Neuronal Expression of Polyglutamine-Expanded Ataxin-2 Increases Aggressive Behavior in Drosophila melanogaster

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Background and Significance

Polyglutamine expansion in human Ataxin-2 (ATXN2) is associated with neurodegenerative diseases, including spinocerebellar ataxia type 2. Affected patients exhibit motor dysfunction as well as changes in sleep and behavioral regulation, including aggression-related behaviors. However, it remains unclear whether neuronal ATXN2-polyQ expression can directly perturb behavior-regulating neural circuits. We addressed this question in Drosophila melanogaster by expressing human ATXN2-polyQ in defined central brain neuronal populations using the GAL4/UAS binary expression system. Adult male flies expressing ATXN2-polyQ were tested in age-matched male-male pairs in a food-cup-based aggression assay and compared with GAL4-only and UAS-only genetic controls. ATXN2-polyQ-expressing experimental males showed a robust increase in aggressive behavior relative to both genetic controls, with significant increases in lunge frequency and wing threat displays. In mixed-genotype assays pairing experimental males with wild-type Canton-S opponents, ATXN2-polyQ-expressing males initiated all fights and performed most aggressive acts, indicating the phenotype was intrinsic to the experimental animal rather than driven by reciprocal escalation. To begin mapping the relevant neurons, we combined the GAL4 driver with a UAS-mCD8::GFP reporter and observed labeled neurons in several regions of the adult central brain, including the mushroom bodies and subesophageal ganglion. We are currently investigating courtship and sleep behavior to distinguish whether ATXN2-polyQ expression selectively increases aggression or produces broader changes in behavioral arousal. Together, these findings establish a fly model for dissecting how ATXN2-polyQ expression in defined neuronal populations alters behavior-regulating circuits, thus providing a framework for understanding how polyglutamine-expanded Ataxin-2 may contribute to behavioral dysregulation in neurodegenerative disease.

Targeted Expression of Human ATXN2-polyQ in Fly Brain Neurons

- Behavioral Testing
- Brain Expression Mapping
- Future RNA-seq

Targeted expression of human ATXN2-polyQ in fly brain neurons. The ATXN-GAL4 driver is crossed with the UAS-ATXN2-polyQ responder to generate progeny chronically expressing full-length human ATXN2-polyQ in the ATXN-GAL4-targeted neuronal population. Experimental applications include behavioral testing, brain expression mapping using a UAS-mCD8::GFP reporter cross, and future RNA-seq analysis.

ATXN-GAL4 Labels Neurons Across Multiple Regions of the Adult Drosophila Brain

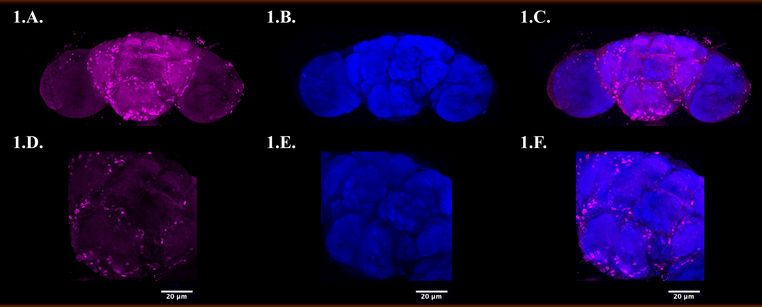
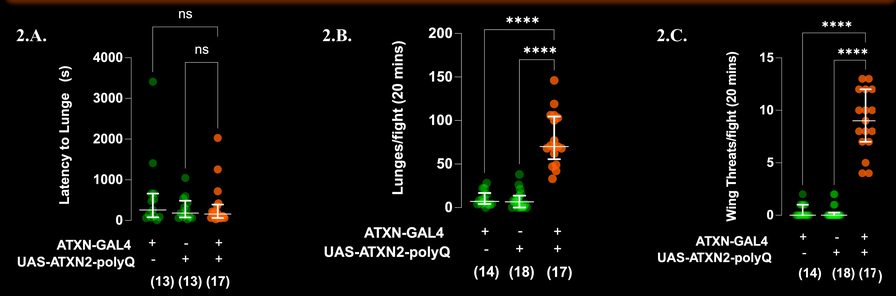


Figure 1. ATXN-GAL4 expression in the adult Drosophila brain. Maximum-intensity projections of confocal z-stacks from adult brains expressing ATXN-GAL4/UAS-mCD8::GFP. **A–C.** Whole-brain images acquired at 20× magnification. **D–F.** Higher-magnification images acquired at 63×. GFP-labeled neurons and processes are shown in magenta, nc82-labeled neuropil in blue, and merged images in the right panels. ATXN-GAL4 labels neurons across multiple brain regions, including the mushroom bodies and subesophageal ganglion. Scale bars in D–F, 20 µm.

Chronic Expression of Human ATXN2-polyQ in ATXN-GAL4 Neurons Escalates Aggression in Genotype-Matched Male Dyads



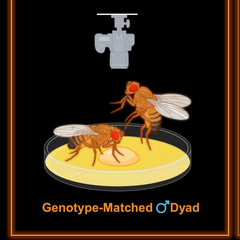


Figure 2. Chronic expression of human ATXN2-polyQ in ATXN-GAL4 neurons enhances aggression in genotype-matched male dyads. A. Latency to the first lunge did not differ significantly among genotypes (Kruskal-Wallis test, $P = 0.6643$). **B.** Males expressing human ATXN2-polyQ in ATXN-GAL4 neurons performed significantly more lunges than either the ATXN-GAL4/+ or UAS-ATXN2-polyQ/+ genetic controls. **C.** Experimental males also displayed significantly more wing threats than both controls. Dunn's multiple-comparisons test was used following the Kruskal-Wallis test for panels B and C; ****adjusted $P < 0.0001$. Each point represents one male dyad, and numbers in parentheses indicate the number of dyads analyzed.

ATXN2-polyQ-Expressing Males Are More Aggressive Than Wild-Type Canton-S Males

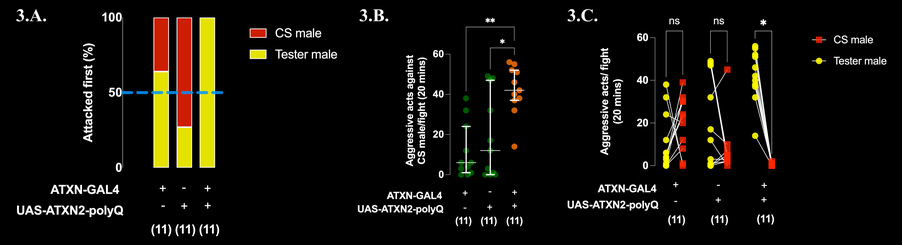
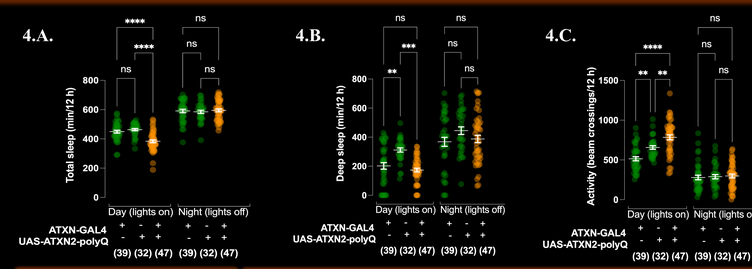




Figure 3. ATXN2-polyQ-expressing males are more aggressive than wild-type Canton-S males. Each tester male was paired with a Canton-S male. **A.** The identity of the male attacking first differed by tester genotype, with ATXN-GAL4/UAS-ATXN2-polyQ males initiating all fights (Fisher's exact test, $P = 0.0017$). **B.** Experimental males performed significantly more aggressive acts against Canton-S males than both genetic controls (Dunn's multiple-comparisons test; adjusted $P = 0.0015$ versus ATXN-GAL4/+ and $P = 0.0140$ versus UAS-ATXN2-polyQ/+). **C.** Within each dyad, experimental males performed significantly more aggressive acts than their Canton-S opponents, whereas neither genetic control differed from Canton-S (Wilcoxon matched-pairs tests with Holm-Šídák correction; adjusted $P = 0.0029$ for experimental males and $P = 0.4918$ for each control). Aggressive acts include lunges and wing threats. Connecting lines link males from the same dyad, and numbers in parentheses indicate the number of dyads analyzed. **D.** Representative 20-minute raster plots show the timing of aggressive acts within a single dyad for each tester genotype paired with Canton-S. A yellow tick indicates an aggressive act performed by the tester male, and a red tick indicates an aggressive act performed by the Canton-S male. In the representative ATXN-GAL4/UAS-ATXN2-polyQ versus Canton-S dyad, the tester male accounts for all aggressive acts.

ATXN2-polyQ Expression Reduces Daytime Sleep and Increases Daytime Activity



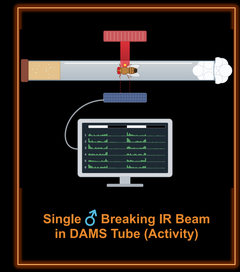


Figure 4. ATXN2-polyQ expression reduces daytime sleep and increases daytime activity. A. Daytime total sleep decreased compared with both controls (genotype × phase interaction, $P < 0.0001$). **B.** Daytime deep sleep decreased compared with the UAS control (Tukey, $P = 0.0001$); the two controls also differed ($P = 0.0049$), but the interaction was not significant ($P = 0.1787$). **C.** Daytime activity increased compared with both controls (interaction, $P < 0.0001$). No significant nighttime differences were detected. Activity was recorded as infrared beam crossings at 1-minute intervals; sleep and deep sleep were defined as ≥5 and ≥60 minutes of continuous inactivity, respectively. Each point represents one fly; numbers in parentheses indicate sample sizes; mean ± SEM. Two-way repeated-measures ANOVA with Tukey's multiple comparisons. **$P < 0.01$; ***$P = 0.0001$; ****$P < 0.0001$; ns, not significant.

ATXN2-polyQ Expression Does Not Impair Climbing or Courtship Initiation

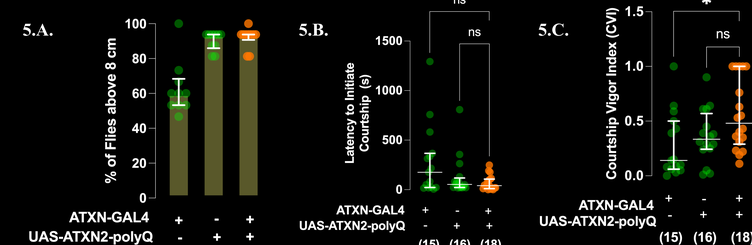


Figure 5. ATXN2-polyQ expression shows no overt climbing deficit or delay in courtship initiation but modestly alters courtship vigor. A. In an initial negative-geotaxis cohort, experimental flies showed no overt climbing deficit across 10 technical replicates; additional biological replicates are underway. Here, data are presented as median with interquartile range. **B.** Courtship-initiation latency did not differ among genotypes (Kruskal-Wallis test, $P = 0.1293$). **C.** Experimental males showed a higher courtship vigor index than ATXN-GAL4/+ controls but not UAS-ATXN2-polyQ/+ controls (Dunn's multiple-comparisons test; adjusted $P = 0.0231$ and 0.1977, respectively). Each point in **A** represents one technical replicate; each point in B–C represents one courtship assay.

Future Directions, Funding Sources, and References

- Test whether ATXN2-polyQ shortens lifespan and whether aggression persists with age.
- Map and validate the ATXN-GAL4 synaptic circuit using MCFO and the male connectome.
- Use CaMPARI2 to test whether ATXN-GAL4 neuron calcium activity changes during fights.

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Illustrations: GAL4/UAS, Fig. 2–3 cartoons created with BioRender.com

Scan for full references