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EXTRA VIEW

Complex roles of myoglianin in regulating adult performance and lifespan

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ABSTRACT

Myoglianin, the *Drosophila* homolog of the secreted vertebrate proteins Myostatin and GDF-11, is an important regulator of neuronal modeling, and synapse function and morphology. While Myoglianin suppression during development elicits positive effects on the neuromuscular system, genetic manipulations of *myoglianin* expression levels have a varied effect on the outcome of performance tests in aging flies. Specifically, Myoglianin preserves jumping ability, has no effect on negative geotaxis, and negatively regulates flight performance in aging flies. In addition, Myoglianin exhibits a tissue-specific effect on longevity, with *myoglianin* upregulation in glial cells increasing the median lifespan. These findings indicate complex role for this TGF-β-like protein in governing neuromuscular signaling and consequent behavioral outputs and lifespan in adult flies.

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Introduction

In vertebrates, growth differentiation factor 11 (GDF-11) and Myostatin (MST, also known as GDF-8) are closely related ligands of the Transforming growth factor-β (TGF-β) superfamily of proteins. While MST functions as a potent negative regulator of skeletal muscle growth,[1,2] GDF-11 is a well-documented suppressor of neurogenesis and neuronal number.[3-5] Both proteins are detected in the blood serum and act primarily by binding to activin type II receptors and eliciting response via intracellular transducers and transcriptional modulators SMAD2 and SMAD3.[6-9] Myoglianin (MYO) is the *Drosophila* homolog of Myostatin and GDF-11, with strong expression detected in muscle and glial cells.[10,11]

Recently, we identified MYO as a regulator of muscle size and body weight in *Drosophila* larvae (Augustin et al.[12]). In addition, genetic attenuation of *myoglianin* (*myo*) in muscles or glial cells strengthens the synaptic transmission and upregulates the density of critical pre- and post-synaptic markers at the fly NMJ (neuromuscular junction) via modulation of Smad2/Smox and GSK-3/shaggy signaling (Fig. 1). The same manipulation improves an electrical synapse output in adult flies, pointing toward a broader mechanism for synapse regulation.[12] These findings led us to believe that Myoglianin in flies could combine some of the main roles of MST and GDF-11 in vertebrates. Downregulation of *myo* throughout development in either muscle or glial compartment leads to improved larval motility (faster crawling speed),[12] demonstrating a way to enhance performance parameters in flies and, possibly, other species. Interestingly, injections of human MST into developing larvae reversed the positive affect of Myoglianin suppression on synaptic function, suggesting the possibility of MST having a similar role in the mammalian nervous system.

Both MST and GDF-11 have been implicated in the control of age-related processes. For example, inhibition of Myostatin increases skeletal muscle mass and strength and improves exercise-induced performance outcome in aging mice.[13,14] Myostatin levels decline with age in healthy men,[15] and MST inhibition is being investigated as a potential treatment of

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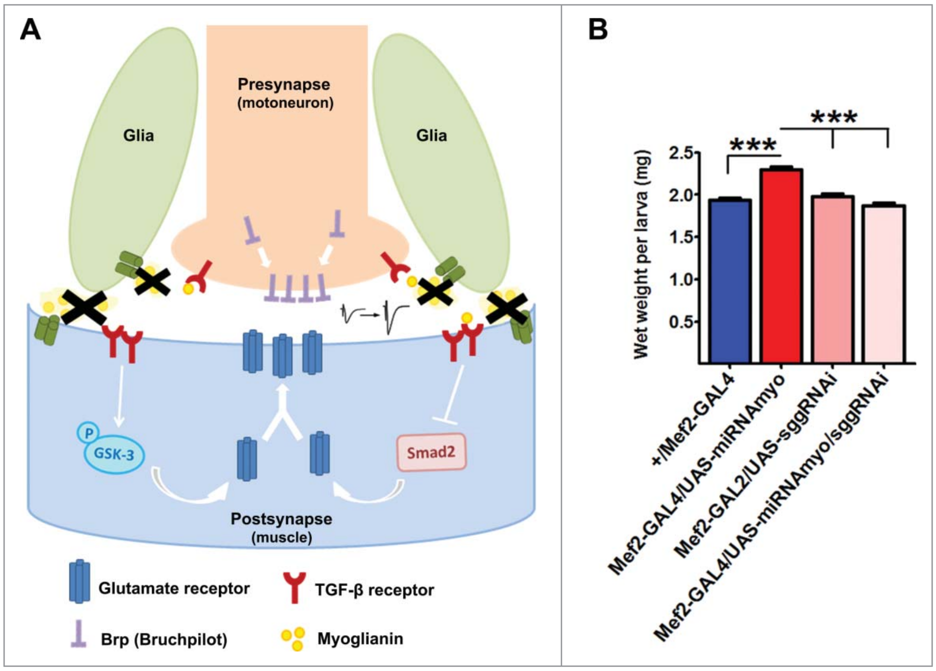

Figure 1. Reduced Myoglianin levels promote membrane targeting of the presynaptic active zone assembly regulator (Brp) and postsynaptic glutamate receptors (type A) via inhibition of Smad2/ Smox and stimulation of GSK-3/shaggy signaling, resulting in enhanced electrophysiological response.

sarcopenia and other muscle-wasting disorders.[16] Conflicting results exist regarding the role of GDF-11 in aging. While early findings indicated a positive effect of GDF-11 on brain[17] and skeletal muscle[18] aging, the subsequent reports identified GDF-11 as a likely pro-aging factor.[19-21]

We therefore wanted to assess the impact of Myoglianin on age-dependent muscle function in adult flies. Myoglianin transcript is detected at low to moderate levels in various fly adult tissues.[22] However, as the muscular system in adult flies appear to be extremely limited in terms of its ability to grow and regenerate,[23] the role of MYO in aging flies might be significantly different from its role in rapidly growing tissues during development.

Results and discussion

In flies with MYO levels reduced in muscles (using the *UAS-miRNAmyo* construct driven by the *Mef2-GAL4* driver), we observed improved flight ability throughout adult life compared with controls, with a small decrease in body weight measured in these flies with age (Fig. 2A). These results parallel the improved motility of 3rd instar larvae with genetically silenced *myo*.[12] Equally, the opposite effect on the flight ability was seen in *myo*-overexpressing flies (*Mef2-GAL4/UAS-myoglianin*, Fig. 2B).

In disagreement with a recent paper reporting impaired climbing in the flies with RNAi-reduced *myo* expression in muscles,[24] we saw no effect on the climbing ability upon muscle-specific *myo* silencing (Fig. 3A). Interestingly, increased *myo* expression prevented age-related decline in the jumping ability, resulting in 'youthful' jump test outcomes even in late adulthood (day 48) (Fig. 3B).

Demontis et al.[24] reported lifespan extension in *myo*-overexpressing flies and reduced longevity in *myo*-silenced animals. In our hands, variable *myo* expression levels in the muscle did not significantly affect lifespan (Fig. 4A). It is possible that these phenotypic differences stem from different muscle drivers and RNAi constructs used to manipulate *myoglianin* levels in adult muscles. For example, while the *MHC-GAL4* driver (used by Demontis et al., 2014[24]) drives strongly in thoracic muscles, the *Mef2-GAL4* drives "far more extensively, in most somatic musculature".[25] Intriguingly, *myo* overexpression in glial cells, previously shown to have a negative impact on the weight, motility and synaptic parameters in *Drosophila* larvae,[12] resulted in significantly increased median lifespan, with glial silencing of *myo* having the opposite effect (Fig. 4B). These findings further our

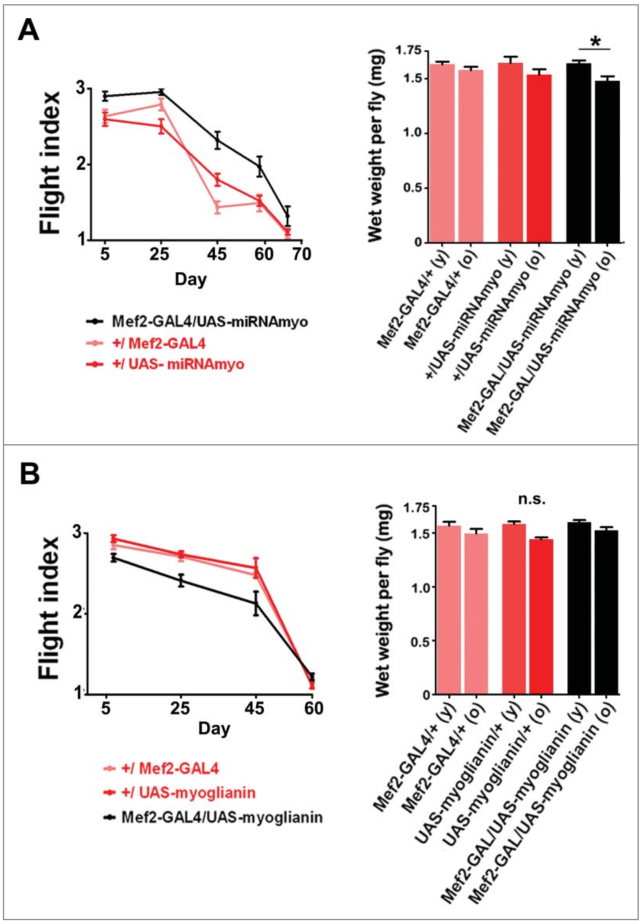

Figure 2. MYO negatively regulates flight performance in adult flies. (A) Left: Flight performance index measured in *Mef2-GAL4/UAS-miRNAmyo* (black) and control (red) flies throughout the adult life (n = 31–33, ANOVA genotype/age interaction *P = 0.0055)*. Right: Total wet weight of individual flies measured in young (day 5) and old (day 50) animals (n = 10–12). (B) Left: Flight performance index measured in *Mef2-GAL4/UAS-myoglianin* (black) and control (red) flies (n = 26–29). The genotype/age interaction was significant *(P=0.0056)*. Right: Total wet weight of individual flies measured in young (day 5) and old (day 50) animals (n = 10–12).

understanding of the lifespan-modulating role of the glial compartment in *Drosophila*.[26,27]

Our results imply a complex role for Myoglianin in modulating neuromuscular system function in adult flies, with different muscles/tissues requiring different levels of MYO for achieving optimal functional output. Furthermore, the combinatorial effect of these inputs is likely to play an important role in determining the overall health- and life-span in aging flies. Overall, in addition to its role as an important regulator of muscle size, body weight, motility, and synaptic composition and function in larvae,[12] Myoglianin appears to have a highly regulated and context-dependent impact on adult tissues and whole organism in *D. melanogaster*.

Materials and methods

Fly stocks and husbandry

Tissue- and cell type-specific expression was achieved with the *GAL4-UAS* system.[28] To standardize genetic background, parental *GAL4* and *UAS* strains used to generate experimental and control genotypes were backcrossed to laboratory control strain w^{Dah} for at least 6 generations. All stocks were maintained and all experiments were conducted at 25°C on a 12h:12h light:dark cycle at constant humidity using standard sugar/yeast/agar (SYA) medium[29] (the food contained 5% sucrose (w/v), 1.5% agar (w/v), 0.3% propionic acid (v/v), 0.3% nipagen (w/v) and either 1% (0.1 × yeast), 5% (0.5 × yeast), 10% autolysed brewer's yeast (w/v) and was prepared as described previously.[30] *UAS-miRNAmyo* line[11] was a gift from T. Awasaki and from the T. Lee laboratory at Janelia Farm; *UAS-myoglianin* (2nd chromosome) was a kind gift from M. O'Connor, University of Minnesota; *Mef2-GAL4* (#27390) and *repo-GAL4* (#7415) were obtained from the Bloomington Stock Center. w^{Dah} was the "wild-type" strain used in all experiments. The *white Dahomey* (w^{Dah}) stock was derived by incorporation of the w^{1118} mutation into the outbred Dahomey background by backcrossing.

Flies were mated for 48 h before separating females from males. Only female flies were used in the experiments.

Flight and climbing tests

Flight testing was done using a described previously protocol.[31] Individual flies were released from the bottom of a perspex ('Sparrow') box 40 cm high with a light source at the top, and scored for flight as follows: top (2 points), middle (1 point) or bottom (no points). The mean point achieved by 3–6 flies was determined; the procedure was repeated 5–10 and mean calculated for each genotype/time point.

For the climbing (negative geotaxis) test, adult female flies were housed at 15 flies/vial and 3 populations were tested for each genotype/experiment. The flies were assayed at 5 time points (days 4, 18, 32, 46 and 70) for climbing activity in a modified 25 ml 'stripette' tube. The flies were gently tapped to the base of the climbing tube and their climbing progress was recorded after 45 s. Each population of flies were assessed 3 times per assay and the average values were used to calculate the performance index as described previously.[32]

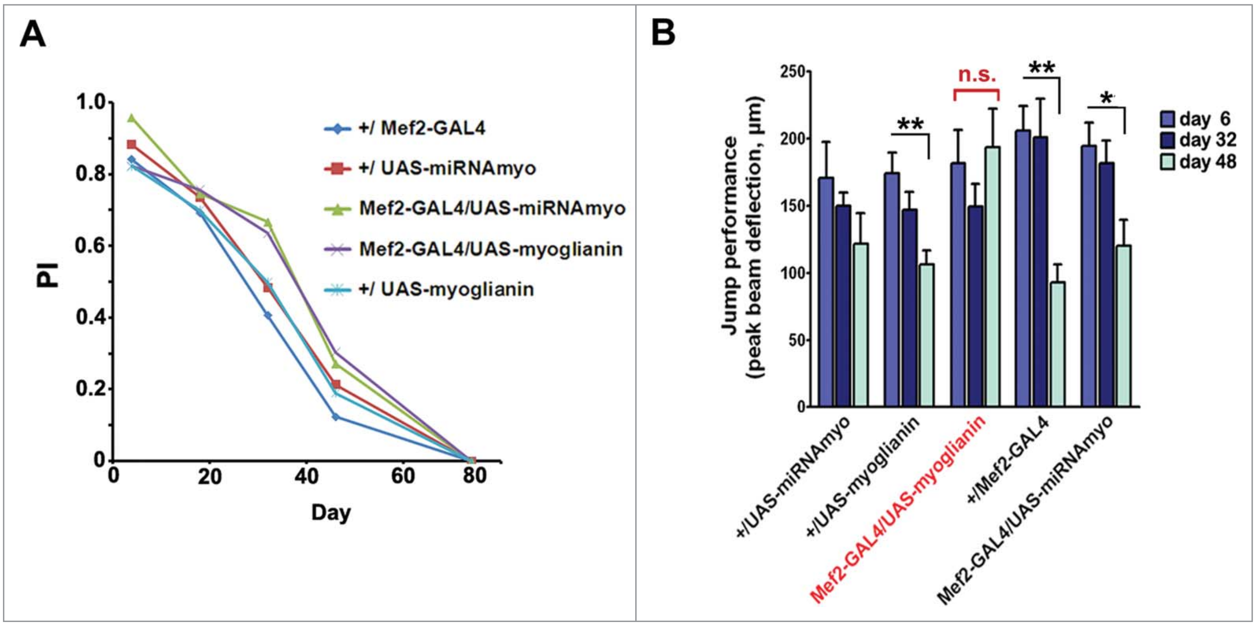

Figure 3. MYO affects jumping ability, but not climbing performance. (A) The climbing ability ('negative geotaxis') of the adult flies of denoted genotypes. No significant difference between the genotypes was observed. (B) Jump muscle performance is sustained by expression of *myoglianin* by *Mef2* (n = 6–19 per genotype/age). In the full data set, including *Mef2-GAL4/UAS-myoglianin*, ANOVA genotype interaction with age *P=0.042*; but without the 48 day-old data, or the *Mef2-GAL4/UAS*-myoglianin data the genotype-age interaction is not significant *(P = 0.57* and *P = 0.49*, respectively).

Lifespan experiments

Flies in lifespan experiments were performed as described previously.[33] Animals were reared at standard larval density and eclosing adults were collected over a 12 h period. Females were mated for 48 h before being separated from the males. Flies were maintained in vials on standard SYA medium at a density of 10 flies per vial and transferred to new vials every 2–3 d and scored for deaths.

Jump muscle performance

Jump muscle performance was determined using a miniature ergometer.[34,35] Briefly, flies were anaesthetized

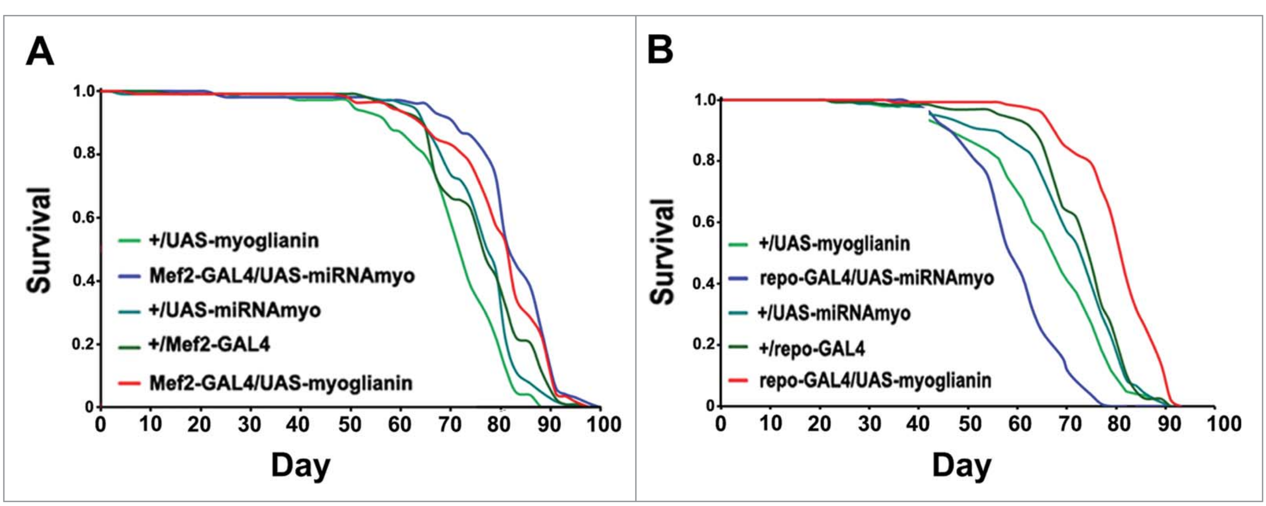

Figure 4. Myoglianin in glia, but not in muscle, affects lifespan. (A) Lifespans in flies expressing various *myo* transgenes in the muscle. Median survival times for female flies (in days) were 82 (*Mef2-GAL4/UAS-miRNAmyo*), 82 (*Mef2-GAL4/UAS-myoglianin*), 71 (*+/UAS-myoglianin*), 78 (*+/UAS-miRNAmyo*) and 76 (*+/Mef2-GAL4*); (B) The survival curves from flies expressing *myo* constructs driven by the glial *repo-GAL4* driver. Median survival times for female flies were 59 (*repo-GAL4/UAS-miRNAmyo*), 80 d (*repo-GAL4/UAS-myoglianin*), 67 (*+/UAS-myoglianin*), 73 (*+/UAS-miRNAmyo*) and 73 d (*+/repo-GAL4*).

with CO_2 and mounted on a tungsten pin and allowed to recover for over 20 min. They were mounted in the apparatus and the jump elicited by electrical stimulation between the eyes. The jump response was determined optically from the movement of the platform below the fly. For each fly the biggest response to 4–10 stimuli was determined. The best response of each fly was used in the calculation of mean and SEM and in statistical evaluations. The experimenter was blind to the exact genotypes during testing.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad Software Inc., USA). A 2 way ANOVA test was used to perform (age x genotype) interaction calculations. For other comparisons between 2 or more groups, a one-way ANOVA followed by a Tukey-Kramer post hoc test was used. In all instances, $P < 0.05$ is considered to be statistically significant ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Log-rank tests were performed for survival. Values are reported as the mean ± SEM.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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