CORL expression and function in insulin producing neurons
reversibly influences adult longevity in Drosophila

Nancy L. Tran\textsuperscript{1}, Samuel L. Goldsmith\textsuperscript{1*}, Agapi Dimitriadou\textsuperscript{2*}, Norma T. Takaesu\textsuperscript{1},
Christos Consoulas\textsuperscript{2} and Stuart J. Newfeld\textsuperscript{1}

* contributed equally

1. School of Life Sciences, Arizona State University, Tempe AZ 85287-4501 USA
2. Medical School, National and Kapodistrian University of Athens, Athens, Greece.

Correspondence: newfeld@asu.edu, phone 480-965-6042, fax 480-965-6899

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Running Head: dCORL impacts insulin neurons and longevity
Summary

CORL proteins (known as SKOR in mice, Fussel in humans and fussel in Flybase) are a family of CNS specific proteins related to Sno/Ski oncogenes. Their developmental and adult roles are largely unknown. A Drosophila CORL (dCORL) reporter gene is expressed in all Drosophila insulin-like peptide 2 (dILP2) neurons of the pars intercerebralis (PI) of the larval and adult brain. The transcription factor Drifter is also expressed in the PI in a subset of dCORL and dILP2 expressing neurons and in several non-dILP2 neurons. dCORL mutant virgin adult brains are missing all dILP2 neurons that do not also express Drifter. This phenotype is also seen when expressing dCORL-RNAi in neurosecretory cells of the PI. dCORL mutant virgin adults of both sexes have a significantly shorter lifespan than their parental strain. This longevity defect is completely reversed by mating (lifespan increases over 50% for males and females). Analyses of dCORL mutant mated adult brains revealed a complete rescue of dILP2 neurons without Drifter. Taken together, the data suggest that dCORL participates in a neural network connecting the insulin signaling pathway, longevity and mating. The conserved sequence and CNS specificity of all CORL proteins imply that this network may be operating in mammals.

Introduction

The majority of secreted Transforming Growth Factor-β (TGF-β) family members belong to either the Decapentaplegic (Dpp)/Bone Morphogenetic Protein (BMP) or the TGF-β/Activin subfamilies. Both subfamilies perform a myriad of tasks during development and homeostasis while mutations disrupting TGF-β pathways can lead to disease. One mechanism of regulating TGF-β functions is modulation of Smad signal transducer activity. An important group of Smad interacting proteins are the Sno/Ski family of oncogenes. Sno/Ski proteins play positive or negative roles in TGF-β signaling depending upon the cellular context. They can also serve as a pathway switch that simultaneously facilitates TGF-β/Activin signaling while antagonizing Dpp/BMP signaling (Takaesu et al. 2006). In addition, Drosophila Snoo (also known as dSno) has a role outside of TGF-β signaling as an antagonist of Wg signaling (Quijano et al. 2010).
mCORL1 (mSKOR1) was identified as a Sno/Ski family member that functions as a transcriptional co-repressor. In embryos mCORL1 is expressed only in dorsal interneurons of the cerebellum (Mizuhara et al. 2005). Developmental studies of mCORL2 (mSKOR2) showed that it is expressed only in Purkinje neurons of the cerebellum (Minaki et al. 2008). Loss of function studies of mSKOR2 revealed a requirement for Purkinje cell differentiation (Miyata et al. 2010; Wang et al. 2011). mSKOR2 knockouts demonstrated that this is accomplished by inhibiting interneuron fate (Nakatani et al. 2014). No knockout studies of mSKOR1 have been reported. mSKOR1 is primarily, though not exclusively, expressed in the cerebellum of adults while mSKOR2 expression is restricted to the cerebellum in adults (Yue et al. 2014).

There are two human Fussel proteins. Fussel15 is homologous to mSKOR1 and Fussel18 is homologous to mSKOR2. The Fussel15 expression pattern is conserved with mSKOR1. It is present primarily in the adult cerebellum. There are also low levels of transcription in the lung and small intestine. The Fussel18 expression pattern is conserved with mSKOR2. It is restricted to the adult cerebellum (Fagerberg et al. 2014). Genome-wide association studies have linked mutations in Fussel15/hSKOR1 to two ataxias (e.g., Li et al. 2017). These ataxias are thought to result from dysfunction in the cerebellum, as that is the site of movement in the brain. No syndromes are yet associated with mutations in Fussel18/hSKOR2.

In the only study of a dCORL mutation, one aspect of its larval brain expression was shown to function downstream of the TGF-β/Activin receptor Baboon in the transcriptional activation of Ecdysone Receptor-B1 in the mushroom body. In parallel, biochemical analyses of mCORL1 revealed a physical interaction with mSmad3 but not other Smads. Taken together the genetic and biochemical data suggested that dCORL is a Smad-interacting protein that facilitates TGF-β/Activin subfamily signaling in the larval mushroom body (Takaesu et al. 2012). Other functions of dCORL remain unknown.

Three other genes with expression in the larval and adult brain are Drifter, dILP2 and dILP5. Drifter is a transcription factor (also known as ventral veinless) that contains both POU and Homeobox domains. It plays numerous roles during development of the CNS including in
medullary neurons of the larval optic lobe (Hasegawa et al. 2011) and projection neurons of the adult antennal lobe (Komiyama and Luo 2007). Interestingly, Drifter’s closest human relative is Oct9/Brn4. This is a CNS specific transcription factor that plays a role in neuronal differentiation of the cochlea. Mutations in Oct9/Brn4 cause an X-linked form of hearing loss (de Kok et al. 1995). Drosophila insulin-like peptides 2 and 5 (dILP2 and dILP5) are secreted hormones with roles in metabolism, growth and longevity (Rulifson et al. 2002; Droujinine and Perrimon 2016). In the larval and adult brain both proteins are detected exclusively in neurosecretory cells of the PI (Broughton et al. 2005). In these cells dILP5 is directly activated by the transcription factor Dachshund (Dac; Okamoto et al. 2012).

Here we identify insulin producing neurons of the larval and adult PI as sites of \textit{dCORL} expression and function. In \textit{dCORL} mutant larval and virgin adult brains we found that all dILP2 neurons that do not also express Drifter are absent. This phenotype is also seen when expressing \textit{dCORL-RNAi} in the PI. \textit{dCORL} mutant virgin adults of both sexes have a significantly shorter then normal lifespan, but this defect is completely reversed by mating (lifespan increases over 50% for males and females). \textit{dCORL} mutant mated adult brains analyzed at three and fifteen days old contain a completely rescued complement of dILP2 neurons lacking Drifter. Overall the data suggest the existence of a neural network connecting \textit{dCORL}, the insulin signaling pathway, longevity and mating.

\textbf{Results}

\textit{dCORL is expressed in all dILP2 neurons of the larval and adult brain}

Previously we showed that \textit{dCORL} displays CNS specific transcription in embryos and third instar larvae. Our clonal analysis of \textit{dCORL} mutant larval brains identified a requirement for \textit{dCORL} in TGF-\(\beta\)/Activin signal transduction in mushroom body neurons (Takaesu et al. 2012). Our recent analysis of reporter genes showed that \textit{dCORL} is expressed in larval brain neurons but not larval mushroom body neurons (Tran et al. 2018). Taken together these results indicated that \textit{dCORL} functions non-autonomously in the mushroom body. This led us to
consider the possibility that dCORL is expressed in the nearby PI, a brain region where neurosecretory cells initiate the insulin signaling pathway.

Initial co-expression studies employing the dCORL reporter AH.lacZ and the transcription factor Drifter (Hasegawa et al. 2011) revealed overlap in a subset of neurons of the PI (roughly 4-5 per hemisphere; Fig. 1A,B). Similar experiments with AH.lacZ and dILP2 (Rulifson et al. 2002) revealed that AH.lacZ is present in every dILP2 expressing neuron in the PI (range 6-8 per hemisphere; Fig. 1C,D). There is a single AH.lacZ neuron located medially to the coexpressing neurons in each brain hemisphere. Coexpression with dILP2 bolstered our hypothesis of dCORL non-autonomous function in the larval mushroom body and suggested a role in the insulin signaling pathway.

The presence of defects in the adult mushroom body of dCORL mutants (Takaesu et al. 2012), led us to examine dCORL reporter adult brain expression. We analyzed virgin adult females and males (one day old) utilizing AH.lacZ. We found that AH.lacZ is strongly expressed in the PI, a topologically recognizable region at the midline of the dorsal anterior-most section of the brain in both sexes (Fig. 2A). Importantly, AH.lacZ in the adult PI does not reflect the expression of twin of eyeless (toy), a divergently transcribed gene immediately distal to dCORL (Tran et al. 2018). Extending the study, no obvious differences were noted in AH.lacZ expression between female virgins at one, five and ten days old. Co-expression analyses of AH.lacZ with Elav and Repo in adults (Fig. 2B,C) were consistent with those in larvae (Tran et al. 2018). AH.lacZ is found in neurons but not glia.

Coexpression studies of AH.lacZ and Drifter in the adult PI revealed that there are more neurons of both types in the adult than in larvae (AH.lacZ both hemispheres mean 18.4 neurons, n=5; Drifter mean 9.8 neurons, n=6; Fig. 2D). Also in the adult, overlap is more extensive with 5-7 neurons co-expressing AH.lacZ and Drifter. Overall, there are 3-4 neurons expressing only AH.lacZ and the same number expressing only Drifter interspersed with the co-expressing neurons. An additional group of 4-6 AH.lacZ neurons form an inverted triangle pointing medially below the coexpressing neurons. We then examined dILP2 and AH.lacZ. We confirmed
the report that dILP2 is expressed in a larger group of neurons in the adult PI then in larvae (Geminard et al. 2009). Consistent with our larval data, AH.lacZ is present in the nuclei of every dILP2 neuron (dILP2 mean 16.7 neurons, n=6; Fig. 2E). Again as in larvae, there is a single AH.lacZ neuron located medially to the coexpressing neurons in each brain hemisphere. Interestingly, the AH.lacZ pattern in the PI (every dILP2 cell plus one per hemisphere) is similar to a subset of the adult expression of Eyeless-Gal4 (OK107.Gal4; Enell et al. 2010).

**dCORL mutant larval & virgin adult brains are missing all dILP2 neurons lacking Drifter**

Given the coincidence in the PI of AH.lacZ, dILP2 and Drifter, we examined dILP2 and Drifter in the brains of larvae and virgin adults that were homozygous for a dCORL deletion (Df(4)dCORL; Takaesu et al. 2012). The goal was to determine if the loss of dCORL had any effect on dILP2 or Drifter expression. We began with third instar larvae. In wild type (yw1w67c23 throughout), we noted that dILP2 neurons form a monolayer (i.e., all visible in the same slice) and that a subset of dILP2 neurons also expressed Drifter (five of eight in Fig. 3A,B). There were diPL2 only neurons (three) and several Drifter only neurons. In a dCORL mutant brain a statistically significant reduction in Df(4)dCORL brain size was noted (roughly 60% smaller than wild type) and the monolayer was disrupted (i.e., not all dILP2 neurons are visible in the same slice). Surprisingly, all dILP2 neurons lacking Drifter were absent (Fig. 3C,D). Neurons expressing Drifter, either with dILP2 or alone did not appear to be affected.

We then compared the expression patterns of dILP2 and Drifter in one day old wild type virgin adult females. dILP2 and Drifter coexpressing neurons occupy a single layer along the apical surface of the PI with a set of dILP2 only neurons forming an inverted triangle pointing medially (Fig. 4A,B). There are also occasional neurons that express Drifter alone in the dILP2-Drifter single layer. Analyzing eight individual slices covering a span of 16 microns confirmed the 3-dimensional structure of the adult PI. The dILP2-Drifter layer of neurons is the apical base of an inverted pyramid with the additional dILP2 only neurons pointed medially (Fig. 4C). This is consistent with previous observations of AH.lacZ and Drifter in the same genotype - AH.lacZ
only neurons form an inverted triangle pointing medially below a single layer of coexpressing neurons (refer back to Fig. 2D).

Examining \(d\text{CORL}\) mutant brains from one day old virgin adult females, we found their size restored to essentially wild type (e.g., roughly 85\% for the example in Fig. 4D). Further, both dILP2 and Drifter are present in the PI but there has been a 35\% decrease in the average number of dILP2 expressing neurons (mean 11.4, \(n=8\); Fig. 4D,E). This number is not significantly different from wild type due to the size of the standard error (Student's T-test \(p=0.07\)). In the \(d\text{CORL}\) mutant, the dILP2-Drifter coexpressing neurons still form a single layer along the apical surface of the PI but the absence of the pyramid of medially located dILP2 only neurons has caused this row to collapse into a V-shape rather than a straight line. There are still occasional neurons that express Drifter alone in the dILP2-Drifter row and the number of Drifter expressing neurons in the mutant is the same as wild type (mean 8.9, \(n=8\); \(p=0.70\)). Analyzing individual slices covering 16 microns confirmed the absence of all medially located dILP2 only neurons in the adult PI of \(d\text{CORL}\) mutants (Fig. 4F). The reduction in dILP2 neurons by 35\% in \(d\text{CORL}\) mutant adults may seem modest but the fact that the missing neurons are exclusively dILP2 neurons lacking Drifter in both larvae and adults is striking.

We then assayed Dac expression in one day old virgin adult female brains as a surrogate for dILP5, another family member produced in neurosecretory cells of the PI. In wild type, Dac expressing neurons are much more numerous than either dILP2 or Drifter, but the topology of Dac cells matches dILP2 by forming an inverted triangle pointing medially (Fig. 5A,B). In addition, every dILP2 neuron also expresses Dac (Fig. 5C). In \(d\text{CORL}\) mutant brains, Dac neurons display a similar phenotype to dILP2. There is a substantial reduction in the number of Dac expressing cells and the inverted pyramid of Dac cells has largely collapsed into a single row forming a V-shape (Fig. 5D,E). In these brains every dILP2 neuron also expresses Dac (Fig. 5F). The data suggests that in \(d\text{CORL}\) mutants there is also an effect on dILP5 neurons.

To assess whether it was the loss of dCORL within Df(4)dCORL that led to the dILP2 and Dac/dILP5 phenotypes we examined OK107.Gal4 driving UAS.dCORL-RNAi in otherwise
wild type one day old virgin adult female brains. Our control for OK107.Gal4 expression was UAS.lacZ and our control for UAS.dCORL-RNAi was adult mushroom body lobe defects previously shown to be due to the loss of dCORL (Takaesu et al. 2012). As expected, expression of lacZ had no effect on the mushroom body (Fig. 6A). There was also no effect on the topology or number of dILP2 cells in the PI (mean 16.3, n=4; Fig 6B,C). In the dCORL-RNAi brains mushroom body defects were plainly visible (Fig. 6D). In the PI, phenotypes resembling Df(4)dCORL were observed. First, the pyramidal shape of dILP2 neurons was not completely lost but the majority of dILP2 neurons formed a row along the apical surface. Second, there was a reduced number of dILP2 neurons (mean 13.0, n=4; Fig. 6E,F). Third and most importantly, all of the dILP2 neurons expressed Drifter as those lacking Drifter were absent. It is the commonality of this latter phenotype between Df(4)dCORL and dCORL-RNAi that establishes this phenotype as dCORL dependent.

Taken together, we conclude from these loss of function experiments that dCORL plays a role in determining the number of dILP2 and Dac/dILP5 cells in the larval and adult PI. Further, dCORL appears to influence the identity of larval and adult dILP2 cells, as when it is lost so are dILP2 cells that do not express Drifter.

**dCORL mutant virgin adult longevity defects are fully rescued by mating in both sexes**

To determine whether any adult lifecycle traits were affected by the loss of this subset of dILP2 expressing neurons we examined fecundity, fertility and longevity (as virgins and mated) in dCORL mutant males and females. In addition to the yw parental strain, six control lines were employed to eliminate the possibility that observed phenotypes for Df(4)dCORL mutant adults were due to loss of one of the other three genes in the deletion. These were Pbac{WH}^{y07015}, Glu-RA^{112b}, Pbac{WH}^{y06253}, Pbac{RB}^{y02096}, and spx^{720RW} (Takaesu et al. 2012). Tested alleles for two of the three genes are demonstrated nulls: Glu-RA^{112} (Bogdanik et al. 2004) and spx^{720RW} (Dai et al. 2008). For the third gene, Pbac{RB}^{y02096}, is expected to disrupt all predicted transcripts of CG32016 and thus should also serve as a null. We showed previously the toy
transcription unit is not affected in the dCORL deletion (Takaesu et al. 2012). We recently found that Toy expression is unaffected in Df(4)dCORL larvae and adults (Tran et al. 2018). Assays of fecundity and fertility will be reported elsewhere.

Assays of longevity turned out quite surprisingly. Studies of virgins showed a statistically significant reduction in lifespan for dCORL mutants versus the parental yw strain. For reference, note that the dCORL mutant strain has a yw background and thus we use yw as a control in these experiments just as in our expression studies. While the yw strain has a lifespan roughly 50% shorter than the wild type strains OregonR and CantonS (Grandison et al. 2009), it is the relative lifespans of matched Df(4)dCORL flies to yw flies that is important. Overall, a majority of the six control lines have yw backgrounds. Genotypes and an initial set of longevity data for eight lines (Df(4)dCORL, yw and the six control lines) are described in Table S1.

We repeated the longevity studies with much larger cohorts of dCORL mutant and yw adults. For dCORL mutants, virgin females lived roughly 19.5 days (Fig. 7A). For yw, virgin females lived on average 31.7 days (Fig. 7B). dCORL mutant virgin females live only 62% as long as yw virgin females (Fig. 7C), a highly significant reduction (yw versus dCORL mutant p<1.0x10^-6, Table 1A). For yw, sibmated females lived roughly 33.9 days (an insignificant increase of 07% over yw virgin females; p=0.14, Table 1A). For dCORL mutants, sibmated females lived on average 33.0 days (an increase of 64% over dCORL mutant virgin females). The increase in lifespan for virgin versus mated dCORL mutant females is highly significant p<1.0x10^-6, Table 1A) and nearly 10-fold greater than the longevity increase of yw virgin versus yw mated females. The lifespan of dCORL mutant mated females is not significantly different from yw mated females (p=0.57; Table 1A). The highly significant reduction in dCORL mutant virgin female lifespan in comparison to yw virgin females is fully rescued by mating; dCORL mutant mated females have the same lifespan as yw mated females (Fig. 7C).

The mating induced lifespan increase for dCORL mutant males is comparable. For dCORL mutants, virgin males lived roughly 23.2 days (Fig. 7A). For yw, virgin males lived on average 38.3 days (Fig. 7B). dCORL mutant virgin males live 61% as long as yw virgin males
(Fig. 7C), a highly significant difference (yw versus dCORL mutant p<1.0x10^{-6}, Table 1A). For yw, sibmated males lived roughly 36.4 days (an insignificant decrease of 05% from yw virgin males, p=0.08, Table 1A). For dCORL mutants, sibmated males lived on average 35.0 days (an increase of 51% over dCORL mutant virgin males). The increase in lifespan for virgin versus mated dCORL mutant males is highly significant (p=<1.0x10^{-6}, Table 1A) and starkly different from the insignificant longevity decrease of yw virgin versus yw mated males. The lifespan of dCORL mutant mated males is not significantly different from yw mated males (p=0.07, Table 1A). The highly significant reduction in dCORL mutant virgin male lifespan in comparison to yw males is fully rescued by mating; dCORL mutant mated males have the same lifespan as yw mated males (Fig. 7C).

Application of the log-rank test (Mantel-Cox) optimized for survival studies arrives at the same statistical significance. Lifespan of dCORL mutant virgins are highly significantly shorter than yw virgins (males p=<1.0x10^{-6}, females p=<1.0x10^{-6}, Table 1B). Lifespan of dCORL mutant mated adults are highly significantly longer then their virgin counterparts (males p=<1.0x10^{-6}, females p=<1.0x10^{-6}, Table 1B). Lifespan of yw mated adults is not significantly different from their virgin counterparts (males p=0.21, females p=0.08, Table 1B). Lifespan of dCORL mutant mated adults are not significantly different from their yw counterparts (males p=0.07, females p=0.39, Table 1B). Application of statistical tests to median lifespans, rather than means as noted above, does not impact the p values in any meaningful way (Table S2). The fact that mating can induce the complete reversal of the lifespan defect in dCORL mutant virgins of both sexes is unprecedented.

**dILP2 neurons lacking Drifter are fully rescued by mating in dCORL mutant adult brains**

In an attempt to understand the basis for the longevity rescue we examined dILP2 and Drifter expression in the brains of sibmated three day old and fifteen day old dCORL mutant adult females (brain size data in Table S3). Right away we noted that the topology of the PI was restored that and that this was due to the presence of dILP2 neurons lacking Drifter (Fig. 8A,B).
Closer examination showed that the arrangement of Drifter expressing neurons in the PI was not completely wild type. There were 2-3 dILP2-Drifter coexpressing neurons in the medial triangle that normally contained dILP2 only neurons. Nevertheless, the number of Drifter neurons in the three day old mated mutants was not significantly different from virgin wild type or virgin \( d\text{CORL} \) mutants (10.3 mean, n=7; versus virgin \( d\text{CORL} \) mutants \( p=0.45 \), Table 2). Analysis of five individual slices covering a span of 10 microns confirmed the rescue of the inverted pyramidal 3-dimensional structure of the PI (albeit more compact) and that this was due to the presence of dILP2 only neurons medially (Fig. 8C). The average number of dILP2 neurons was increased in mated versus virgin mutants but not with statistical significance again due to the large standard error (mean 13.6 for three day mated versus 11.4 for virgin; \( p=0.39 \), Table 2).

Observations in mated fifteen day old adult \( d\text{CORL} \) mutants showed that the atypical presence of Drifter neurons medially persists (Fig. 8D,E). The number of Drifter neurons remains not significantly different from any other analyzed groups (mean 8.4, n=8; versus mated three day old mutants \( p=0.51 \), Table 2). Analysis of twelve individual slices covering a span of 24 microns revealed a spatial expansion of the inverted pyramidal 3-dimensional structure of the PI in comparison to three day old mated mutants (Fig. 8F). The number of dILP2 only neurons in the PI of mated fifteen day old adult \( d\text{CORL} \) mutants was essentially unchanged from three day old mated mutants (mean 12.8, n=8; \( p=0.67 \), Table 2). Overall the data from mated \( d\text{CORL} \) mutants reveals that after the rapid rescue of dILP2 neurons in the PI by mating (within three days), these neurons are maintained over at least the next twelve days.

**Discussion**

Data supporting a connection between \( d\text{CORL} \) expression and function, insulin neurons, longevity and mating is: 1) \( d\text{CORL} \) reporter expression in all dILP2 neurons of the larval and adult PI, 2) \( d\text{CORL} \) mutant virgin adults display significant lifespan reduction and have lost all dILP2 neurons lacking Drifter in their PI, and 3) \( d\text{CORL} \) mutant adult lifespan is fully rescued by mating as are dILP2 neurons lacking Drifter in their brains.
We attribute the phenotypes of \textit{Df(4)dC\textsc{or}l} to loss of \textit{dC\textsc{or}l} for three reasons. First, the presence of the \textit{dC\textsc{or}l} reporter AH.lacZ in all dILP2 cells of the larval and adult brain that are the same cells affected in \textit{Df(4)dC\textsc{or}l} brains. While circumstantial, Tran et al. (2018) further illustrates the fidelity of AH.lacZ and \textit{dC\textsc{or}l} brain expression (e.g., AH.lacZ does not overlap with neighboring Toy in larvae or adults). Second, the published brain expression patterns for \textit{Glu-RA} (anterior \textit{ad} neurons in larvae and antennal+optic lobes in adults; Ramaekers et al. 2001) and for \textit{sphinx} (Chen et al. 2011; antennal lobes in larvae and the \textit{ab1} class of large basiconic sensillum of the antenna in adults) do not correspond to insulin producing cells. No images of CG32016 brain expression are available, but two lines of evidence suggest it is not present in larval or adult insulin producing cells. It was found in a cell-based overexpression screen to bind Orb2 (a mushroom body specific protein; White-Grindley et al. 2014) and in an adult odor aversion RNAi screen (Walkinshaw et al. 2015). Third, and most importantly the phenocopy of the \textit{Df(4)dC\textsc{or}l} dILP2 phenotype by expression of \textit{dC\textsc{or}l}-RNAi in the PI.

It was shown some time ago that mating reduces female lifespan in Drosophila due to exposure to sex-peptide in the male ejaculate (Chapman et al. 1995). Yet \textit{Df(4)dC\textsc{or}l} exhibits the opposite phenotype. In the 20+ years since the original sex-peptide paper, numerous reports have shown the situation is more complex than initially described. For example, Fricke et al. (2010) showed that diet altered the presence, magnitude and sign of the effects of sex-peptide on a variety of phenotypic traits including lifespan. Altering the "sign" of the effect means that lifespan was increased by sex-peptide under some circumstances. In an second example, Grandison et al. (2009) reported that the Dahomey strain utilized in the 1995 paper is among the longest-lived wild type lines in the lab; Dahomey lifespan is more than 40\% longer than OregonR and CantonS. Effects of mating on this outlier strain may not be readily transferrable to strains such as \textit{Df(4)dC\textsc{or}l}. Further, the fact that \textit{Df(4)dC\textsc{or}l} males and females are similarly affected suggests a sex-peptide independent mechanism.

The fact that there are likely several mechanisms connecting mating and longevity is a conclusion of Fricke et al. (2010) and connections between mating and longevity independent of
insulin have been seen (e.g., Bowman and Tatar 2016). Alternatively, there are mutants affecting the insulin signaling pathway that extend lifespan independent of mating (e.g., Huang et al. 2015), but no previous data has connected insulin, longevity and mating. If \textit{dCORL} regulates insulin producing neuron formation, maintenance, identity or function downstream of TGF-β/Activin signaling (it modulates Activin signaling in the mushroom body), that suggests a mechanism for the connection. For example, Activin is a major metabolic regulator via functional links with insulin and dFOXO and these interactions influence longevity (Bai et al. 2013; Ghosh and O’Connor 2014). Also midgut-derived Activin elicits responses in the fat body that modulate the metabolism of sugars and triglycerides (Song et al. 2017a,b). The hypotheses that \textit{dCORL} function in the PI is influenced by, or contributes to Activin signaling, are currently being tested. A TGF-β independent function for \textit{dCORL} in the insulin pathway is also possible, as suggested by the TGF-β independent role of \textit{dSno} in Wg signaling (Quijano et al. 2010).

Another intriguing issue is the mechanism that allows \textit{dCORL} mutant virgin longevity defects to be fully rescued by mating in both sexes. Two papers have shown a connection between changes in female reproductive status (i.e., pre- and post-mating) and intestinal remodeling, presumably to more efficiently nourish the growth of oocytes (Cognigni et al. 2011; Reiff et al. 2015). The second paper identifies Juvenile Hormone as an integral part of the process. The presence of Juvenile Hormone in both sexes provides a point of departure for characterizing the mechanism of interaction between dCORL, insulin, longevity and mating. One hypothesis for \textit{dCORL} mutant longevity reversal is that a gender-neutral mating-responsive neural network employs Juvenile Hormone in response to reproductive activity to influence insulin neuron identity in the PI.

The rapid rescue of dILP2 neurons lacking Drifter in \textit{dCORL} mutant adults (within three days of mating) lends itself to two hypotheses. The first hypothesis is that dILP2 only neurons are never actually lost in \textit{dCORL} mutants. What is lost is dILP2 expression in non-Drifter expressing PI neurons. In this case what is rescued by mating is dILP2 expression. Alternatively, rescued dILP2 only neurons are born via adult brain neurogenesis triggered by mating in \textit{dCORL}
mutants. The neurogenesis hypothesis is consistent with the observed regeneration of PI topology in mated \textit{dCORL} mutants. The possibility of adult neural regeneration in the brain of \textit{dCORL} mutants leads to the essentially unexplored area of adult brain neuroblast division in Drosophila. A Flybase search identified only a single paper describing the activation of adult brain neuroblast cell division in response to acute damage (Fernandez-Hernandez et al. 2013). In contrast there is widespread interest in understanding the origins of adult born neurons in the mammalian brain (e.g., Ernst et al. 2014; generation of striatal neurons in adult humans). Interest in mammalian adult neurogenesis is stimulated by the potential for harnessing this process therapeutically in traumatic brain injury and disease (e.g., Iqubal et al. 2014). For example, dopamine derived from adult born striatal neurons regulates systemic glucose metabolism in humans and stimulation of this process reduced the insulin dependence of a diabetic patient (ter Horst et al. 2018). \textit{dCORL} mutants may provide a non-invasive, genetic model for analyses of adult brain neurogenesis and any connections to the insulin signaling pathway. A link between insulin signaling and ventral cord neuroblast reactivation after hatching has been reported (Otsuki and Brand 2018).

Our \textit{dCORL} mutant brain and longevity data generate numerous hypotheses for our colleagues studying human and mouse SKOR1/2 regarding their function in neural development and adults. During development, the coincidence of SKOR1/2 and CNS specific homologs of Drifter could be examined (POU3F2 and POU3F4). In adults, while the brain is not the site of insulin production, certainly there are signals from the brain that influence insulin signaling and/or longevity (e.g., Templeman et al. 2017). An relationship between insulin and longevity in humans is shown by the reduced lifespan of patients with insulin resistance syndrome (e.g., Ranieri et al. 2013). One approach could be genome wide association studies for connections between insulin resistance or longevity and single nucleotide polymorphisms in hSKOR1/2.

In summary, our data on \textit{dCORL} expression and function suggest this gene participates in a previously unknown neural network connecting the insulin signaling pathway, longevity and mating. The conserved sequence and CNS specificity of all CORL proteins plus the obvious
adaptive benefit to the offspring of parents living longer implies that this network exists in mammals. Further studies of CORL family members will advance our understanding of their expression and function relevant to human homeostasis and disease.

Material and Methods

**Drosophila genetics:** Fly stocks are as described: AH.lacZ (Tran et al. 2018), \textit{Df(4)CORL} (Takaesu et al. 2012), OK107.Gal4 (Enell et al. 2010), \textit{Glu-RA}^{1/2b} and \textit{Glu-RA}^{2b} (Bogdanik et al. 2004), \textit{Pbac\{RB\}e02096}, \textit{Pbac\{WH\}e07015} and \textit{Pbac\{WH\}e06233} (Exelixis collection at Harvard Medical School; Takaesu et al. 2012), \textit{sphinx}^{720RW} (Dai et al. 2008), \textit{UAS.dCORL-RNAi} (Takaesu et al. 2012) and \textit{yw} (y\textsuperscript{1w67c23} employed as wild type throughout). \textit{Glu-RA}^{1/2} and \textit{sphinx}^{720} are demonstrated null alleles. \textit{Pbac\{RB\}e02096} is expected to disrupt all predicted transcripts of CG32016 and thus would also serve as a null. For antibody staining larvae that have stopped wandering but not yet begun pupariation (prior to anterior spiracle eversion - equivalent to 122 hours at 25°C for \textit{yw}) were picked individually, sorted by sex and their CNS dissected in groups of 9-12. Adult virgins were collected either prior to sexual maturation, segregated by sex and aged to one, five and ten days post-eclosion, or aged three and fifteen days post-eclosion with males and females together. Brains were dissected in groups of 9-12. Counting neurons in the PI was conducted by scrolling through slices in ImageJ. For longevity assays, two protocols were used. Data reported in Table 1 is derived from 100 flies per trial, housed as ten flies per vial (male or female virgins or males+females). Data reported in Tables S1 and S2 are derived from 16 flies per trial, housed as four flies per vial (male or female virgins) or four sibmated pairs per vial. In all cases, vials were observed daily and each death was noted until none survived. Medians, means, standard deviations, and pairwise p values employing Student's T-test (independent, two tailed) were generated in Excel. D'Agostino-Pearson normality tests and Mantel-Cox log-rank tests were conducted via onlinestatbook.com.

**Immunohistochemistry:** AH.lacZ is a nuclear-lacZ reporter (high levels of expression yield nuclear and cytoplasmic staining) that contains a region of genomic DNA 7-11kb upstream
of the \textit{dCORL} transcription start as described (Tran et al. 2018). Two independent insertions were analyzed to eliminate position effects (lines 1A and 3A). For 3-color confocal detection of antibodies, tissues were fixed in 4\% formaldehyde, rinsed and stored in methanol until staining. Primary antibodies were: rabbit and rat $\alpha$-lacZ (Organon Teknika, Durham; MBL, Nagoya), guinea pig $\alpha$-Toy (gift of Uwe Walldorf, Saarland Univ.), mouse $\alpha$-Dac (DSHB 1-1-c), mouse $\alpha$-Fas2 (DSHB 1D4), rat $\alpha$-Elav (DSHB 7E8A10), mouse-$\alpha$ Repo (DSHB 8D12), guinea pig $\alpha$-Drifter (gift of Makoto Sato, Kanazawa Univ.) and rat $\alpha$-dILP2 (gift of Pierre Leopold, Nice Univ.). Secondary antibodies were: goat $\alpha$-mouse, $\alpha$-rabbit, $\alpha$-guinea pig or $\alpha$-rat Alexa Fluor 488, 546, and 633 (Molecular Probes). Tissues were imaged on Leica SP5 or SP8 confocal microscopes with slices acquired every 2\(\mu\)m.

\textbf{Data Availability Statement}

Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

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\textbf{Author contributions}

N.L.T., S.L.G., A.D. and C.C. collected and analyzed data, N.T.T. generated reagents. S.J.N. conceived the project, designed experiments, analyzed data and wrote the paper.
References

Bai H, Kang P, Hernandez AM, Tatar M. (2013) Activin signaling targeted by insulin/dFOXO regulates aging and muscle proteostasis in Drosophila. PLoS Genet. 9:e1003941.

Bogdanik L, Mohrmann R, Ramaekers A, Bockaert J, Grau Y, et al. (2004) Drosophila Glu-RA regulates activity-dependent synaptic facilitation and morphology. J Neurosci. 24:9105-9116.

Bowman E, Tatar M. (2016) Reproduction regulates Drosophila nutrient intake through independent effects of egg production and sex peptide: Implications for aging. Nutr. Healthy Aging 4:55-61.

Broughton S, Piper M, Ikeya T, Bass T Jacobson J, et al. (2005) Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. Proc. Natl. Acad. Sci. USA 102:3105-3110.

Chen Y, Dai, H, Chen S, Zhang I, Long, M. (2011) Highly tissue specific expression of Sphinx supports its male courtship role in Drosophila melanogaster. PLoS One 6:e18853.

Cognigni P, Bailey AP, Miguel-Aliaga I. (2011) Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. Cell Metab. 13:92-104.

Dai H, Chen Y, Chen S, Mao Q, Kennedy D, et al. (2008) The evolution of courtship behaviors through the origination of a new gene in Drosophila. Proc. Natl. Acad. Sci. USA 105:7478-7483.

de Kok Y, van der Maarel S, Bitner-Glindzicz M, Huber I, Monaco A. (1995) Association between X-linked mixed deafness and mutations in the POU domain gene POU3F4. Science 267:685-688.

Droujinine I, Perrimon N. (2016) Interorgan communication pathways in physiology: focus on Drosophila. Annu. Rev. Genet. 50:539-570.

Dulcis D, Levine RB, Ewer J. (2005) Role of the neuropeptide CCAP in Drosophila cardiac function. J Neurobiol. 64:259-274.

Enell LE, Kapan N, Söderberg JA, Kahsai L, Nässel DR. (2010) Insulin signaling, lifespan and stress resistance are modulated by metabotropic GABA receptors on IPCs in the brain of Drosophila. PLoS One 5:e15780.

Ernst A, Alkass K, Bernard S, Salehpour M, Perl S, et al. (2014) Neurogenesis in the striatum of the adult human brain. Cell 156:1072-1083.

Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, et al. (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol. Cell. Proteomics 13:397-406.

Fernández-Hernández I, Rhiner C, Moreno E. (2013) Adult neurogenesis in Drosophila. Cell Rep. 3:1857-1865.

Fricke C, Bretman A, Chapman T. (2010) Female nutritional status determines the magnitude and sign of responses to a male ejaculate signal in Drosophila melanogaster. J. Evol. Biol. 23:157–165.

Géminard C, Rulifson EJ, Léopold P, (2009) Remote control of insulin secretion by fat neurons in Drosophila. Cell Metab. 10:199-207.
Ghosh AC, O’Connor MB. (2014) Systemic Activin signaling independently regulates sugar homeostasis, cellular metabolism, and pH balance in Drosophila. Proc. Natl. Acad. Sci. U S A. 111:5729-5734.

Grandison R, Wong R, Bass T, Partridge L, Piper M. (2009) Effect of a standardized dietary restriction protocol on multiple laboratory strains of Drosophila. PLoS One 4:e4067.

Hasegawa E, Kitada Y, Kaido M, Takayama R, Awasaki T, et al. (2011) Concentric zones, cell migration and neuronal circuits in the Drosophila visual center. Development 138:983-993.

Huang CW, Wang HD, Bai H, Wu MS, Yen JH, et al. (2015) Tequila regulates insulin-like signaling and extends life span in Drosophila. J. Gerontol. 70:1461-1469.

Iqbal K, Kazim SF, Bolognin S, Blanchard J. (2014) Shifting balance from neurodegeneration to regeneration of the brain: a novel therapeutic approach to Alzheimer’s disease and related neurodegenerative conditions. Neural Regen. Res. 9:1518-1519.

Komiyama T, Luo L. (2007) Intrinsic control of precise dendritic targeting by an ensemble of transcription factors. Curr. Biol. 17:278-285.

Li G, Tang H, Wang C, Qi X, Chen J, et al. (2017) Association of BTBD9 and MAP2K5/SKOR1 With Restless Legs Syndrome in Chinese Population. Sleep. 40:zxs028.

Minaki Y, Nakatani T, Mizuhara E, Inoue T, Ono Y. (2008) Identification of a novel transcriptional corepressor Corl2 as a cerebellar Purkinje cell-selective marker. Gene Expr. Patterns 8:418-423.

Miyata T, Ono Y, Okamoto M, Masaoka M, Sakakibara A, et al. (2010) Migration, early axonogenesis, and Reelin-dependent layer-forming behavior of early/posterior Purkinje neurons in the developing mouse lateral cerebellum. Neural Dev. 5:1-21.

Mizuhara E, Nakatani T, Minaki Y, Sakamoto Y, Ono Y. (2005) Corl1 a novel neuronal lineage-specific transcriptional corepressor for the homeodomain transcription factor Lbx1. J. Biol. Chem. 280:3645-3655.

Nakatani T, Minaki Y, Kumai M, Nitta C, Ono, Y. (2014) The c-Ski family member and transcriptional regulator Corl2/Skor2 promotes early differentiation of cerebellar Purkinje neurons. Dev. Biol. 388:68-80.

Okamoto N, Nishimori Y, Nishimura T. (2012) Conserved role for Dachshund with Drosophila Pax6 homolog Eyeless in insulin expression Proc. Natl. Acad. Sci. USA 109:2406-2411.

Otsuki L, Brand A. (2018) Cell cycle heterogeneity directs the timing of neural stem cell activation from quiescence. Science 360:99–102.

Quijano J, Stinchfield M, Zerlanko B, Gibbens Y, Takaesu N, et al. (2010) The Sno oncogene antagonizes Wingless signaling in wing disks of Drosophila. PLoS One 5:e11619.

Ramaekers A, Parmentier, M, Lasnier C, Bockaert, J, Grau, Y. (2001) Distribution of metabotropic glutamate receptor DmGlu-RA in Drosophila melanogaster central nervous system. J Comp. Neurol. 438:213-225

Ranieri S, Fusco S, Pani, G. (2013) p66Sca1, Linking mammalian longevity with obesity-induced insulin resistance. Vitamins Hormones 91:219-241.
Reiff T, Jacobson J, Cognigni P, Antonello Z, Ballesta E, et al. (2015) Endocrine remodeling of the adult intestine sustains reproduction in Drosophila. Elife 4:e06930.

Rulifson, E., Kim, S., Nusse, R. (2002) Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296:1119-1120.

Song W, Cheng D, Hong S, Sappe B, Hu Y, et al. (2017a) Midgut-derived Activin regulates glucagon-like action in the fat body and glycemic control. Cell Metab. 25:386-399.

Song W, Owusu-Ansah E, Hu Y, Cheng D, Ni X, et al. (2017b) Activin signaling mediates muscle-to-adipose communication in a mitochondria dysfunction-associated obesity model. Proc. Natl. Acad. Sci. USA 114:8596–8601.

Takaesu NT, Hyman-Walsh C, Ye Y, Wisotzkey RG, Stinchfield MJ, et al. (2006) dSno facilitates Baboon signaling in the Drosophila brain by switching the affinity of Medea away from Mad and toward dSmad2. Genetics 174:1299-1313.

Takaesu NT, Stinchfield MJ, Shimizu K, Arase M, Quijano JC, et al. (2012) Drosophila CORL is required for Smad2-mediated activation of Ecdysone Receptor expression in the MB. Development 139:3392-3401.

Templeman NM, Flibotte S, Chik JHL, Sinha S, Lim GE, et al. (2017) Reduced circulating insulin enhances insulin sensitivity in old mice and extends Lifespan. Cell Rep. 20:451-463.

Tran N, Takaesu N, Cornell E, Newfeld SJ. (2018) CORL expression in the Drosophila central nervous system is regulated by stage specific interactions of intertwined activators and repressors. G3: Genes, Genomes, Genetics 8:2527-2536.

Walkinshaw E, Gai Y, Farkas C, Richter D, Nicholas E, et al. (2015) Identification of genes that promote or inhibit olfactory memory formation in Drosophila. Genetics 199:1173-1182.

Wang B, Harrison W, Overbeek PA, Zheng H. (2011) Transposon mutagenesis with coat color genotyping identifies an essential role for Skor2 in sonic hedgehog signaling and cerebellum development. Development 138:4487-4497.

White-Grindley E, Li L, Khan R, Ren F, Saraf A, et al. (2014) Contribution of Orb2A stability in regulated amyloid-like oligomerization of Drosophila Orb2. PLoS Biol. 12:e1001786.

Yue F, Cheng Y, Breschi A, Vierstra J, Wu W, et al. (2014) A comparative encyclopedia of DNA elements in the mouse genome. Nature 515:355-364.
Table 1. dCORL mutant virgin adult longevity defects are fully rescued by mating in both sexes.a

A. T-test statisticsb

| Genotype, Lifespan | Mean | Genotype, Lifespan | Mean | Δ | P-value |
|--------------------|------|--------------------|------|---|---------|
| Df(4) virgin female | 19.5 +/- 3.4 | Df(4) mated female | 33.0 +/- 6.7 | + 64% | <1.0x10^-6 |
| Yw^c virgin female | 31.7 +/- 7.0 | yw mated female | 33.9 +/- 7.3 | + 07% | 0.14 |
| Df(4) virgin female | 19.5 +/- 3.4 vs. Df(4) mated female | 33.0 +/- 6.7 vs. Df(4) mated female | for virgins: yw significantly longer than Df(4) |
| yw virgin female | 31.7 +/- 7.0 | yw mated female | 33.9 +/- 7.3 | 0.57 |
| Df(4) virgin male | 23.2 +/- 3.5 | Df(4) mated male | 35.0 +/- 7.1 | + 51% | <1.0x10^-6 |
| yw male | 38.3 +/- 6.8 | yw mated male | 36.4 +/- 7.7 | - 05% | 0.08 |
| Df(4) virgin male | 23.2 +/- 3.5 vs. Df(4) mated male | 35.0 +/- 7.1 vs. Df |
| yw virgin male | 38.3 +/- 6.8 | yw mated male | 36.4 +/- 7.7 | 0.07 |
| for mated: no difference yw and Df(4) |

B. Χ² and log-rank statistics

| X² value; log-rank test P value | Df(4) male | Df(4) female | yw male | yw female | X² value; log-rank test P value | yw male | yw female | yw male | yw female |
|---------------------------------|------------|-------------|---------|-----------|--------------------------------|---------|----------|---------|----------|
| Df(4) male virgin | 135.9 <1.0x10^-6 | Df(4) female virgin | 158.3 <1.0x10^-6 | yw male virgin | 1.6 0.21 | Df(4) female mated | 3.1 0.08 | yw female virgin | 0.75 0.39 |
| Df(4) female | 158.3 <1.0x10^-6 | Df(4) female virgin | 141.0 <1.0x10^-6 | yw male | 1.6 0.21 | Df(4) female mated | 3.1 0.08 | yw female | 0.75 0.39 |
| yw male | 1.6 0.21 | Df(4) female | 3.1 0.08 | yw female | 0.75 0.39 |
| yw female | 0.75 0.39 |

a. Data set of eight experiments including six additional controls shown in Table S1.
b. All data were normally distributed per D’Agostino-Pearson omnibus test.
c. Note that yw is the genetic background of Df(4)dCORL.

Table 2. Missing dILP2 cells in dCORL mutant virgin adults are fully rescued by mating.

| Genotype, virgin/mated days post-eclosion | n | dILP2 PI cell counts | P value vs. yw virgin | P value vs. Df(4) virgin | P value vs. Df(4) 3 day mated |
|------------------------------------------|---|----------------------|-----------------------|------------------------|-----------------------------|
| yw, virgin | 6 | 16.7 ± 1.8 | 0.075 | 0.061 | 0.673 |
| Df(4), virgin, 1 day | 8 | 11.8 ± 4.3 | 0.248 | 0.445 | 0.262 |
| Df(4), mated, 3 day | 7 | 13.6 ± 4.0 | 0.611 | 0.663 | 0.075 |
| Df(4), mated, 15 day | 8 | 12.8 ± 2.8 | 0.611 | 0.663 | 0.075 |
| Df(4), mated, 3 day | 7 | 10.3 ± 6.3 | 0.611 | 0.663 | 0.075 |
| Df(4), mated, 15 day | 8 | 8.4 ± 3.5 | 0.611 | 0.663 | 0.075 |
| Df(4), virgin, 1 day | 8 | 8.5 ± 1.1 | 0.702 | 0.663 | 0.075 |
| Df(4), mated, 3 day | 7 | 10.3 ± 6.3 | 0.611 | 0.663 | 0.075 |
| Df(4), mated, 15 day | 8 | 8.4 ± 3.5 | 0.611 | 0.663 | 0.075 |
**Fig. 1.** *dCORL* is expressed in all dILP2 neurons of the larval brain. Dorsal view of AH.lacZ transgenic larval female brains with anterior up. A) AH.lacZ (green-nuclear), Fas2 (blue–marks a distinct subset of neurons including the optic lobe) and Drifter (red-nuclear) at 20X. AH.lacZ is a nuclear-lacZ reporter containing genomic DNA upstream of *dCORL*. Expression of both lacZ and Drifter in the PI is seen. B) 40X stack and single slice of the PI in the left hemisphere from the same brain. Co-expression of AH.lacZ and Drifter in a subset of neurons is visible. C) AH.lacZ (green), Fas2 (blue) and dILP2 (red-cytoplasmic) at 20X. Coexpression of AH.lacZ and dILP2 in the PI is evident (neurons with red cytoplasm have green nuclei). Axons from the coexpressing neurons in both hemispheres extend medially. D) 40X stack and single slice of the PI in the left hemisphere from the same brain. Coexpression of AH.lacZ and dILP2 in all dILP2 neurons of the PI is confirmed (all neurons with red cytoplasm have green nuclei). An additional AH.lacZ neuron sits just medial to the co-expressing neurons. Note that the bottom four panels in Fig. 5 of Tran et al. (2018) are intentionally similar to the bottom four panels here. The point of the similarity is to document the continuous thread of logic between that paper and this one.
Fig. 2. *dCORL* is expressed in all dILP2 neurons of the adult brain. One day old AH.lacZ transgenic virgin adult female brains in dorsal view with anterior up. A) AH.lacZ (green), Fas2 (blue marks mushroom body lobes employed to determine orientation) and Toy (red-nuclear) at 20X. AH.lacZ expression is clearly visible in the PI. A’) 40X views in 3-color and as single channels. An arbitrary line in the 3-color view surrounding the PI serves as a landmark in the individual channels. Strong AH.lacZ expression in neurons of the PI overflows the nucleus into the descending axon bundle. A few scattered neurons in the posterior display AH.lacZ expression, as do 2-3 neurons at the border of the optic lobe. No coexpression between AH.lacZ and Toy in the PI is noted as Toy expressing neurons are located ventral to AH.lacZ neurons and their nuclei are a different size. B) AH.lacZ (green), Fas2 (blue) and Elav (red-nuclear) show coexpression (yellow) in neurons of the PI. C) AH.lacZ (green), Toy (blue) and Repo (red-nuclear) shows no coexpression in glia of the PI (red and green neurons are adjacent). D) AH.lacZ (green), Fas2 (blue) and Drifter (red) shows coexpression of AH.lacZ and Drifter (yellow) in a subset of neurons within the PI. E) AH.lacZ (green), Fas2 (blue) and dILP2 (red) shows coexpression in all dILP2 neurons of the PI (all neurons with red cytoplasm have green nuclei). An additional AH.lacZ neuron without dILP2 is also visible in the PI of each hemisphere, sitting medially.
Fig 3. *dCORL* mutant larval brains are missing all dILP2 neurons lacking Drifter. Dorsal view of larval female brains with anterior up stained with Drifter (green - nuclear), Fas2 (blue) and dILP2 (red - cytoplasmic). A) Wild type (y¹w⁶⁷c²) at 20X shows the presence of dILP2 and Drifter in the PI. B) 40X stack and single slice of the PI of the left hemisphere from the same brain. The single slice view shows that the dILP2 neurons are essentially in a monolayer (all 8 neurons are visible in a single slice). There are neurons that express Drifter alone, three neurons expressing dILP2 alone (white arrowheads), and five neurons that express both. C) Df(4)dCORL brain (larvae was aged to the same developmental point as wild type before dissection) at 20X shown to scale. Notwithstanding the complete disarray of Fas2 and Drifter staining as well as the statistically significant reduction in Df(4)dCORL brain size, both dILP2 and Drifter are present in the PI. D) 40X stack and single slice of the PI of the left hemisphere from the same brain. The single slice view shows that there are just 5 dILP2 neurons (versus 8 in wild type) and that their topology is altered. These are no longer in a monolayer (only 3 neurons are visible in a single slice). There are no neurons expressing dILP2 alone as all 5 dILP2 neurons express Drifter.
**Fig 4.** *dCORL* mutant virgin adult brains are missing all dILP2 neurons lacking Drifter.

One day old virgin adult female brain in dorsal view with anterior up displaying Drifter (green), Fas2 (blue) and dILP2 (red). A) Wild type (*y¹w¹⁶⁷c²⁵*) at 20X shows dILP2 and Drifter in the PI. B) 40X stack of the PI from the same brain: (top) 2-color, (middle and bottom) single channel of dILP2 (red) and Drifter (green). The 2-color view shows that dILP2-Drifter coexpressing neurons form a single straight row along the apical surface of the PI with additional dILP2 neurons forming an inverted triangle. There are neurons that express Drifter alone in the apical row. C) Three slices show the topology of the PI (not all dILP2 neurons are visible in the same slice). Four dILP2 neurons that do not express Drifter are indicated (white arrowheads). D) *Df(4)dCORL* brain at 20X shown to scale with dILP2 and Drifter in the PI. E) 40X stack of the PI from the same brain: (top) 2-color, (middle and bottom) single channel of dILP2 (red) and Drifter (green). The 2-color view shows there has been a decrease in the number of dILP2 expressing neurons. The dILP2-Drifter coexpressing neurons still form a single row but the absence of additional dILP2 neurons medially caused the apical row to collapse into a V-shape. There are neurons that express Drifter alone in the dILP2-Drifter V-shape and their number has not changed. F) Three slices show that the V-shaped row of dILP2-Drifter neurons has wild type topology. No dILP2 neurons that do not express Drifter are present.
**Fig 5.** *dCORL* mutant virgin adult brains are missing Dac neurons in the PI. One day old virgin adult female brains in dorsal view with anterior up stained with Drifter (green), Dac (blue - nuclear) and dILP2 (red). A) Wild type (y¹-w¹67-c3) at 20X shows the presence of all proteins in the PI. B) 40X stack of the PI from the same brain: (top) 2-color view, (middle and bottom) single channel views of dILP2 (red) and Dac (blue). The 2-color view shows dILP2-Dac coexpressing neurons are surrounded by many Dac neurons that form an inverted triangle. C) Three slices covering 8 microns show that all dILP2 neurons express Dac. D) Df(4)dCORL brain at 20X shown to scale. All proteins are present in the PI. E) 40X stack of the PI from the same brain: (top) 2-color view, (middle and bottom) single channel views of dILP2 (red) and Dac (blue). The 2-color view shows that there has been a modest decrease in the number of dILP2-Dac coexpressing neurons and a substantial decrease in Dac neurons. The dILP2-Dac coexpressing neurons and the majority of remaining Dac neurons form a single row along the apical surface of the PI (with the exception of a single dILP2 cell). The absence of additional dILP2-Dac and Dac only neurons medially has caused the apical row to collapse into a V-shape. F) Three slices covering a span of 8 microns shows that all dILP2 neurons express Dac.
Fig. 6. dCORL-RNAi in the PI phenocopies the $Df(4)dCORL$ dILP2 phenotype. One day old virgin adult female brains in dorsal view with anterior up. A) Wild type ($y^{1}w^{67c23}$) with OK107.Gal4 driving UAS.dCORL-lacZ at 20X displaying lacZ (green), Fas2 (blue) and dILP2 (red) shows the presence of lacZ with Fas2 in the mushroom body lobes and lacZ with dILP2 in the PI. B) 40X stack of the PI from the same brain: (top) 2-color view, (middle) single channel view of dILP2 (red). Both views show the wild type pyramidal structure of lacZ and dILP2 expressing neurons in the PI. C) One slice showing the same topology. D) Wild type ($y^{1}w^{67c23}$) with OK107.Gal4 driving UAS.dCORL-RNAi displaying Drifter (green), Fas2 (blue) and dILP2 (red). Mushroom body defects due to loss of dCORL in $Df(4)dCORL$ adult brains are visible that verify dCORL-RNAi expression. E) 40X stack of the PI from the same brain: (top) 2-color view, (middle and bottom) single channel views of dILP2 (red) and Drifter (green). These views show that there has been a modest decrease in the number of dILP2 expressing neurons and that their topology has flattened out though not to the extent seen in $Df(4)dCORL$. F) One slice shows that all dILP2 neurons express Drifter, a phenocopy of the $Df(4)dCORL$ dILP2 phenotype.
Fig 7. *dCORL* mutant virgin adult longevity defects are fully rescued by mating in both sexes. Cohorts of 100 flies of each genotype and mating status were analyzed in parallel under identical conditions. Mortality assessments were made daily. Numerical data from this experiment is in Table 1 with a much larger set of genotypic controls shown in Table S1. Note that *Df(4)dCORL* has a *yw* background. A) *Df(4)dCORL* virgins display a relatively short lifespan but upon mating there is a highly significant extension of lifespan for both sexes. B) *yw* virgins and mated flies of both sexes show no lifespan differences. C) *Df(4)dCORL* virgins of both sexes show a highly significant lifespan reduction versus *yw*. Upon mating, the lifespan reduction of *Df(4)dCORL* virgins is completely rescued as mated *Df(4)dCORL* adults of both sexes are not different from *yw*.
Fig 8. dILP2 neurons lacking Drifter are fully rescued by mating in dCORL mutant adult brains. Df(4)dCORL mated adult female brains shown as in Fig. 4. A) Three day old at 20X shows rescue of PI topology compared to dCORL mutant virgin females. B) 40X stack of the PI from the same brain: (top) 2-color view, (middle and bottom) single channel views of dILP2 (red) and Drifter (green). Three day old mated females display an increase in dILP2 expressing neurons versus virgin females due to the presence of rescued neurons expressing dILP2 only. C) Three slices at 40X covering 10 microns show the 3-dimensional structure of the PI is similar to wild type though more compact. Multiple dILP2 neurons lacking Drifter are present (white arrowheads). D) Fifteen day old at 20X shows that the rescue of PI topology persists. E) 40X stack of the PI from the same brain: (top) 2-color view, (middle and bottom) single channel views of dILP2 (red) and Drifter (green). Fifteen day old mated female also displays an increase in dILP2 expressing neurons versus virgins indicating that rescued neurons expressing dILP2 only persist. F) Three slices covering a span of 24 microns show the 3-dimensional structure of the PI is similar to wild type though more expansive. Multiple rescued dILP2 neurons lacking Drifter (white arrowheads) are present after two weeks.
Table S1. *dCORL* mutant virgin and mated adult longevity defects compared to seven control lines.

| Genotypea | Mean Lifespan | P-value | Genotype | Mean Lifespan | Change | P-value |
|-----------|---------------|---------|-----------|---------------|--------|---------|
| Df(4) virgin female 12.0 +/- 1.14 | Df(4) mated female 24.9 +/- 1.74 | +108% | 0.02 | Mated longer |
| yw virgin | 27.3 +/- 1.17 | yw mated | 29.4 +/- 1.69 | + 08% | 0.52 | No diff |
| PB707015 virgin | 41.7 +/- 1.95 | PB70015 mated | 57.0 +/- 1.97 | + 37% | 0.04 | Mated longer |
| Glu-RA12b virgin | 72.1 +/- 20.9 | Glu-RA12b mated | 64.6 +/- 19.9 | - 10% | 0.32 | No diff |
| Glu-RA2b virgin | 57.7 +/- 25.8 | Glu-RA2b mated | 69.6 +/- 11.9 | + 20% | 0.12 | No diff |
| PB06235 virgin | 27.0 +/- 18.9 | PB06235 mated | 25.5 +/- 17.3 | - 06% | 0.82 | No diff |
| PB02096 virgin | 36.9 +/- 15.6 | PB02096 virgin | 36.1 +/- 11.3 | - 02% | 0.89 | No diff |
| spx720RW virgin | 48.3 +/- 22.9 | spx720RW mated | 52.3 +/- 19.8 | + 09% | 0.62 | No diff |

for virgins: yw significantly longer than Df(4)

| Df(4) virgin female 12.0 +/- 1.14 vs. Df(4) | Df(4) mated female vs. Df(4) |
|-------------------------------------------|----------------------------------|
| yw virgin 27.3 +/- 1.17 vs. Df(4)          | yw mated 29.4 +/- 1.69 vs. Df(4) |
| PB707015 virgin 31.7 +/- 1.95 vs. Df(4)   | PB70015 mated 57.0 +/- 1.97 vs. Df(4) |
| Glu-RA12b virgin 72.1 +/- 20.9 vs. Df(4) | Glu-RA12b mated 64.6 +/- 19.9 vs. Df(4) |
| Glu-RA2b virgin 57.7 +/- 25.8 vs. Df(4)  | Glu-RA2b mated 69.6 +/- 11.9 vs. Df(4) |
| PB06235 virgin 27.0 +/- 18.9 vs. Df(4)   | PB06235 mated 25.5 +/- 17.3 vs. Df(4) |
| PB02096 virgin 36.9 +/- 15.6 vs. Df(4)   | PB02096 mated 36.1 +/- 11.3 vs. Df(4) |
| spx720RW virgin 48.3 +/- 22.9 vs. Df(4) | spx720RW mated 52.3 +/- 19.8 vs. Df(4) |

for mated: no difference yw and Df(4)

| Df(4) virgin female 12.0 +/- 1.14 vs. Df(4) |
|-------------------------------------------|
| yw virgin 21.0 +/- 15.6 vs. Df(4)          |
| yw virgin 29.5 +/- 16.0 vs. Df(4)          |
| PB707015 virgin 40.1 +/- 31.0 vs. Df(4)   |
| Glu-RA12b virgin 67.2 +/- 15.4 vs. Df(4) |
| Glu-RA2b virgin 63.3 +/- 14.3 vs. Df(4)  |
| PB06235 virgin 26.2 +/- 22.2 vs. Df(4)   |
| PB02096 virgin 44.1 +/- 27.1 vs. Df(4)   |
| spx720RW virgin 47.6 +/- 21.2 vs. Df(4) |

for virgins: yw significantly longer than Df(4)

| Df(4) virgin male 21.0 +/- 15.6 vs. Df(4) |
|-------------------------------------------|
| yw virgin 29.5 +/- 16.0 vs. Df(4)          |
| PB707015 virgin 40.1 +/- 31.0 vs. Df(4)   |
| Glu-RA12b virgin 67.2 +/- 15.4 vs. Df(4) |
| Glu-RA2b virgin 63.3 +/- 14.3 vs. Df(4)  |
| PB06235 virgin 26.2 +/- 22.2 vs. Df(4)   |
| PB02096 virgin 44.1 +/- 27.1 vs. Df(4)   |
| spx720RW virgin 47.6 +/- 21.2 vs. Df(4) |

for mated: Df(4) significantly longer than yw

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a. Mutation locations shown in genomic map in Tran et al. 2018.
b. Note that yw is the background of Df(4)dCORL, *Pbac{WH}707015*, Glu-RA12b and Glu-RA2b.

*Pbac{WH}6253* and *Pbac{RB}02096* have only a w mutation on their X chromosomes. *spx720RW* has a wild type X chromosome.
Table S2. Mean & median longevity of dCORL virgin and mated adults with seven controls.

| Genotype          | Mean Lifespan | Median Lifespan | Δ   | Genotype          | Mean Lifespan | Median Lifespan | Δ   |
|-------------------|---------------|-----------------|-----|-------------------|---------------|-----------------|-----|
| Df(4) virgin female | 12.0±/-11.4   | 10.0            | -2  | Df(4) mated female | 24.9±/-17.4   | 33.5            | +8.6|
| yw virgin         | 27.3±/-11.7   | 25.5            | -1.8| yw mated          | 29.4±/-16.9   | 35.0            | +5.6|
| PB07015 virgin    | 41.7±/-19.5   | 36.0            | +5.7| PB07015 mated     | 57.0±/-19.7   | 61.0            | +4.0|
| Glu-RA112b virgin | 72.1±/-20.9   | 79.0            | +6.9| Glu-RA112b mated  | 64.6±/-19.9   | 70.5            | +5.9|
| Glu-RA2b virgin   | 57.7±/-25.8   | 65.0            | +7.3| Glu-RA2b mated    | 69.6±/-11.9   | 72.5            | +2.9|
| PB06235 virgin    | 27.0±/-18.9   | 24.5            | -2.5| PB06235 mated     | 25.5±/-17.3   | 23.5            | -2.0|
| PB02096 virgin    | 36.9±/-15.6   | 43.0            | +6.1| PB02096 virgin    | 36.1±/-11.3   | 35.5            | -0.6|
| spx720RW virgin   | 48.3±/-22.9   | 47.0            | -1.3| spx720RW mated    | 52.3±/-19.8   | 60.0            | +7.7|
|                   |               |                 |     |                   |               |                 |     |
| Df(4) virgin male | 21.0±/-15.6   | 23.5            | +2.5| Df(4) mated male  | 36.9±/-05.0   | 37.0            | +0.1|
| yw virgin         | 29.5±/-16.0   | 26.0            | -3.5| yw mated          | 25.7±/-15.0   | 27.5            | +1.8|
| PB07015 virgin    | 40.1±/-31.0   | 38.0            | -2.1| PB07015 mated     | 49.8±/-12.9   | 52.0            | +2.2|
| Glu-RA112b virgin | 67.2±/-15.4   | 66.0            | -1.2| Glu-RA112b mated  | 54.1±/-15.8   | 57.5            | +3.4|
| Glu-RA2b virgin   | 63.3±/-14.3   | 70.0            | +6.7| Glu-RA2b mated    | 52.7±/-21.4   | 52.0            | -0.7|
| PB06235 virgin    | 26.2±/-22.2   | 25.0            | -1.2| PB06235 mated     | 30.4±/-15.3   | 33.5            | +3.1|
| PB02096 virgin    | 44.1±/-27.1   | 44.0            | -0.1| PB02096 mated     | 25.1±/-10.4   | 28.0            | +2.9|
| spx720RW virgin   | 47.6±/-21.2   | 47.0            | -0.6| spx720RW mated    | 46.6±/-13.5   | 41.5            | -5.1|

a. All means and medians are within 20% of each other except Df(4) mated females have a median lifespan 36% longer than their mean lifespan.

Table S3. Significant brain size reduction is present in Df(4)dCORL larvae but not adults.

| Genotype, virgin/mated, age in days post-eclosion | Larval or adult brain size (pixels) | P-value vs. yw virgin | P-value vs. Df(4) virgin | P-value vs. Df(4) 3 day mated |
|--------------------------------------------------|------------------------------------|-----------------------|--------------------------|-------------------------------|
| Third instar larvae                               | n                                  |                       |                          |                               |
| yw                                               | 8                                  | 31682 ± 3037          |                          |                               |
| Df(4)dCORL                                       | 7                                  | 20304 ± 5718          | 0.001                    |                               |
| Adult                                            |                                    |                       |                          |                               |
| yw, virgin                                       | 6                                  | 49382 ± 3276          |                          |                               |
| Df(4), virgin, 1 day                             | 8                                  | 47181 ± 3688          | 0.305                    |                               |
| Df(4), mated, 3 day                              | 7                                  | 48228 ± 4281          | 0.630                    | 0.644                         |
| Df(4), mated, 15 day                             | 8                                  | 50802 ± 2807          | 0.435                    | 0.058                         | 0.217 |