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# Regulation of neuron growth and development by the matricellular protein *dCCN*

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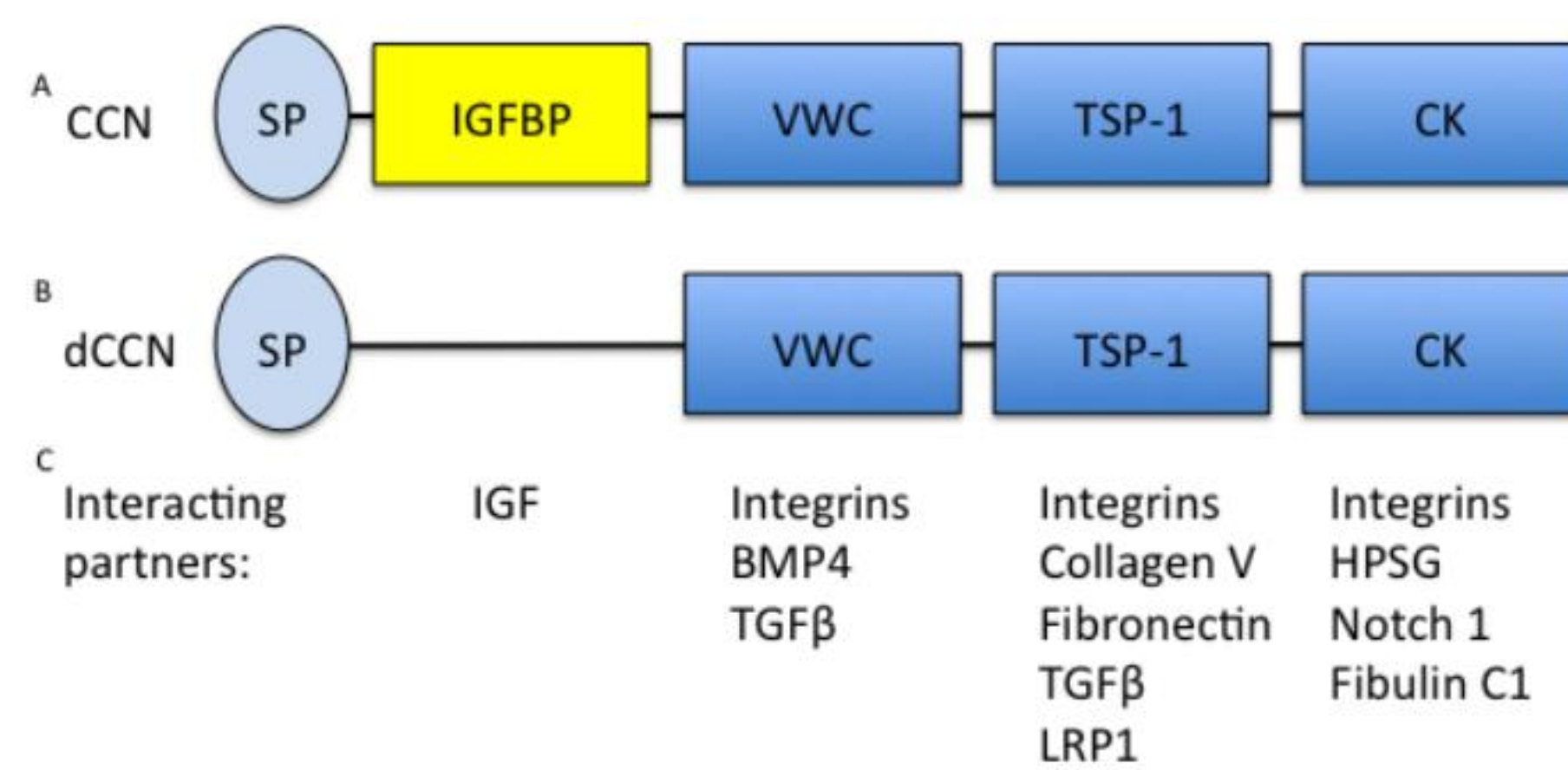
## 1 – Introduction

The extracellular matrix (ECM) provides critical biochemical and physical signals to initiate and support a diverse array of cellular functions. The CCN (*CCN1*, *CCN2*, *CCN3*) family of proteins functions in a primarily regulatory role rather than structural. CCN members are highly expressed in the central nervous system and contribute to neuron development, differentiation, and synaptic plasticity. However, the contributions of CCN family members and matricellular proteins to neuron communication and synaptic transmission remain understudied. Here we examine the role of the sole *Drosophila* CCN (*dCCN*) family member in the developing and mature nervous system. *dCCN* is expressed in motorneurons and interneurons throughout embryonic, larval, and adult stages. In the adult, *dCCN* expression is found in peripheral neurons located in the legs, wing, and proboscis that respond to sensory information and in the ventral nerve cord in neurons that innervate the reproductive system and motorneurons. In the central brain, our results demonstrate *dCCN* is expressed in octopamine (OA) neurons, dopamine (DA) neurons, and neurons that express the male form of Fruitless (FruM), a key regulator of male-specific behavior.

Hypotheses:

- *dCCN* is required for neuron function
- Loss of *dCCN* will result in alterations to neuron communication and changes in behavior

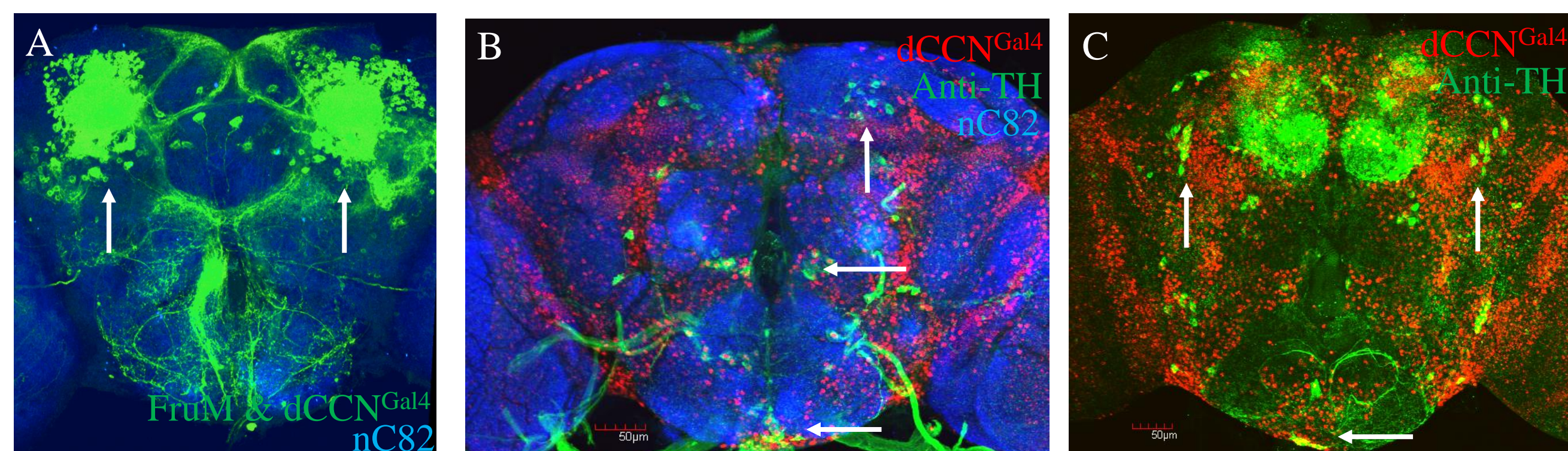
The *dCCN* protein has three of the four well conserved domains.



(A) The four well-conserved domains of CCN. (B) The three well-conserved domains found in *dCCN*. Insulin-like growth factor binding protein (IGFBP), a von Willebrand factor type C repeat (VWC), thrombospondin type-1 repeat (TSP-1), and a cysteine knot-containing domain (CK). (C.) The many interacting partners of the CCN and *dCCN* domains. Bone morphogenetic protein 4 (BMP4), transforming growth factor  $\beta$  (TGF $\beta$ ), lipoprotein receptor-related protein 1 (LRP1), and Heparin sulfate proteoglycans (HPSG).

## 4 – *dCCN* expression

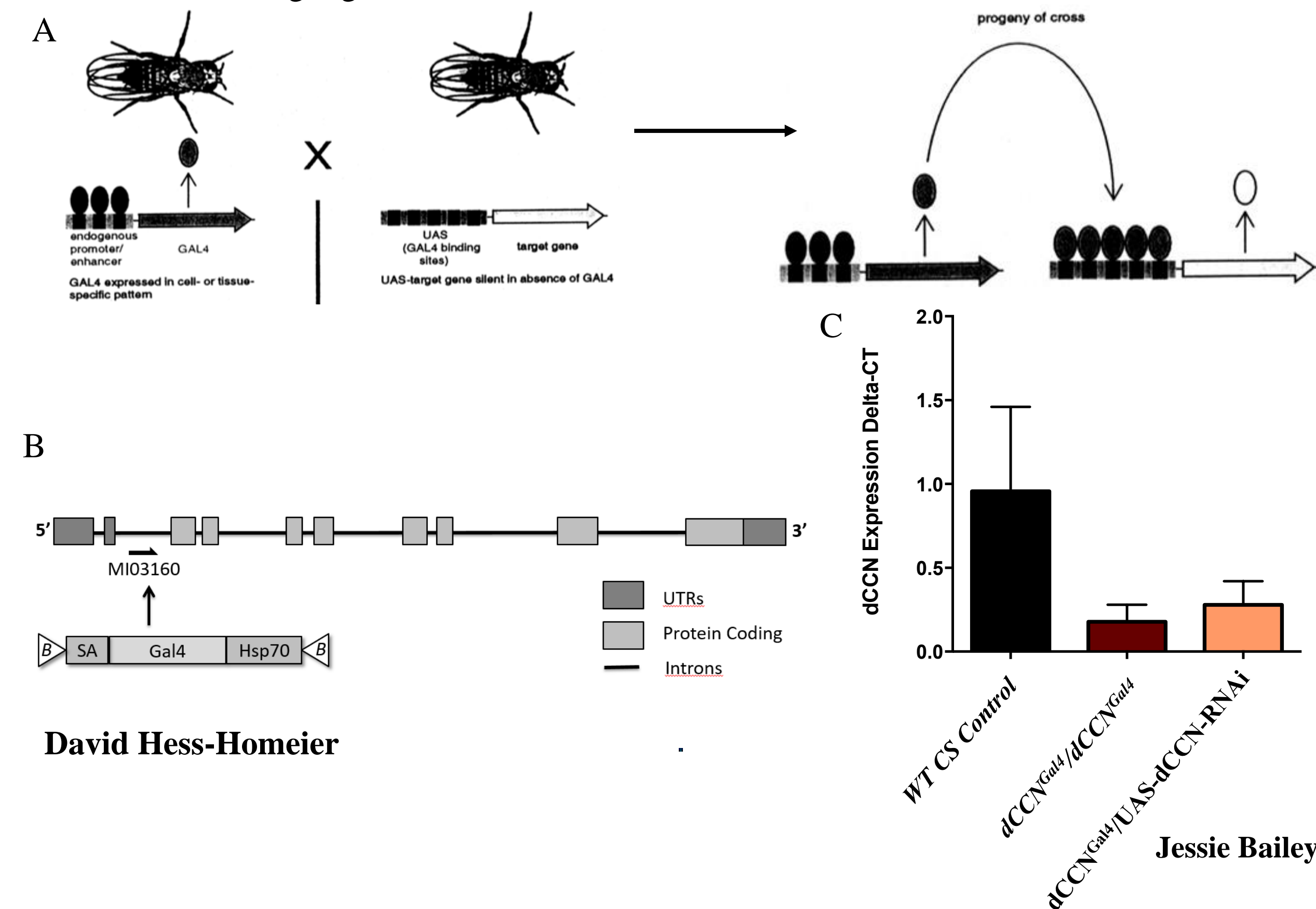
*dCCN<sup>Gal4</sup>* is expressed in FruM, OA, and DA neurons. FruM neurons are required for male behavior such as courting or aggressive behavior. Previous work of the Certel lab has demonstrated that OA null mutants display low levels of aggression and high levels of courtship. These findings suggest a role for *dCCN* among amine neurons, differentiation, and/or function of sex-specific circuitry. We next examined aggression as a readout for neuron function.



(A) *dCCN<sup>Gal4</sup>* is expressed in the majority of FruM expressing neurons, including the Kenyon cells that comprise the mushroom bodies (arrow, *UAS-stop>CD8:GFP; dCCN<sup>Gal4</sup>/fru-flp*). (B) The majority of OA neurons (arrows) co-express *dCCN* (*dCCN<sup>Gal4</sup>/UAS-dsRed* with anti-tdc2 staining). (C) *dCCN<sup>Gal4</sup>* is expressed in some DA neurons (arrows) distal of the mushroom body area (*dCCN<sup>Gal4</sup>/UAS-dsRed* with anti-TH staining).

## 2 – Tools to examine CCN in *Drosophila melanogaster*

To manipulate gene expression, the Gal4/UAS binary system will be utilized. The Gal4/UAS system is used every day in *Drosophila* labs around the world to activate, knockdown, or inhibit gene expression. Gal4, a yeast transcriptional activator, is expressed under control of a cell or tissue-specific promoter. Upon expression, Gal4 binds to the upstream activating sequence, UAS, to activate transcription of the gene or RNAi sequences downstream of the UAS sequence. To reduce *dCCN* function, a *Drosophila dCCN<sup>Gal4</sup>* line was generated in the Certel lab. This line contains an insertion of Gal4 into the coding region of *dCCN*. The resulting line drives Gal4 expression under the *dCCN* promoter thus faithfully recapitulating endogenous expression patterns, as well as creating a loss-of-function allele due to disruption of the *dCCN* coding region.

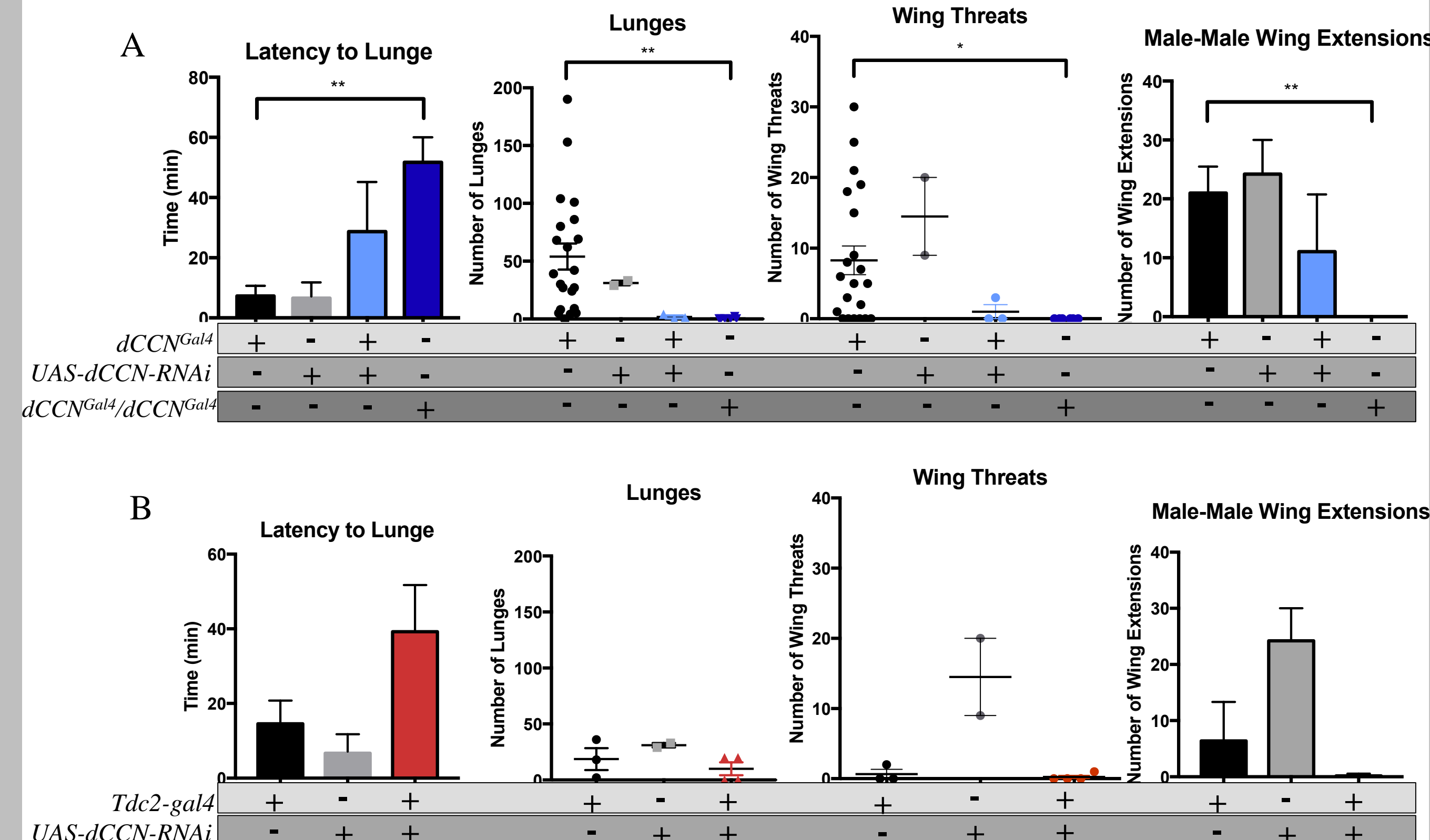


David Hess-Homeier  
Jessie Bailey

*dCCN<sup>Gal4</sup>* is a strong hypo-morphic allele constructed by Gal4 replacement of a MiMiC insertion. (A) A schematic of the Gal4/UAS binary system in *Drosophila*. (B) A schematic detailing the Gal4 construct insertion. (C) *Drosophila* CCN transcript levels via PCR analysis.

## 5 – Loss of *dCCN* results in decrease in aggressive behavior

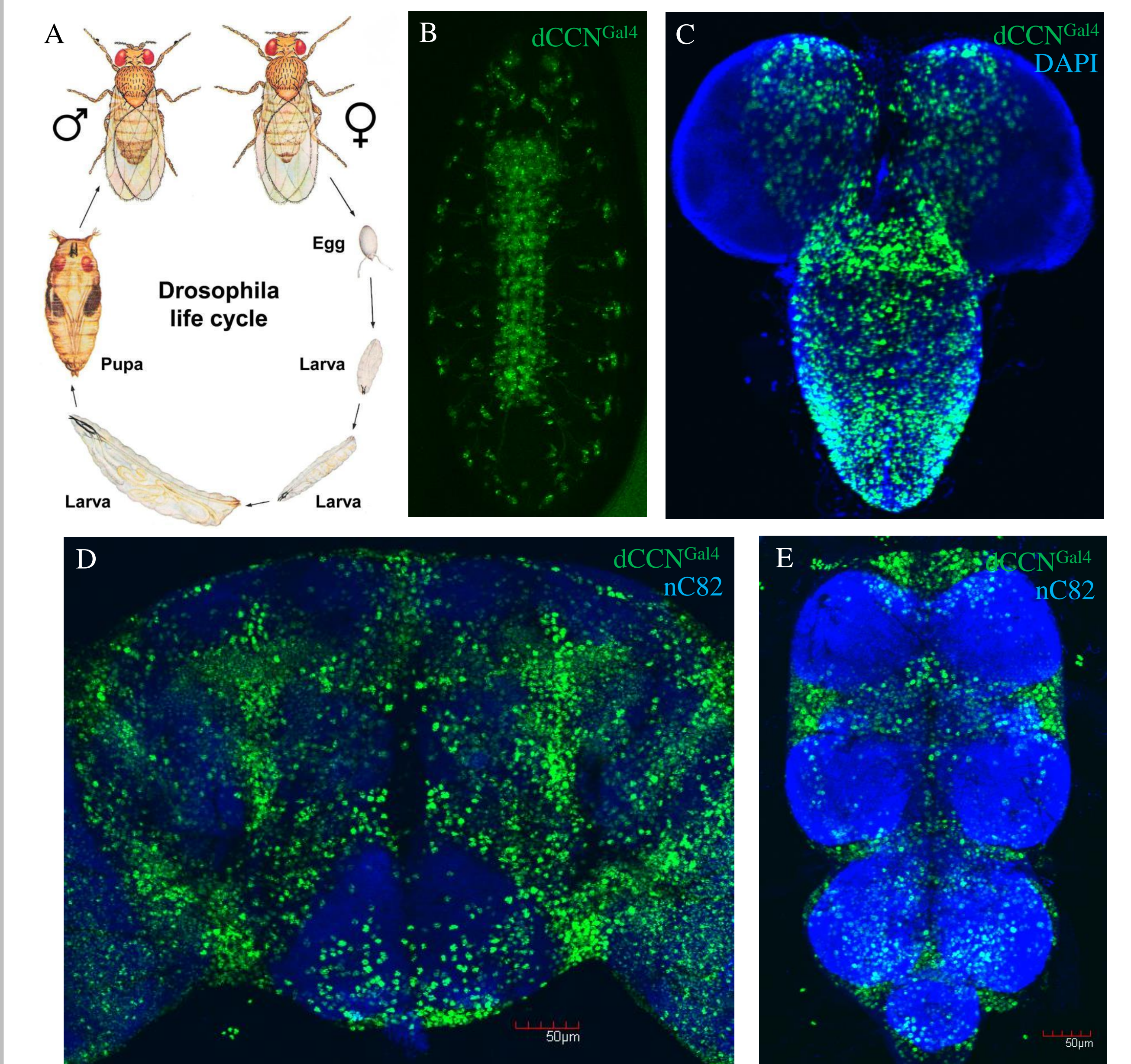
Using aggression as a readout for neuron function, decreased levels of aggression are observed when *dCCN* is lost or reduced.



(A.) An increase in latency, and a decrease in lunges, wing threats, and male-male wing extensions are observed in *dCCN*-RNAi lines and homozygous *dCCN<sup>Gal4</sup>* mutant flies (*dCCN<sup>Gal4</sup>/UAS-dCCN-RNAi*, *dCCN<sup>Gal4</sup>/dCCN<sup>Gal4</sup>*). (B.) An increase in latency and male-male wing extensions, along with reductions of lunges and wing threats is observed when *dCCN* is reduced in OA neurons (*tdc2-gal4; UAS-dCCN-RNAi*). (A-B). Aggression data sets for latency, lunging, wing threats, and male-male courting wing extensions. A Kruskal-Wallis multiple comparisons non-parametric test was used to determine statistical significance. Error bars report SEM. \* equals  $p \leq 0.05$ , \*\* equals  $p \leq 0.01$ .

## 3 – *dCCN* expression

*Drosophila melanogaster* undergo morphological changes throughout development within their life cycle, which is comprised of 4 major stages: embryo, larval, pupa, and adult stage.



(A) A schematic of the *Drosophila melanogaster* life cycle. (B) A green fluorescent protein is expressed in all *dCCN<sup>Gal4</sup>* containing cells within an embryo (*dCCN<sup>Gal4</sup>/20X-UAS-6X-GFP*). (C) Expression of dsRed turned green in the nuclei of *dCCN*-expressing neurons in larvae through *dCCN<sup>Gal4</sup>* (*dCCN<sup>Gal4</sup>/UAS-dsRed*). (D-E) Expression of dsRed turned green in the nuclei of *dCCN*-expressing neurons in the adult brain and ventral nerve cord through *dCCN<sup>Gal4</sup>* (*dCCN<sup>Gal4</sup>/UAS-dsRed*).

## 6 - Conclusions

How does the loss of *dCCN* affect neuron function and behavioral circuitry in *Drosophila melanogaster*?

- ❖ *dCCN<sup>Gal4</sup>* expression identified throughout developmental stages in many tissue types

- ❖ *dCCN<sup>Gal4</sup>* expressed in FruM, OA, and DA neurons

- ❖ Loss of *dCCN* results in changes in male aggressive behavior

- ❖ *dCCN* critical for development and neuron function

Future Directions

- ❖ Examine changes in aggression when *dCCN* is reduced in specific subsets of neurons

- ❖ Identify and quantify expression of *dCCN* in other amine neurons such as serotonin

- ❖ Examine subsets of *dCCN* neurons to investigate morphology and synaptic connections

