODUCTION & AIMS

Studies conducted by Prof. McCabe's Lab has revealed that the LOF (loss-of-function) of *commis sureless 2* (*comm2*) [1] causes **extensive branching** at the larval neuromuscular junction (NMJ) leading to the hypothesis that the mutant flies would present an aberrant level of muscle activity and **less efficient motor neuron circuitry**. To investigate our hypothesis, we have recorded adult flies on a treadmill to **analyze kinematics** of the *Drosophila Melanogaster*'s legs, representative of motor neuron activity and also tested their climbing abilities. Thus, the goal of this experiment is to characterize how synaptic arborisation regulation by *comm2* at the NMJ affects the **locomotion of adult flies**. It should enable us to gain a **better understanding of locomotor systems**, fundamental to our comprehension of motor neuron diseases.

To what extent does the lack of comm2 protein impact the locomotion of adult flies ?

METHODS



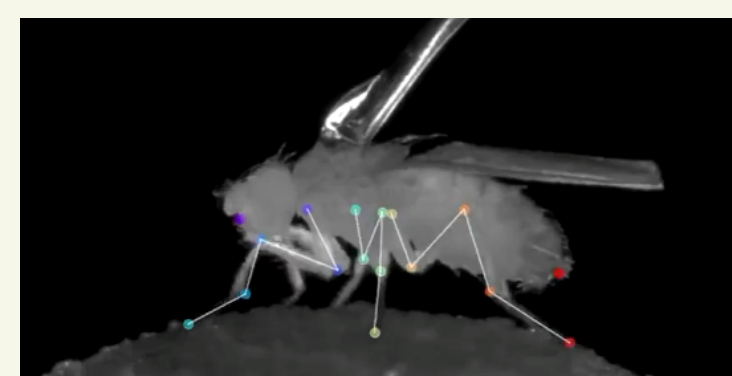
Climbing - Flies are put in transparent vials and tapped three times against a table, to activate their survival reflex of climbing up. The different genotypic groups' vertical velocity are then computed using *FreeClimber* [2].

Fly treadmill - Flies glued dorsally to the thorax are placed on a ball where they can walk freely. Five surrounding cameras record the flies' fine motor movements in 3 x 60 R60s sessions.

1 Run climbing and fly treadmill experiments to record flies' motor movements

2 Automatic tracking of joint positions over time by *DeepLabCut* [3]

3 Analyzing the data obtained by extracting the average rest time of each genotypic group using a tailored Python script



DeepLabCut video - Each bodypart gets labelled by the software, before its spatial coordinates are transcribed in a csv file.

DISCUSSION



Merits

- Development of a program for **multidimensional analysis** of the data extracted by the software
- First characterization of the previously unstudied phenotype of the *comm2* gene **paving the way for future investigations** into its specific function.

Limitations



- Limited number of flies tested (5 for each group)
- *DeepLabcut* neural network presenting a **lack of accuracy when labelling** unusual behaviour, leading to imprecise results
- Data analysis focused on **only two behavioural features**

RESULTS

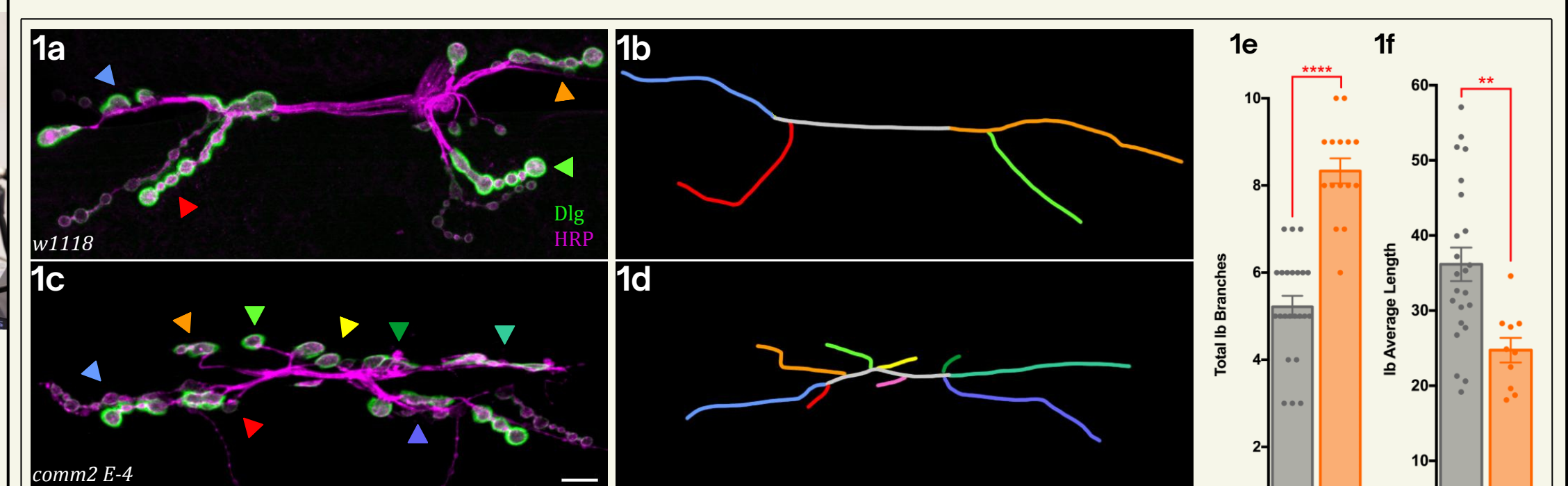


Fig. 1 : LOF of *comm2* increases the number of branches and their length in lb motor NMJs in *Drosophila* 3rd instar larvae

(a) : lb branches (arrowheads) of *w1118* larvae in m6/7 NMJ.
(c) : NMJ image of *comm2* null mutant larvae clearly shows that the no. of branches are significantly increased compared to control NMJ. It was also observed that mutant lb branches are shorter compared to controls (f).
(b) & (d) : schematic diagram depicting the branching pattern of aforementioned (a) & (c) NMJ image.
(e) : No. of total lb branches in *w1118* and *comm2* quantification. t-test
(f) : Quantification of branches' average length in *w1118* and *comm2*. t-test

Dlg: postsynaptic marker
HRP: Neuronal membrane marker
DPE: Days Post Ecllosion
LOF: loss-of-function
n= biologically independent samples
Scale bar: 10µm.

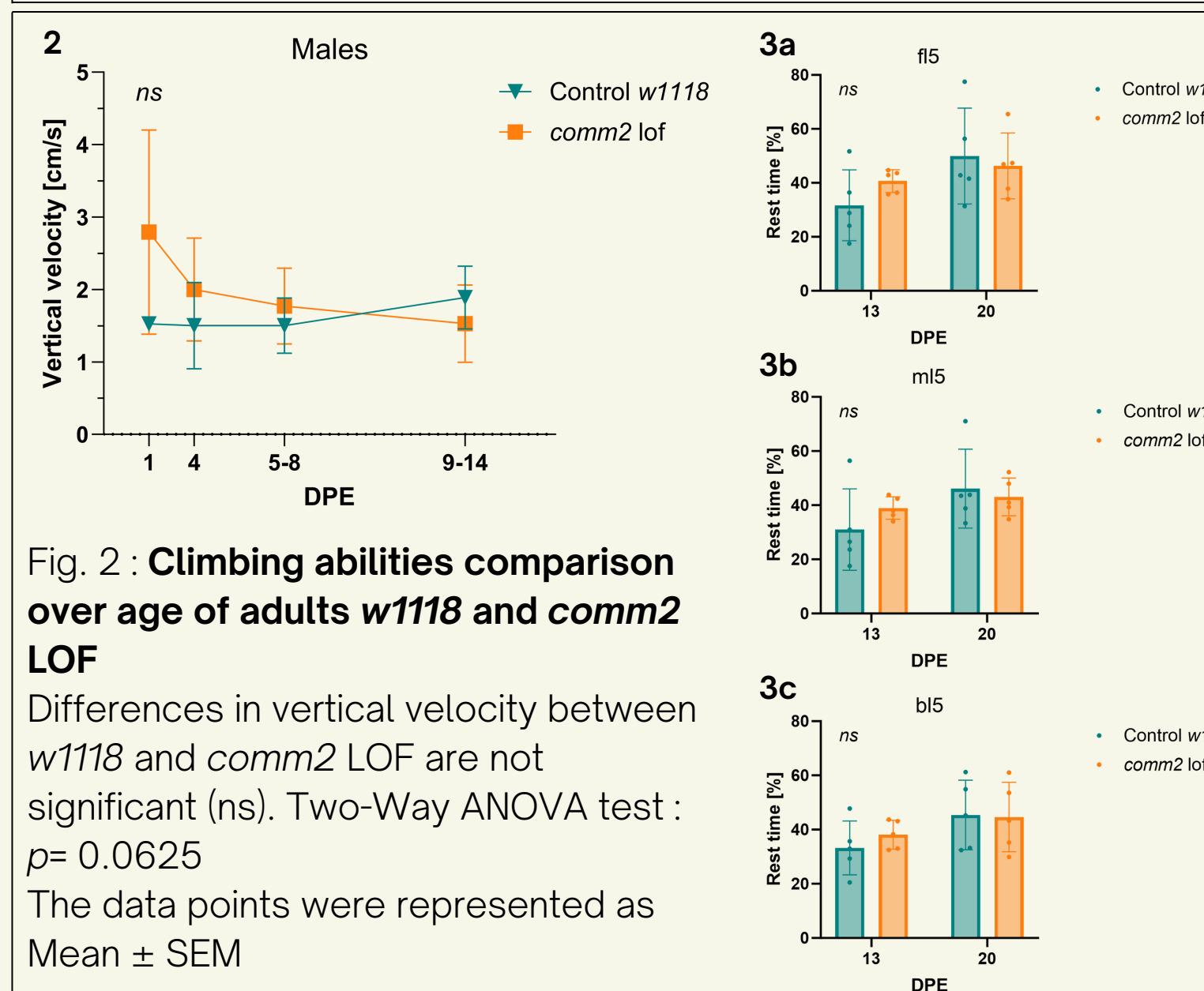


Fig. 2 : Climbing abilities comparison over age of adults *w1118* and *comm2* LOF

Differences in vertical velocity between *w1118* and *comm2* LOF are not significant (ns). Two-Way ANOVA test : $p=0.0625$
The data points were represented as Mean \pm SEM

Fig. 3: Proportion of resting time for each left leg comparison between *w1118* and *comm2* LOF groups at two different ages

Not significant results in regards of the variables tested (genotype, age and body part). Two-Way ANOVA test
(a) : $p=0.6456$
(b) : $p=0.6327$
(c) : $p=0.6741$
The data points were represented as Mean \pm SEM

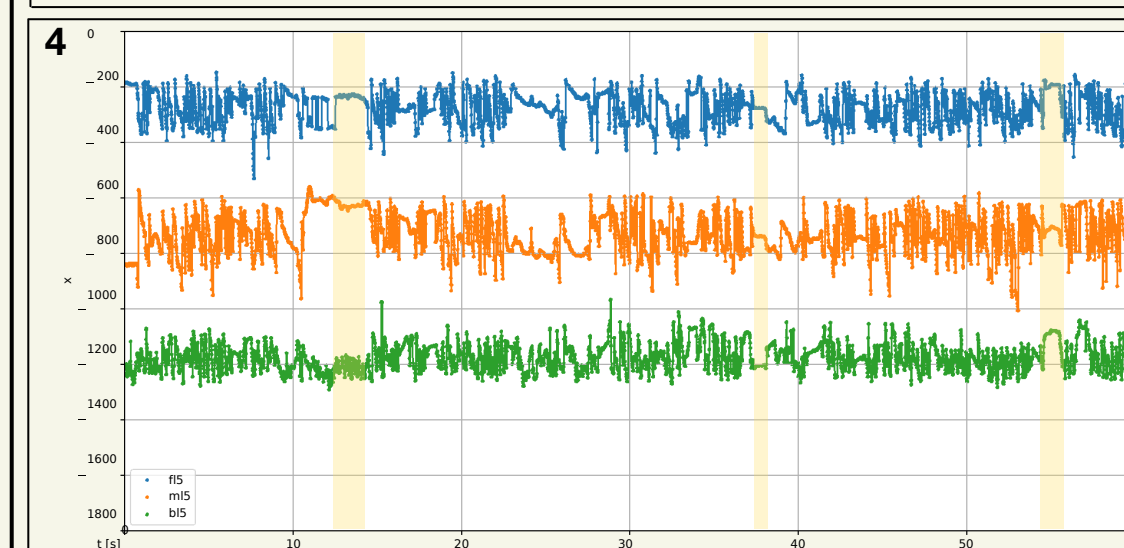


Fig. 4: Position x of the left legs' distal tip over time

Derivation over time dx/dt was used to determine joints horizontal speed. Rest time intervals were defined as when the speed was close to zero (highlighted in yellow).

fl5/ ml5/ bl5: front/ middle/ back left leg's distal tip

CONCLUSION & PERSPECTIVE

The purpose of this investigation was to determine *comm2* necessity in an organism. Even though its role has been linked to an **overbranching phenotype** in 3rd instar larvae, the experiments undertaken did **not reveal a subsequent motor movement defect** in adult flies. However, it should be noted that the *comm2* LOF group showed a tendency towards longer periods of inactivity.

Moreover further research may be needed, in particular by studying a **larger population of flies** and focusing on a **wider scope of behavioural characteristics**. It may also be interesting to extend the age parameters of the experiment to encompass older flies. This may allow us to better understand how the mutant group evolves with age.

REFERENCES

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- [3] Mathis A, Mamidanna P, Cury KM, Abe T, Murthy VN, Mathis MW, Bethge M. DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat Neurosci*. 2018 Sep;21(9):1281-1289. doi: 10.1038/s41593-018-0209-y. Epub 2018 Aug 20. PMID: 30127430.