nature COMMUNICATIONS

ARTICLE

Received 18 Jul 2013 | Accepted 23 Dec 2013 | Published 4 Feb 2014

DOI: 10.1038/ncomms4177

Tailless and Atrophin control *Drosophila* aggression by regulating neuropeptide signalling in the *pars intercerebralis*

Shaun M. Davis[1,*], Amanda L. Thomas[1,*], Krystle J. Nomie[1,*], Longwen Huang[3] & Herman A. Dierick[1,2,3,4]

Aggressive behaviour is widespread throughout the animal kingdom. However, its mechanisms are poorly understood, and the degree of molecular conservation between distantly related species is unknown. Here we show that knockdown of *tailless* (*tll*) increases aggression in *Drosophila*, similar to the effect of its mouse orthologue *Nr2e1*. Tll localizes to the adult *pars intercerebralis* (*PI*), which shows similarity to the mammalian hypothalamus. Knockdown of *tll* in the *PI* is sufficient to increase aggression and is rescued by co-expressing human *NR2E1*. Knockdown of *Atrophin*, a Tll co-repressor, also increases aggression, and both proteins physically interact in the *PI*. *tll* knockdown-induced aggression is fully suppressed by blocking neuropeptide processing or release from the *PI*. In addition, genetically activating *PI* neurons increases aggression, mimicking the aggression-inducing effect of hypothalamic stimulation. Together, our results suggest that a transcriptional control module regulates neuropeptide signalling from the neurosecretory cells of the brain to control aggressive behaviour.

[1] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA. [2] Department of Pathology and Immunology, Baylor College of Medicine, Houston, Texas 77030, USA. [3] Department of Neuroscience, Baylor College of Medicine, Houston, Texas 77030, USA. [4] Program in Developmental Biology, Houston, Texas 77030, USA. * These authors contributed equally to this work. Correspondence and requests for materials should be addressed to H.A.D. (email: dierick@bcm.edu).

Aggression is a complex social behaviour that is pervasive across the animal kingdom and has important societal consequences[1]. For many species, aggressive behaviour is important for the acquisition of resources, such as food, territory and mates; however, the behaviour can be energetically costly and can result in damage to the organism. The elucidation of the genes and neuronal circuitry that regulate aggression is key to the understanding of its mechanisms. The fruit fly *Drosophila melanogaster* has recently emerged as a model system to study aggression[2–12]. Several conserved neuromodulators have been shown to play a role in fly aggression[5–7]. For example, serotonin plays a critical role in the modulation of aggression across a broad range of species but the effect is different in vertebrates and invertebrates[13,14]. In addition, fly-specific pheromonal cues have been identified that affect aggression in *Drosophila*[10–12]. What remains unclear is whether the molecular mechanisms that control aggression in flies are mechanistically conserved with those in mammals. Transcriptional control modules that consist of transcription factors, their co-factors, DNA-binding sites and target genes are often conserved in the control of developmental and behavioural processes across a broad range of species[15,16]. Such transcription modules are therefore ideal to investigate the question of evolutionary conservation of molecular control mechanisms of specific phenotypes. A classical example in behaviour is the transcriptional negative feedback loop that controls circadian behaviour in most animal species[16]. Circadian mutants were first isolated in a screen for *Drosophila* eclosion rhythm[17], and the molecular clock was elucidated in the following decades[16] and found to be remarkably conserved across many species[18].

In mice, a transcription factor, called Nr2e1 or Tlx, was identified almost 20 years ago as having a major effect on aggressive behaviour[19]. Nr2e1 is a transcriptional repressor that belongs to the family of orphan nuclear receptors[20,21] and is an orthologue of *Drosophila tailless* (*tll*), which is critical for early embryonic development[22,23]. Mammalian Nr2e1 and *Drosophila* Tll have a conserved binding site[24] and share a conserved co-repressor[25], encoded by the *Atrophin* locus. In addition, some of the known targets are conserved between mice and flies[26]. A deletion of the mouse gene causes abnormal brain development and extreme aggression[19,27–29]. These phenotypes are rescued with a human transgene covering the human genomic locus demonstrating molecular conservation between mice and humans even at the level of the regulatory regions of this locus[29]. In addition, single nucleotide polymorphisms in the human *NR2E1* locus have been associated with schizophrenia, bipolar disorder and psychopathy[30]—three human disorders associated with excessive aggressive tendencies. However, the mechanism of action of this gene with respect to its extreme aggression phenotype is still not well understood.

Here we show that pan-neuronal knockdown of *tailless* in *Drosophila* induces a strong aggression phenotype similar to what is observed in mice, and that adult-specific knockdown is sufficient to cause the phenotype. In the adult fly brain, Tailless localizes to the neurosecretory cells of the *pars intercerebralis* (*PI*), a brain region with functional, developmental and structural similarities to the mammalian hypothalamus[31]. Knockdown of *tll* specifically in the *PI* causes aggression and can be rescued by co-expression of human NR2E1. Knockdown of *Atrophin* (*Atro*), which encodes a co-repressor of Tll, also increased aggression. We further show that Tll and Atro physically interact *in vitro* and in transgenic animals expressing tagged variants of the two proteins. To explore the role of the *PI* neurons in the regulation of aggression, we genetically activated these neurons by expressing the bacterial sodium channel, NaChBac[32]. Flies with genetically activated *PI* neurons show an increased aggression phenotype, similar to electrical stimulation of the mammalian hypothalamus, which also induces aggression in a wide range of mammalian species[33–35]. To characterize the neuronal mechanism of *tll* knockdown-induced aggression, we blocked neuropeptide processing and release from these neurons and fully suppressed the aggression phenotype. Finally, we show that knockdown of *tll* in the *PI* leads to increased release of a neuropeptide reporter, suggesting that Tll controls aggression by regulating the release of neuropeptides from the neurosecretory cells of the *PI*. Taken together, our results suggest that Tll is part of a transcriptional module that controls aggression in the neurosecretory cells of the *pars intercerebralis* by regulating the release of neuropeptides.

Results

Knockdown of *tailless* in adult males increases aggression. We first asked whether transcriptional control of aggression is conserved between fruit flies and mice and focused on the role of the orphan nuclear receptor orthologue of Nr2e1 in this complex behaviour. In mice, a deletion of the *Nr2e1* locus results in brain developmental defects and extreme aggression, although it is not known whether the developmental and behavioural effects are directly linked[19,27–29]. The *Drosophila* genome harbours two *Nr2e1* orthologues[20,21], *tailless* (*tll*) and *dissatisfaction* (*dsf*) (Supplementary Fig. 1 and Supplementary Table 1). Null mutations in *tll* are embryonic lethal[22,23]. In contrast, null mutations in *dsf* are adult viable and have male courtship defects[36]. We analysed existing null alleles of *tll* ($tll^{LE3}/+$) and *dsf* (dsf^{1}) but neither showed an increase in aggressive behaviour (Fig. 1a). We also knocked down both genes using RNA interference (RNAi) and assayed for aggression[37]. We used the *GAL4/UAS* system[38] to drive RNAi alleles, which have been inserted in the same genomic locus[39]. Using the pan-neuronal driver, *elav-GAL4* (ref. 40), only males in which *tll* was knocked down showed significantly increased fighting frequencies compared with control animals (Fig. 1a, Kruskal–Wallis analysis of variance (ANOVA), $P<0.001$). Knockdown was verified using western blotting, which shows that Tll levels are significantly reduced in the brains of the knockdown males (Supplementary Fig. 2). These results suggest that *tll*, but not its paralogue *dsf*, plays a role in aggression.

Loss of Nr2e1 in mice causes abnormal brain development in addition to extreme aggression[19,27–29]. However, it is unclear whether these brain defects cause the aggression phenotype in mice or whether the behavioural abnormalities are independent from the developmental defects. In flies, we found no obvious anatomical defects in aggressive *elav-GAL4>tll*^{RNAi} male brains when we immunostained with the neuropil marker Dlg. To address whether the behavioural phenotype in *elav-GAL4>tll*^{RNAi} males is indeed due to a post-developmental role of Tll, we tested adult-specific knockdown of *tll* using the TARGET system[41]. This system adds temporal control to the *GAL4/UAS* system by using an ubiquitously expressed temperature-sensitive GAL4 inhibitor, *TubP-GAL80*^{ts}, to block GAL4-induced activation at the permissive temperature (18 °C) and allowing GAL4 induction at the restrictive temperature (25–30 °C)[41]. Only the *elav-GAL4>UAS-tll*^{RNAi}*; TubP-GAL80*^{ts} flies reared and maintained at 25 °C (Fig. 1b, Kruskal–Wallis ANOVA, $P=0.0246$) or reared at 18 °C and shifted to 25 °C after eclosion (Supplementary Fig. 3) showed significantly increased fighting frequencies compared with the control animals, indicating that adult knockdown is necessary and sufficient to increase aggression.

Tll localizes to the adult *PI*. The adult-specific behavioural effects of *tll* knockdown prompted us to investigate Tll protein

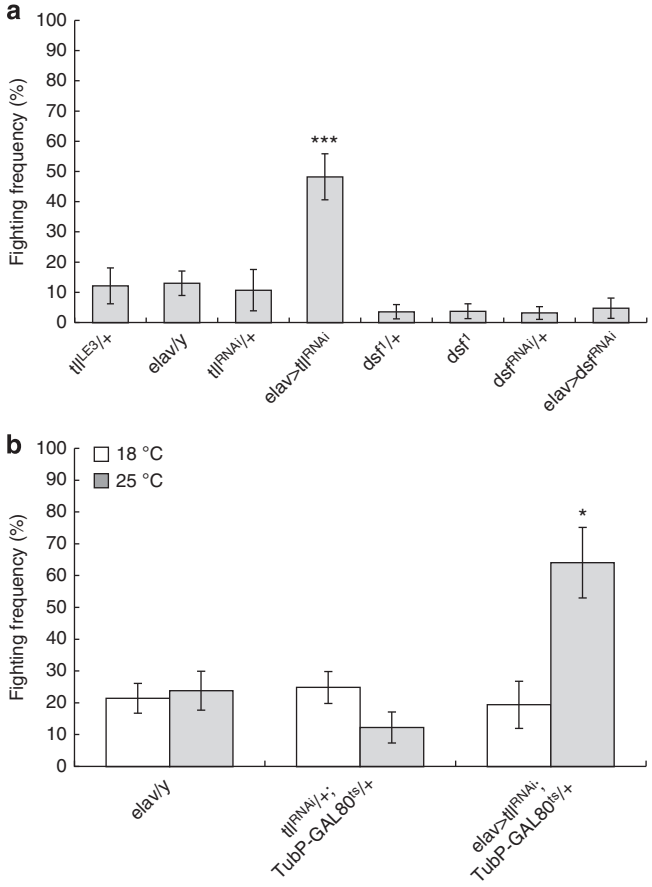

Figure 1 | Knockdown of *tll* in the adult fly increases aggression. (**a**) Fighting frequencies of flies of the following genotypes: $tll^{Le3}/+$, *elav-GAL4/+*, *UAS-tll*$^{RNAi}/+$, *elav-GAL4; UAS-tll*^{RNAi}, $dsf^{1}/+$, dsf^{1}, $dsf^{RNAi}/+$ and *elav-GAL4; UAS-dsf*^{RNAi}. Only *elav>tll*^{RNAi} males have significantly higher median fighting frequencies (Kruskal–Wallis ANOVA, $^{***}P<0.001$, n = minimum of 70 pairs for each genotype). (**b**) Fighting frequencies of *elav-GAL4>UAS-tll*^{RNAi} in a *TubP-GAL80*^{ts} background and control lines containing only one component of the binary expression system (*elav-GAL4/+* and *TubP-GAL80*$^{ts}/+$; *UAS-tll*$^{RNAi}/+$). All flies were reared at 25 °C and on the day of eclosion were either moved to 18 °C (open bars) or maintained at 25 °C (grey bars) for 7 days. Only *elav>tll*^{RNAi}; *TubP-GAL80*^{ts} males that were maintained at 25 °C after eclosion had significantly higher median fighting frequencies (Kruskal–Wallis ANOVA, $^{*}P=0.025$, n = minimum of 60 pairs for each genotype). Bar graphs are presented as means ± s.e.m.

localization in the adult fly brain. We performed immunohistochemistry using two different antibodies, one raised against *Drosophila* Tll[42] and one raised against human NR2E1 (Sigma) that is specific to the highly conserved 50 amino-terminal residues (76% identical to fly Tll). Both antibodies revealed localization of Tll in the *PI* (Fig. 2a,b and Supplementary Fig. 4), a brain region composed of ~200 neurosecretory cells that has been suggested to be functionally equivalent to the hypothalamus based on molecular, structural and developmental data[31]. Interestingly, mouse Nr2e1 is expressed in the adult hypothalamus[28], which is known for its important regulatory role in mammalian aggression[43,44]. To confirm that the signal observed in the *PI* was specific to Tll and not to its paralogue, Dsf, we immunostained flies in which the *dsf* locus is deleted. The signal in the *PI* was indistinguishable from wild-type and heterozygous animals (Supplementary Fig. 4), further confirming that Tll is expressed in the *PI*.

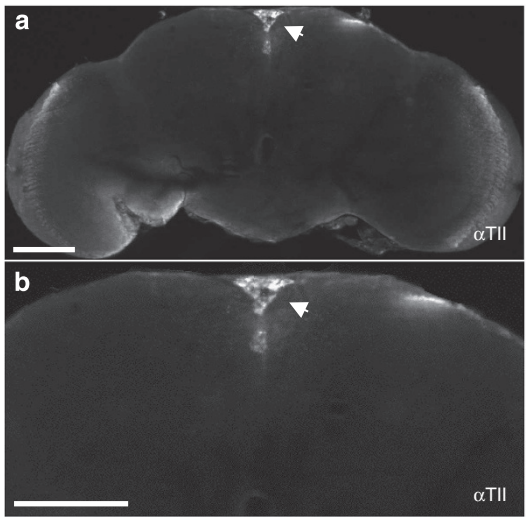

Figure 2 | Tll localizes to the adult *pars ntercererbralis*. (**a**,**b**) Immunofluorescence image of a wild-type adult brain with anti-Tll antibody. Tll localizes to the *pars intercerebralis* (arrowhead). Some staining is also observed in the optic lobes. (**b**) Close-up view of the staining in the *PI*. Scale bar = 100 μm.

***PI*-specific knockdown of *tll* induces aggression**. To assess whether Tll expression in the *PI* plays a critical role in aggression, we used three GAL4 driver lines (*c929* (ref. 45), *50Y* (ref. 46), *dIlp2* (ref. 47)) with overlapping expression in the *PI* (Fig. 3a–c) to drive *tll*^{RNAi} specifically in these neurons. Males derived from these crosses displayed significantly increased fighting frequencies (Fig. 3d, Kruskal–Wallis ANOVA, $P<0.001$). In contrast, we did not observe increased aggression when we used GAL4 drivers to knockdown *tll* in the eye, central complex or circadian LNv neurons (Fig. 3d). These results suggest that Tll affects aggression by acting in the *PI* neurons. To exclude the possibility that the effect of Tll on aggression is due to expression outside the brain, we blocked *GAL4* expression in areas below the head using *tsh-GAL80* (ref. 48) and found no change in fighting frequencies (Fig. 3e). However, when we blocked *50Y-GAL4* with a ubiquitously expressed *TubP-GAL80* or a pan-neuronally expressed *elav-GAL80* (ref. 49), we observed complete suppression of *tll* knockdown-induced aggression (Fig. 3e, Kruskal–Wallis ANOVA, $P<0.001$). To further show that this effect is due to the specific knockdown of *tll*, we tested a second RNAi line directed against *tll*^{50} and found a similar increase in aggression when we drove it in *PI* neurons (Supplementary Fig. 5). We also performed a rescue experiment by co-expressing fly *UAS-tll* and human *UAS-NR2E1* with *tll*^{RNAi} in the *PI*. We observed strong suppression of the fighting frequencies in the rescued males (black bars, *50Y>UAS-tll; tll*^{RNAi} and *50Y>UAS-NR2E1; tll*^{RNAi}) compared with *tll* knockdown males (*50Y>tll*^{RNAi}) (Fig. 3f, Kruskal–Wallis ANOVA, $P<0.001$). These results indicate that the increased aggression phenotype caused by *tll* knockdown in the *PI* is specific to *tll* and that the human gene can functionally substitute for the fly gene in its behavioural regulation. As the *PI* has been implicated in other behaviours, we also tested whether *PI*-specific knockdown of *tll* affected courtship, mating and locomoter activity (Supplementary Fig. 6). We observed no differences in courtship and mating behaviour between the *tll* knockdown males and their controls; however, we did found a significant decrease in activity in the knockdown males (Supplementary Fig. 6d, ANOVA, $P<0.01$). It has been previously argued that aggressive behaviour read-outs should be adjusted for activity in flies because increased activity is associated

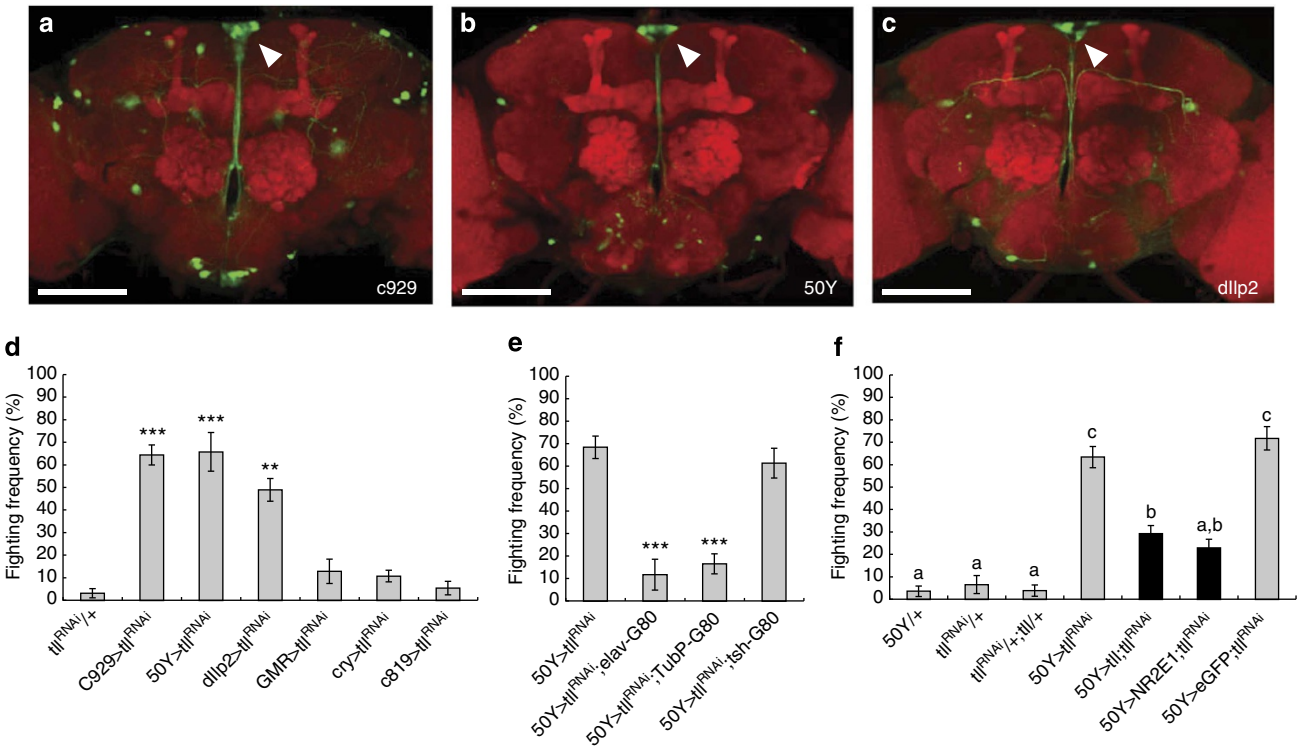

Figure 3 | Knockdown of *tailless* in the *pars intercerebralis* increases aggression. (**a**–**c**) The GAL4 expression patterns, detected by *UAS-eGFP* (green), in the adult brain of three different drivers that primarily express in the *pars intercerebralis*: (**a**) *c929-GAL4*, (**b**) *50Y-GAL4* and (**c**) *dllp2-GAL4*. Neuropil is marked with an anti-Dlg antibody (red). Arrowheads denote the *pars intercerebralis*. Scale bar = 100 μm. (**d**) Fighting frequencies of flies expressing *UAS-tll*^{RNAi} in different regions of the fly brain using *c929-GAL4*, *50Y-GAL4*, *dllp2-GAL4*, *GMR-GAL4*, *cry-GAL4* and *c819-GAL4* and a control line of *UAS-tll*^{RNAi}/+. Only *c929>tll*^{RNAi}, *50Y>tll*^{RNAi} and *dllp2>tll*^{RNAi} males showed statistically significantly higher median fighting frequencies (Kruskal–Wallis ANOVA, $^{**}P<0.01$ and $^{***}P<0.001$, n = minimum of 70 pairs for each genotype). (**e**) Fighting frequencies of flies with *tailless* knocked down in *50Y* neurons or of flies co-expressing *tsh-GAL80*, *elav-GAL80* or *TubP-GAL80* (*GAL80* abbreviated as G80). Asterisks denote statistically significant rescue in *elav-GAL80* and *TubP-GAL80* co-expressing males (Kruskal–Wallis ANOVA, $^{***}P<0.001$, n = minimum of 60 pairs for each genotype). (**f**) Fighting frequencies of flies with *tailless* knocked down in *50Y* neurons and control lines of flies expressing a component of the *GAL4/UAS* system. Fighting frequencies of flies co-expressing *tll*^{RNAi} and either fly *tll* or human *NR2E1* and *eGFP* in *50Y* neurons and control lines expressing only one component of the *GAL4/UAS* system. Letters denote groups with statistically significant different median fighting frequencies. Black bars show statistically significant rescue in males co-expressing *UAS-Tll* or *UAS-NR2E1* compared with *50Y>tll*^{RNAi} and *50Y>tll*^{RNAi} males co-expressing *UAS-eGFP* (Kruskal–Wallis ANOVA, n = minimum of 70 pairs for each genotype). Bar graphs represent means ± s.e.m.

with increased aggression[6]. Given that the *tll* knockdown males have decreased activity, that interpretation would suggest that we may be underestimating aggression in the *tll* knockdown males.

Tll interacts with Atro to regulate aggression. Tll is a known transcriptional repressor and has evolutionarily conserved co-repressors in flies and mice[25]. We tested whether loss of two previously described Tll co-repressors, Scribbler (Sbb)[51] and Atrophin (Atro)[52], also affect aggression. Similar to *tll*, both genes are embryonic lethal, and we therefore used RNAi to silence them and assayed for aggression. We found that *50Y>sbb*^{RNAi} males did not show significantly increased fighting frequencies, while *50Y>Atro*^{RNAi} males did (Fig. 4a, Kruskal–Wallis ANOVA, $P<0.001$). We also knocked down *Atro* with another previously validated RNAi allele (*Atro*^{IR})[52], and again observed a significant increase in fighting frequency compared with controls (Fig. 4a, Kruskal–Wallis ANOVA, $P<0.01$). Pan-neuronal knockdown with *Atro*^{IR} significantly decreases the level of Atro (Supplementary Fig. 7). Consistent with the stronger behavioural effect of *Atro*^{RNAi} expression in the *PI*, *elav-GAL4>Atro*^{RNAi} flies were lethal and could not be evaluated for knockdown efficiency. We next determined Atro localization and found that it is ubiquitous throughout the brain including the *PI* neurons (Fig. 4b and Supplementary Fig. 8). To evaluate whether Tll and Atro may act in the same pathway, we used a genetic and cell biological approach. We simultaneously silenced *tll* and *Atro* by driving *tll*^{RNAi} and *Atro*^{IR} in the *PI* neurons and found that the double knockdown animals showed the same level of aggression as the single knockdown *50Y>tll*^{RNAi} males (Fig. 4c, Kruskal–Wallis ANOVA, $P<0.001$). Although neither is a null allele, the data suggest that Tll and Atro work together to affect aggression rather than in parallel pathways. To examine whether Tll and Atro physically interact, we performed bimolecular fluorescent complementation[53]. We tagged *tll* with the C-terminal portion of yellow fluorescent protein (YFP) and tagged the C-terminal part of Atro that was shown to interact with Tll *in vitro*[54] with the N-terminal portion of YFP. We first transfected both constructs in *Drosophila* S2 cells and observed nuclear YFP signal only when we expressed both constructs together (Fig. 5j,n compared to b,f and Supplementary Fig. 9). We next made transgenic lines expressing both constructs from an *UAS* promoter and we observed YFP signal in the *PI* also only when we expressed both construct simultaneously with the *50Y* driver (Fig. 6a–c and Supplementary Fig. 10). Together, these results suggest that Tll and Atro function together to regulate aggression through the neurosecretory cells of the *PI*.

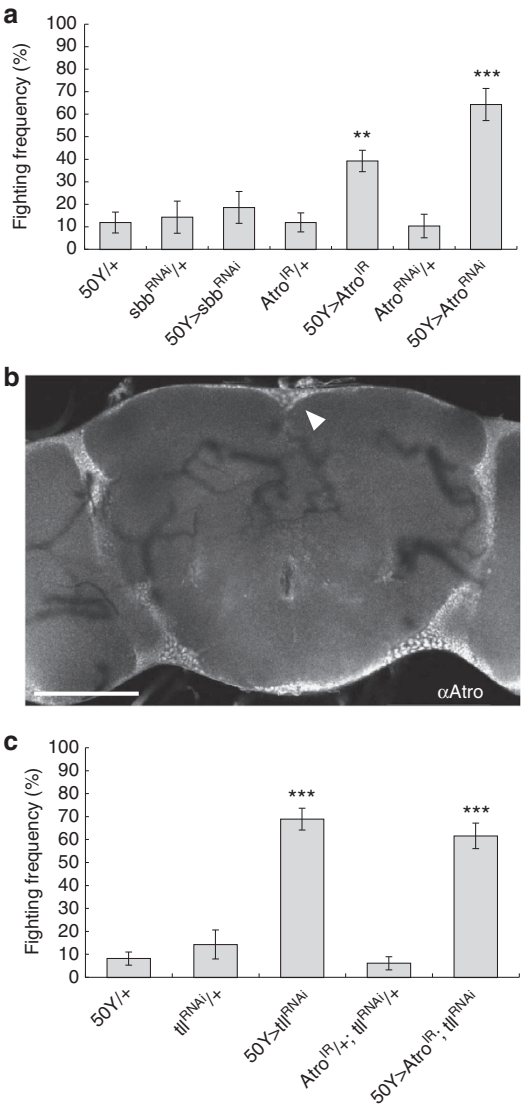

Figure 4 | Knockdown of *Atrophin* in the *PI* promotes aggressive behaviour. (**a**) Fighting frequencies of flies with *sbb* or *atro* knocked down in the *PI* using the *50Y* driver and control lines containing only one component of the binary expression system. Only *50Y>Atro^{IR}* and *50Y>Atro^{RNAi}* flies have significantly increased fighting frequencies (Kruskal–Wallis ANOVA, $^{**}P<0.01$ and $^{***}P<0.001$, $n=$minimum of 70 pairs for each genotype). (**b**) Immunofluorescence image of a slice of an adult male brain stained with anti-Atro antibody. The *PI* is indicated with a white arrowhead. Scale bar = 100 μm. (**c**) Fighting frequencies of flies in which *tll* alone is knocked down, in which *tll* and *atro* are simultaneously knocked down in the *pars intercerebralis* using the *50Y* driver, and control lines containing only one component of the GAL4/UAS system. Asterisks denote statistically significant higher medians in the *tll* and *tll/Atro* knockdown males compared with the single binary component controls (Kruskal–Wallis ANOVA, $^{***}P<0.001$, n = minimum of 60 pairs for each genotype). Bar graphs represent means ± s.e.m.

Tll regulates neuropeptide release from the adult *PI*. Finally, we investigated the underlying neuronal mechanism in the *PI* that may lead to an increased aggression response in flies. We wondered whether increasing electrical activity in *PI* neurons may be responsible for the increased aggressive behaviour, similar to the increased aggression phenotype that is observed in mammals when the hypothalamus is electrically or optogenetically stimulated[33–35,55]. We expressed the bacterial sodium channel, *UAS-NaChBac*[32], to increase electrical activity in *PI* neurons, and observed a modest but significant increase in aggression in *50Y>UAS-NaChBac* males (Fig. 7a, Kruskal–Wallis ANOVA, $P=0.0059$). We observed an even stronger effect when we drove expression of *UAS-NaChBac* with the *dIlp2* driver that is expressed almost exclusively in the *PI* neurons[47] (Fig. 3c) (Supplementary Fig. 11, Kruskal–Wallis ANOVA, $P<0.001$). Recently, *NaChBac* expression in the *PI* was shown to increase neuropeptide release from these neurons[56], and we therefore tested whether we could block *tll* knockdown-induced aggression by disrupting neuropeptide function. Neuropeptides that are released from neurosecretory cells typically require processing of precursor forms by the proprotein convertase encoded by *amontillado* (*amon*)[57]. Following processing, mature peptides are packaged into dense core vesicles, which require the function of Caps (calcium-activated protein for secretion) for subsequent release of these vesicles[58]. When we co-expressed a previously validated RNAi allele directed against *amon*[57] (Supplementary Fig. 12) together with *tll^{RNAi}*, we could fully suppress *tll* knockdown-induced aggression (Fig. 7b, Kruskal–Wallis ANOVA, $P<0.001$). Similarly, a homozygous transposon insertion in *Caps* fully suppressed the *tll* knockdown-induced aggression (Fig. 7c, Kruskal–Wallis ANOVA, $P<0.001$). Co-expression of *amon^{RNAi}* also fully suppressed the *NaChBac*-mediated activation of the *PI* neurons (Supplementary Fig. 11). To visualize the effect of *tll* knockdown on neuropeptide release, we co-expressed a heterologous green-fluorescent protein (GFP)-tagged neuropeptide reporter[59] in the *PI*. Compared with controls (Fig. 7d), GFP staining in the cell bodies of the *PI* was strongly diminished when we also knocked down *tll* (Fig. 7e). Together with the neuropeptide processing and release suppression results, these staining results suggest that Tll regulates aggressive behaviour through the release of neuropeptides from the *PI* (Fig. 7f).

Discussion

We set out to explore whether the molecular mechanisms underlying aggressive behaviour in the fruit fly are conserved with mechanisms that play a role in mammals. We chose to focus our attention on a transcription factor with a strong effect on aggression in mice that has a clear conserved counterpart in *Drosophila*. Our rationale was based on the idea that conserved transcription modules—which consist of a transcription factor with conserved co-factors, conserved binding site and conserved targets—exist in the evolutionarily conserved control of development[15] and behaviour[16,18]. We found that a conserved transcription factor, Tll, and its conserved co-repressor, Atro, indeed affect aggression in flies and that this transcriptional repressor complex acts through a set of adult neurosecretory cells known as the *PI*, which has remarkable similarity to the mammalian hypothalamus[31], a brain region known for its critical role in the control of aggressive behaviour in mammals[33–35,44]. Moreover, we found that electrical activation of these neurons is sufficient to cause an increase in aggression in flies, just like electrical stimulation of the hypothalamus does in mammals[33–35]. In addition, we were able to block the behavioural effect of *PI* neuronal activation by blocking neuropeptide processing in these neurosecretory cells. Interestingly, neuropeptide release from the mammalian hypothalamus is also known to play a role in aggressive behaviour[44]. Thus, we have shown that three key mechanisms involved in the control of mammalian aggression are also involved in the control of fly aggression and that they may in fact be a single mechanism.

The orphan nuclear receptor Nr2e1 or Tailless is known to have conserved co-repressors[25], binding sites[24] and targets[26], and loss of

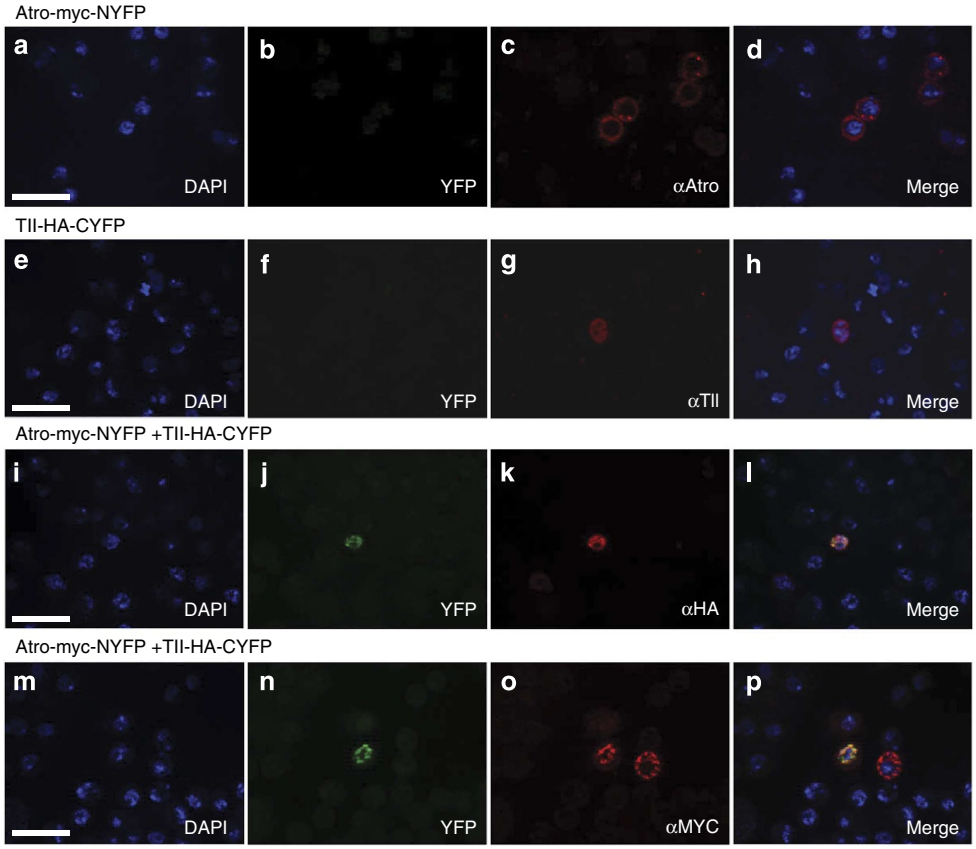

Figure 5 | Atrophin interacts with Tll in the nucleus in S2 cells. (**a**–**d**) Transfection of a single bimolecular fluorescent complementation (BiFC) component, NYFP-MYC-tagged Atro, into S2 cells shows no YFP signal (**b**) in transfected cells that stain for MYC (**c**). Red Atro staining labels the cytoplasm, while nuclei are stained in blue with 4′,6-diamidino-2-phenylindole (DAPI) (**a**). Merged images show red Atro staining around blue nuclei (**d**). (**e**–**h**) Transfection of the other BiFC component, CYFP-HA-tagged Tll, into S2 cells shows no YFP signal (**f**) in transfected cells that stain for HA (**g**). Red Tll staining is localized in the nuclei stained in blue with DAPI (**e**). Merged image shows overlapping signal of red and blue (**h**). (**i**–**p**) Co-transfection of NYFP-MYC-tagged Atro together with CYFP-HA-tagged Tll reconstitutes green YFP signal in the nucleus of co-transfected cells (**j**,**n**). Co-transfected cells also stain with an antibody against HA in red (**k**) or an antibody against MYC in red (**o**). Red HA signal labels nuclei that are yellow in the merged image (**l**). Red MYC staining localizes in the cytoplasm and nucleus of co-transfected cell but only in the cytoplasm of cells that only harbour the NYFP-MYC-tagged Atro construct (the lower cell in **o** is not green in **n**). Merged image shows yellow signal in the nucleus in the upper cell but only red signal in the lower cell (**p**). Scale bar = 20 μm.

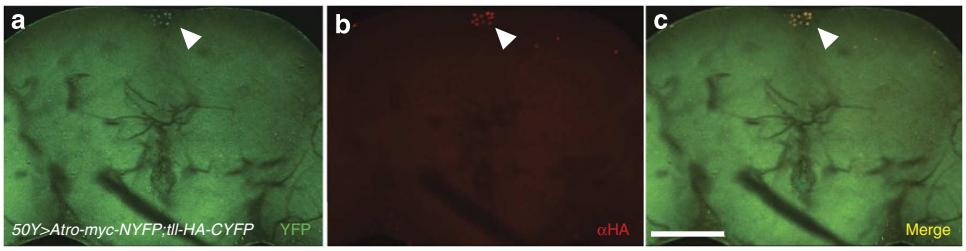

Figure 6 | Atrophin interacts with Tll in the nucleus in the *PI*. (**a**) YFP signal in adult brains of flies co-expressing NYFP-MYC-tagged Atro and CYFP-HA-tagged Tll in the *PI* with the 50Y driver. (**b**) Same brain as in **a** stained with αHA shows CYFP-HA-tagged Tll expression in the *PI*. (**c**) Merge between unstained image in **a** and HA-stained image in **b**. No signal is observed when only one component of BiFC is expressed (Supplementary Fig. 10). Scale bar = 100 μm.

function of this gene in mice has a strong aggression phenotype[19,27–29] that can be rescued with a human genomic transgene[29]. The fly harbours two *Nr2e1* orthologues[20,21], *tailless* (*tll*) and *dissatisfaction* (*dsf*), the former best known for its essential role in early embryogenesis[22,23] the latter known for its effect on courtship[37]. When we knocked down *tll*, we observed a strong aggression phenotype similar to the phenotype observed in mice, while we found no effect in *dsf* mutants. In mice, loss of Nr2e1 also causes severe brain defects[19,27–29] but it is unknown whether these developmental brain abnormalities are required for the extreme behavioural response. We show that adult-specific knockdown of *tll* is sufficient to cause aggression, thereby uncoupling the developmental and behavioural defects. It will be interesting to examine whether this adult role of Tll holds true in mice.

To further test the idea of a conserved transcription module controlling aggressive behaviour, we also examined the role of a

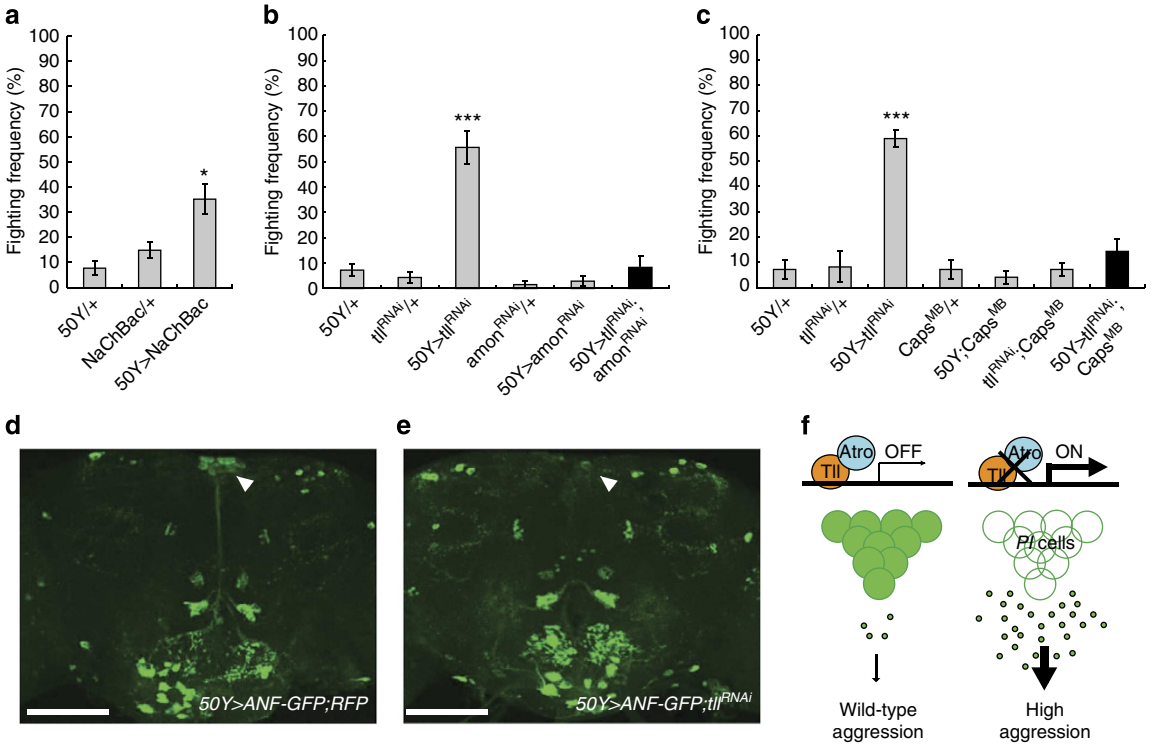

Figure 7 | Tailless affects aggression through a neuropeptide-based mechanism. (**a**) Fighting frequencies of flies expressing *UAS-NaChBac* in the *pars intercerebralis* with *50Y-GAL4* and control lines containing only one component of the *GAL4/UAS* system (Kruskal–Wallis ANOVA, $*P=0.0059$, $n=$minimum of 90 pairs for each genotype). (**b**) Fighting frequencies of flies expressing *UAS-tll*^{RNAi} and/or *UAS-amon*^{RNAi} in the *pars intercerebralis* with *50Y-GAL4* and control lines containing only one component of the binary expression system. Knockdown of *tll* in the *PI* induces aggression, which is fully suppressed when *amon* is simultaneously knocked down (black bar, Kruskal–Wallis ANOVA, $***P<0.001$, $n=$minimum of 60 pairs for each genotype). (**c**) Fighting frequencies of flies expressing *UAS-tll*^{RNAi} in the *pars intercerebralis* with *50Y-GAL4* crossed into a homozygous transposon insertion into *Caps*, *Caps*^{MB03912}. Knockdown of *tll* in the *PI* induces aggression, which is fully suppressed by a homozygous mutation in *Caps* (black bar, Kruskal–Wallis ANOVA, $***P<0.001$, $n=$minimum of 60 pairs for each genotype). (**d**) Co-expression of *UAS-nRFP* and the heterologous neuropeptide reporter *UAS-ANF-GFP* (the prepro form of atrial natriuretic factor fused to emerald *GFP*) in *PI* neurons with *50Y-GAL4* shows strong expression in the *PI* and its projections. (**e**) Co-expression of *UAS-tll*^{RNAi} and the heterologous neuropeptide reporter *UAS-ANF-GFP* in *PI* neurons with *50Y-GAL4* strongly decreases staining in the cell bodies of the *PI* but not its projections, suggesting increased release. (**f**) Schematic diagram depicting the regulation of aggression mediated by a Tailless/Atrophin complex acting in the *pars intercerebralis* on neuropeptide release. Scale bar = 100 μm.

known co-factor of Tll. We found that knockdown of a conserved co-repressor of Tll in mice and flies[25], encoded by the *Atrophin* locus, also caused an increase in aggression. *Drosophila Atro* (also known as *Grunge* (*Gug*)) shows similarity to two mouse paralogues[25], *atrophin 1* (*atn1*) and *atrophin 2* (*atn2*, also known as *Rere*). A knockout allele of the former has no obvious phenotypic effects[60], while a null allele of the latter is embryonic lethal[61]. A polyQ expansion of human *atn1* causes a dominant neurodegenerative disease, called dentatorubral pallidoluysian atrophy[62,63]. Patients with this disease develop neurodegenerative symptoms as well as psychiatric problems including excessive aggressive behaviour[64], in some cases before the onset of neurodegeneration[65]. The mouse model expressing the human mutation recapitulates many of these phenotypes, including excessive aggression[66]. Fly *Atro* is more similar to *atn2/rere* than the shorter *atn1* gene but contains several stretches of polyQs that are only found in *atn1* (ref. 25). In mice, Atn1 and Atn2 can dimerize and the expanded polyQ variant of Atn1 binds more strongly to Atn2 (ref. 67). It is intriguing to speculate that excessive aggression in patients with dentatorubral pallidoluysian atrophy may involve a transcriptional module affecting the function of an NR2E1/ATN2 repressor complex in the hypothalamus.

To further explore the cellular mechanism of action of Tll, we investigated its expression pattern and found strong expression of Tll in the neurosecretory region of the *PI*. These neurons have been suggested to be part of an ancient neuro-endocrine axis shared between vertebrates and invertebrates[68]. Multiple lines of evidence have suggested that the *PI–corpora cardiaca* system has structural, developmental and functional similarities with the mammalian hypothalamic-pituitary axis[31,68]. Electrical and optogenetic stimulation of the mammalian hypothalamus leads to a strong increase in aggression in a wide range of mammalian species[33–35]. Release of neuropeptides from the mammalian hypothalamus also plays an important role in aggression[44]. On the basis of these two mammalian mechanisms involved in aggression and the known similarity between these neurosecretory neuronal anatomies in mammals and flies, the *Drosophila PI* is an excellent candidate for a role in aggression regulation. When we knocked down *tll* in the *PI* neurons, we observed a strong increase in aggression that we could rescue by co-expressing the human *NR2E1* gene, suggesting mechanistic conservation between flies and mammals.

If *PI* neurons are indeed functionally similar to the mammalian hypothalamus in their control of aggressive behaviour, stimulation of these neurons would be predicted to increase aggressive behaviour just as electrical stimulation of the hypothalamus does in mammals. We tested this idea by genetically activating the *PI* with the bacterial sodium channel, NaChBac, which is known to increase electrical activity in the cells where it is expressed[32]. As

expected, we observed an increase in aggression in males with NaChBac-activated *PI* neurons. Recently, it was shown that NaChBac expression in the *PI* increases neuropeptide release[56]. When we blocked neuropeptide processing by inhibiting the neuropeptide preproconvertase Amon[57], we could fully suppress this response. We next explored whether the mechanism of *tll* knockdown-induced aggression in the *PI* is similarly based on increased neuronal activation and neuropeptide release from the *PI*. Indeed, when we blocked neuropeptide processing through Amon function, or neuropeptide release by reducing Caps, which is required for dense core vesicle release[58], we could fully suppress the *tll* knockdown-induced aggression response. Finally, we used a heterologous neuropeptide GFP reporter[59] to visualize the effect of *tll* knockdown on neuropeptide release from *PI* neurons and showed that the reporter nearly completely vanishes from *PI* neurons when *tll* is knocked down. The identity of the specific neuropeptide(s) that regulate aggression through this transcriptional module is currently not known. There are at least 40 different neuropeptide-encoding genes with corresponding receptors in the *Drosophila* genome[69], and it will be interesting not only to identify the causative peptide but to show whether it is conserved in mammals and whether it is directly regulated by the Tll/Atro transcriptional repressor complex.

The well-established role of Tll as a transcription factor[20,21,26,70] makes it likely that the behavioural effects that we observe are caused by changes in transcription, although we have not identified direct targets of Tll in this novel adult process. However, together our results suggest that Tll and Atro are part of the transcription module that controls the activity of *PI* neurons affecting the release of neuropeptides that regulate aggressive behaviour. Our findings in flies suggest that three mechanisms that are involved in the regulation of aggression in mammals—transcriptional control by Nr2e1 (refs 19,27–29), electrical[33–35] or optogenetic[55] stimulation of hypothalamic neurons and neuropeptide release from the neurosecretory cells of the hypothalamus[44]—may in fact be a single mechanism that may represent a core aggression regulatory mechanism in all animals.

Methods

Fly stocks and rearing conditions. The following fly strains were obtained from the Bloomington stock center: *UAS-tll*^{JF02537} (*tll*^{RNAi}, BL27242); *UAS-dsf*^{JF02537} (*dsf*^{RNAi}, BL29373); *UAS-sbb*^{JF02375} (*sbb*^{RNAi}, BL27049); *UAS-Atro*^{HMS00756} (*Atro*^{RNAi}, BL32961); *UAS-EGFP* (BL5431); *UAS-NaChBac* (BL9466); *UAS-amon*^{RNAi} 78b (BL29009); *Caps*^{MB03912} (BL24279); and *UAS-preproANF-EMD* (*ANF-GFP*, BL7001). *tll*^{LE3} was a gift from J. Merriam (University of California, Los Angeles). *tll*^{RNAi2} was a gift from Tzumin Lee (Janelia Farm Research Campus, Virginia). *Df(2L)clot7*, *Df(2L)dsf*^{3} and *dsf*^{1} were gifts from K. Finley (San Diego State University). *c929* and *cry-GAL4* were gifts from P. Shaw (Washington University St. Louis Medical School). *50Y* was a gift from R. Greenspan (University of California, San Diego). *elav-GAL4*^{c155} and *dIlp2-GAL4* were gifts from S. Pletcher (University of Michigan). *GMR-GAL4* was a gift from H. Bellenaylor College of Medicine). *c819-GAL4*, *tubP-Gal80* and *tubP-GAL80*^{ts} were gifts from G. Roman (University of Houston). *tsh-GAL80* was a gift from G. Miesenbock (Cambridge University). *elav-GAL80* was a gift from L. Jan (University of California, San Francisco) and *UAS-Atro*^{IR1} was a gift from C.-C. Tsai (University of New Jersey Medical School). All flies were reared on yeast, cornmeal, molasses and agar food at room temperature (22.5 ± 0.5 °C) on a 12-h light/12-h dark cycle. The sequences that are targeted by the different RNAi constructs against *tll* and *Atro* are shown in Supplementary Information (Supplementary Fig. 13).

Aggression assay. All aggression analyses were performed using the arena assay as previously described with minor modifications[37]. Briefly, one pair of 7- to 8-day-old males was introduced into each of the 35 cells of the arena chamber and filmed for 20 min immediately upon loading. All males were collected on the day of eclosion and were isolated 2.5 days before the assay. We typically tested 70 pairs of flies per genotype and the different genotypes were tested side-by-side. In the analysis, we primarily focused on fighting frequency (the percentage of males that establish a clear dominance hierarchy in the 20-min observation interval). Fighting frequencies correlate very well with other aggression parameters such as lunge number[37]. We scored only unambiguous stereotypical fighting actions such as wing threat, charging/lunging, tussling and boxing. Average fighting frequencies from 70 pairs of flies are plotted with s.e.m.

Courtship and mating behaviour. For courtship analysis, 10 2- to 5-day-old males of each genotype were tested against CantonS control virgin females that were collected on the morning of the experiment. Flies were videotaped for 10 min and all courtship parameters were recorded. Courtship index was calculated as the percentage of courting over the total observation time. Courtship latency was calculated as the time until the first courtship element. For mating parameters, 30 4-day-old males of each genotype were group-mated with 2- to 4-day-old virgin CantonS females. Latency to copulation and total mating duration were measured and averaged for all the pairs.

Sleep and activity analysis. Sleep and activity data were analysed in trikinetics monitors (TriKinetics). Four-day-old males were loaded into glass tubes, with food on one end, sealed with wax to prevent desiccation of the food and a foam plug on the other end. Tubes were loaded into the monitors and recorded for 4 days using the DAMSystem data acquisition software (TriKinetics), which records how many times a fly crosses an infrared beam throughout the observation period. Rest/activity data were analysed in Excel[3]. Thirty-two males of each genotype were tested for 3 consecutive days after 1 baseline day. All flies were tested between 4 and 8 days of age.

Quantitative RT-PCR. Twenty larval brains were dissected from *Elav-Gal4; UAS-amon7B*, *Elav-Gal4/Y* and *UAS-amon7B/+* male larvae. The tissue was homogenized in 1 ml TriZol extraction buffer and RNA-extracted according to the manufacturer's instructions. Total RNA (500 ng–1 μg) was used to generate cDNA using SuperScript III First-Strand Synthesis Kit and Oligo dT primer. Quantitative RT–PCR (PCR with reverse transcription) was performed using PowerSYBR Green (Applied Biosystems) to amplify *amon* using the following primer sets Forward 5′-AAGAACACGGGTCAGAATGG-3′ and Reverse 5′-GGATACGGAAAGGGA TCGTT-3′. For normalization, *rp49* was also quantified using the following primers Forward 5′-ACTCAATGGATACTGCCCAAGA-3′ and Reverse 5′-CAAGGTGT CCCACTAATGCAT-3′. Three biological replicates for each sample were analysed. Results were obtained and quantified using Applied Biosystems 7900 HT machine and SDS 2.3 software.

Molecular cloning and transgenic strains. Fly *tll* cDNA was obtained from the BDGP (clone no. IP01133) and was digested with EcoRI and XhoI (NEB), creating a fragment containing the 5′-untranslated region (UTR), open reading frame and 3′-UTR. The open reading frame human *NR2E1* was PCR-amplified from a human brain cDNA library (kindly provided by J. Neul, Baylor College of Medicine). The fly *tll* cDNA and human PCR product were then cloned into pUAST-*attB* site (kindly provided by K. Venken, Baylor College of Medicine) using EcoRI and XhoI. Bimolecular complementation vectors were obtained from S. Bogdan (University of Munster, Berlin, Germany). Full-length *tll* was cloned in frame into the HA (haemagglutinin)-CYFP156-239 (ref. 53) vector as an EcoRI fragment deleting the stop codon of *tll*. The C-terminal 870-bp fragment of *Atro*[9] was cloned in frame downstream of the NYFP1-173-MYC[53] vector as an XhoI–XbaI fragment. Reciprocal constructs were also generated for *tll* and *Atro* but they yielded no interaction *in vitro* in S2 cells. All the constructs were verified by sequence analysis and subsequently injected into fly embryos containing an *attP* site on the second or third chromosome and a ϕC31 integrase on the first chromosome (kindly provided by K. Venken, Baylor College of Medicine). Transformants were crossed to a cantonized *w*; *Gla*/*CyO* (for second chromosome inserts) or *w*; *Sb*/*TM6, Tb* (for third chromosome inserts) stock to remove the ϕC31 integrase chromosome and to balance and homozygose the transgene.

Immunohistochemistry and western blotting. For immunohistochemistry, adult brains were dissected in ice-cold 4% paraformaldehyde–phosphate-buffered saline (PBS) and were further fixed for a total of 60 min. Next, the brains were rinsed quickly three times with PBS-0.5% Triton X-100 (PBT) and then washed three times for 20 min in PBT at room temperature. The brains were then blocked in 5% normal goat serum–PBT for 1 h at room temperature. Samples were incubated in 5% normal goat serum–PBT with primary antibody for two nights at 4 °C. After three 20-min washes with PBT, the brains were incubated in 5% normal goat serum–PBT with secondary antibody for two nights at 4 °C. The brains were then washed four times for 20 min and then overnight at 4 °C. Finally, brains were mounted in SlowFade mounting medium (Invitrogen) and covered with a no. 0 glass coverslip. The immunostained brains were imaged with an inverted Zeiss Confocal Microscope (Axiovert 100 M). The following primary antibodies were used for immunofluorescence: mouse anti-Dlg (1:100; Developmental Studies Hybridoma Bank); rabbit anti-GFP (1:100; Invitrogen) or mouse anti-GFP (1:200; NeuroMab); rabbit anti-Tll[42] (1:100; gift from J. Reinitz, University of Chicago); rabbit anti-human NR2E1 (SAB2101643, 1:100; Sigma); and rabbit anti-Atro[52] (1:2,000; gift from C.-C. Tsai, University of New Jersey Medical School). Mouse anti-HA (16B12, 1:100, Covance) and mouse anti-MYC (9E10, 1:10; Developmental Studies Hybridoma Bank). Alexafluor secondary antibodies were

obtained from Invitrogen and were used at a final concentration of 1:500. Images were analysed with AxioVision Software (Version 4.8.2.0, Zeiss) and further processed using Adobe Photoshop CS3 Extended (Version 10.01, Adobe). Western blotting was performed using standard protocols using rabbit anti-NR2E1 (1:1,000; Sigma), rabbit anti-Atro (1:5,000) and rabbit anti-Dlg (1:2,000; Millipore). Horseradish peroxidase-conjugated secondary antibodies were used at a concentration of 1:2,000 (Millipore). Full-length images of western blots are shown in Supplementary Information (Supplementary Figs 14,15).

Transfection and immunofluorescence of cultured S2 cells. *Drosophila* S2 cells (1×10^6 cells per well) were added to a six-well plate with a 22×22 mm glass coverslip in 2 ml of culture media. After 24 h of growth, cells were transfected with the *Act5C-GAL4* driver vector and the *tll-HA-CYFP* and *Atro-MYC-NYFP* bimolecular fluorescent complementation vectors. Two micrograms of each vector were resuspended in a 100-µl volume to which 3 µl of FuGENE HD Transfection Reagent (Promega) was added and incubated for 15 min at room temperature. The transfection complex was added to the cells with a gentle swirl and left to grow at room temperature. Forty-eight hours post transfection, the coverslips were moved to a new six-well plate for immunofluorescent staining. The cells were fixed in 1 ml of 4% formaldehyde in PBS for 20 min on a gentle shaker at room temperature. The cells were permeabilized and washed twice in 1 ml PBT (1 × PBS with 0.1% (w/v) BSA and 0.1% Triton) for 15 min. All washes were performed at room temperature on a gentle shaker. The cells were then blocked for 1 h at room temperature in 1 ml of PBT with 5% normal goat serum (NGS, Abcam) while shaking followed by incubation with primary antibody in PBT with 5% NGS for 1 h at room temperature by placing the coverslip (cell side down) on 40 µl antibody solution on parafilm. Cells were washed four times for 30 min in PBT. Subsequently, the cells were incubated with secondary antibody for 2 h using the same procedure as for the primary antibodies. Finally, the cells were washed three times for 20 min and mounted in 15 µl Slowfade Gold (Life Technologies) with 1 µl 4′,6-diamidino-2-phenylindole. The immunostained S2 cells were imaged with an Axioplan2 Zeiss microscope equipped with an ApoTome. Images were captured and analysed with AxioVision Software (Version 4.8.2.0, Zeiss) and further processed using Adobe Photoshop CS3 Extended (Version 10.01, Adobe).

Statistical analysis. Aggression data are typically not normally distributed and for these data medians were statistically compared using the non-parametric Kruskal–Wallis ANOVA for unpaired groups. We used the Mann–Whitney *U*-test for *post hoc* comparisons to identify those groups that differed to a statistically significant extent. Mating and courtship and sleep and activity data were analysed by ANOVA. Statistically significantly different groups were identified using Tukey–Kramer *post hoc* tests. Behavioural data are presented as bar graphs representing the means with s.e.m.

References

1. Corso, P. S., Mercy, J. A., Simon, T. R., Finkelstein, E. A. & Miller, T. R. Medical costs and productivity losses due to interpersonal violence and self-directed violence. *Am. J. Prev. Med.* **32,** 474–482 (2007).
2. Chen, S., Lee, A. Y., Bowens, N. M., Huber, R. & Kravitz, E. A. Fighting fruit flies: a model system for the study of aggression. *Proc. Natl Acad. Sci. USA* **99,** 5664–5668 (2002).
3. Dierick, H. A. & Greenspan, R. J. Molecular analysis of flies selected for aggressive behavior. *Nat. Genet.* **38,** 1023–1031 (2006).
4. Edwards, A. C., Rollmann, S. M., Morgan, T. J. & Mackay, T. F. Quantitative genomics of aggressive behavior in *Drosophila melanogaster*. *PLoS Genet.* **2,** e154 (2006).
5. Dierick, H. A. & Greenspan, R. J. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat. Genet.* **39,** 678–682 (2007).
6. Hoyer, S. C. *et al.* Octopamine in male aggression of *Drosophila*. *Curr. Biol.* **18,** 159–167 (2008).
7. Zhou, C., Rao, Y. & Rao, Y. A subset of octopaminergic neurons are important for Drosophila aggression. *Nat Neurosci.* **11,** 1059–1067 (2008).
8. Alekseyenko, O. V., Lee, C. & Kravitz, E. A. Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male *Drosophila melanogaster*. *PLoS One* **5,** e10806 (2010).
9. Edwards, A. C., Zwarts, L., Yamamoto, A., Callaerts, P. & Mackay, T. F. Mutations in many genes affect aggressive behavior in *Drosophila melanogaster*. *BMC Biol.* **7,** 29 (2009).
10. Wang, L. & Anderson, D. J. Identification of an aggression-promoting pheromone and its receptor neurons in *Drosophila*. *Nature* **463,** 227–231 (2010).
11. Wang, L. *et al.* Hierarchical chemosensory regulation of male-male social interactions in *Drosophila*. *Nat. Neurosci.* **14,** 757–762 (2011).
12. Liu, W. *et al.* Social regulation of aggression by pheromonal activation of Or65a olfactory neurons in *Drosophila*. *Nat. Neurosci.* **14,** 896–902 (2011).
13. Kravitz, E. A. & Huber, R. Aggression in invertebrates. *Curr. Opin. Neurobiol.* **13,** 736–743 (2003).
14. Popova, N. From genes to aggressive behavior: the role of the serotonergic system. *Bioessays* **28,** 495–503 (2006).
15. Gehring, W. J. & Ikeo, K. Pax 6: mastering eye morphogenesis and eye evolution. *Trends Genet.* **15,** 371–377 (1999).
16. Hall, J. C. Molecular neurogenetics of biological rhythms. *J. Neurogenet.* **12,** 115–181 (1998).
17. Konopka, R. J. & Benzer, S. Clock mutants of *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **68,** 2112–2116 (1971).
18. Rosato, E. & Kyriacou, C. P. Flies, clocks and evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **356,** 1769–1778 (2001).
19. Monaghan, A. P. *et al.* Defective limbic system in mice lacking the tailless gene. *Nature* **390,** 515–517 (1997).
20. King-Jones, K. & Thummel, C. S. Nuclear receptors--a perspective from *Drosophila*. *Nat. Rev. Genet.* **6,** 311–323 (2005).
21. Gui, H., Li, M. L. & Tsai, C. C. A tale of tailless. *Dev. Neurosci.* **33,** 1–13 (2011).
22. Strecker, T. R., Merriam, J. R. & Lengyel, J. A. Graded requirement for the zygotic terminal gene, tailless, in the brain and tail region of the *Drosophila* embryo. *Development* **102,** 721–734 (1988).
23. Pignoni, F. *et al.* The *Drosophila* gene tailless is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell* **62,** 151–163 (1990).
24. Yu, R. T., McKeown, M., Evans, R. M. & Umesono, K. Relationship between *Drosophila* gap gene tailless and a vertebrate nuclear receptor Tlx. *Nature* **370,** 375–379 (1994).
25. Wang, L. & Tsai, C. C. Atrophin proteins: an overview of a new class of nuclear receptor corepressors. *Nucl. Recept. Signal.* **6,** e009 (2008).
26. Yu, R. T. *et al.* The orphan nuclear receptor Tlx regulates Pax2 and is essential for vision. *Proc. Natl Acad. Sci. USA* **97,** 2621–2625 (2000).
27. Young, K. A. *et al.* Fierce: a new mouse deletion of Nr2e1; violent behaviour and ocular abnormalities are background-dependent. *Behav. Brain Res.* **132,** 145–158 (2002).
28. Roy, K., Thiels, E. & Monaghan, A. P. Loss of the tailless gene affects forebrain development and emotional behavior. *Physiol. Behav.* **77,** 595–600 (2002).
29. Abrahams, B. S. *et al.* Pathological aggression in 'fierce' mice corrected by human nuclear receptor 2E1. *J. Neurosci.* **25,** 6263–6270 (2005).
30. Kumar, R. A. *et al.* Initial association of NR2E1 with bipolar disorder and identification of candidate mutations in bipolar disorder, schizophrenia, and aggression through resequencing. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **147B,** 880–889 (2008).
31. Hartenstein, V. The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. *J. Endocrinol.* **190,** 555–570 (2006).
32. Nitabach, M. N. *et al.* Electrical hyperexcitation of lateral ventral pacemaker neurons desynchronizes downstream circadian oscillators in the fly circadian circuit and induces multiple behavioral periods. *J. Neurosci.* **26,** 479–489 (2006).
33. Kruk, M. R. *et al.* Comparison of aggressive behaviour induced by electrical stimulation in the hypothalamus of male and female rats. *Prog. Brain Res.* **61,** 303–314 (1984).
34. Gregg, T. R. & Siegel, A. (2001Brain structures and neurotransmitters regulating aggression in cats: implications for human aggression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **25,** 91–140 (2001).
35. Lipp, H. P. & Hunsperger, R. W. Threat, attack and flight elicited by electrical stimulation of the ventromedial hypothalamus of the marmoset monkey *Callithrix jacchus*. *Brain Behav. Evol.* **15,** 260–293 (1978).
36. Finley, K. D. *et al.* Dissatisfaction encodes a tailless-like nuclear receptor expressed in a subset of CNS neurons controlling *Drosophila* sexual behavior. *Neuron* **21,** 1363–1374 (1998).
37. Dierick, H. A. A method for quantifying aggression in male *Drosophila melanogaster*. *Nat. Protoc.* **2,** 2712–2718 (2007).
38. Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118,** 401–415 (1993).
39. Ni, J. Q. *et al.* A *Drosophila* resource of transgenic RNAi lines for neurogenetics. *Genetics* **182,** 1089–1100 (2009).
40. Lin, D. M. & Goodman, C. S. Ectopic and increased expression of Fasciclin II alters motoneuron growth cone guidance. *Neuron* **13,** 507–523 (1994).
41. McGuire, S. E., Mao, Z. & Davis, R. L. Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in *Drosophila*. *Sci. STKE.* **220,** l6–25 (2004).
42. Kosman, D., Small, S. & Reinitz, J. Rapid preparation of a panel of polyclonal antibodies to *Drosophila* segmentation proteins. *Dev. Genes Evol.* **208,** 290–294 (1998).
43. Siegel, A., Roeling, T. A., Gregg, T. R. & Kruk, M. R. Neuropharmacology of brain-stimulation-evoked aggression. *Neurosci. Biobehav. Rev.* **23,** 359–389 (1999).
44. Caldwell, H. K., Lee, H. J., Macbeth, A. H. & Young, 3rd W. S. Vasopressin: behavioral roles of an 'original' neuropeptide. *Prog. Neurobiol.* **84,** 1–24 (2008).

45. Hewes, R. S., Gu, T., Brewster, J. A., Qu, C. & Zhao, T. Regulation of secretory protein expression in mature cells by DIMM, a basic helix-loop-helix neuroendocrine differentiation factor. *J. Neurosci.* **26,** 7860–7869 (2006).
46. Foltenyi, K., Greenspan, R. J. & Newport, J. W. Activation of EGFR and ERK by rhomboid signaling regulates the consolidation and maintenance of sleep in *Drosophila*. *Nat. Neurosci.* **10,** 1160–1167 (2007).
47. Rulifson, E. J., Kim, S. K. & Nusse, R. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* **296,** 1118–1120 (2002).
48. Clyne, J. D. & Miesenböck, G. Sex-specific control and tuning of the pattern generator for courtship song in *Drosophila*. *Cell* **133,** 354–363 (2008).
49. Xiang, Y. *et al.* Light-avoidance-mediating photoreceptors tile the *Drosophila* body wall. *Nature* **16,** 921–926 (2010).
50. Lin, S., Huang, Y. & Lee, T. Nuclear receptor unfulfilled regulates axonal guidance and cell identity of *Drosophila* mushroom body neurons. *PLoS One* **4,** e8392 (2009).
51. Haecker, A. *et al. Drosophila* brakeless interacts with atrophin and is required for tailless-mediated transcriptional repression in early embryos. *PLoS Biol.* **5,** e145 (2007).
52. Wang, L., Charroux, B., Kerridge, S. & Tsai, C. C. Atrophin recruits HDAC1/2 and G9a to modify histone H3K9 and to determine cell fates. *EMBO Rep.* **9,** 555–562 (2008).
53. Gohl, C., Banovic, D., Grevelhörster, A. & Bogdan, S. WAVE forms hetero- and homo-oligomeric complexes at integrin junctions in *Drosophila* visualized by bimolecular fluorescence complementation. *J. Biol. Chem.* **285,** 40171–40179 (2010).
54. Wang, L., Rajan, H., Pitman, J. L., McKeown, M. & Tsai, C. C. Histone deacetylase-associating Atrophin proteins are nuclear receptor corepressors. *Genes Dev.* **20,** 525–530 (2006).
55. Lin, D. *et al.* Functional identification of an aggression locus in the mouse hypothalamus. *Nature* **470,** 221–226 (2011).
56. Rajan, A. & Perrimon, N. *Drosophila* cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* **151,** 123–137 (2012).
57. Rhea, J. M., Wegener, C. & Bender, M. The proprotein convertase encoded by amontillado (amon) is required in *Drosophila* corpora cardiaca endocrine cells producing the glucose regulatory hormone AKH. *PLoS Genet.* **6,** e1000967 (2010).
58. Renden, R. *et al.* Drosophila CAPS is an essential gene that regulates dense-core vesicle release and synaptic vesicle fusion. *Neuron* **31,** 421–437 (2001).
59. Rao, S., Lang, C., Levitan, E. S. & Deitcher, D. L. Visualization of neuropeptide expression, transport, and exocytosis in *Drosophila melanogaster*. *J. Neurobiol.* **49,** 159–172 (2001).
60. Shen, Y., Lee, G., Choe, Y., Zoltewicz, J. S. & Peterson, A. S. Functional architecture of atrophins. *J. Biol. Chem.* **282,** 5037–5044 (2007).
61. Zoltewicz, J. S., Stewart, N. J., Leung, R. & Peterson, A. S. Atrophin 2 recruits histone deacetylase and is required for the function of multiple signaling centers during mouse embryogenesis. *Development* **131,** 3–14 (2004).
62. Nagafuchi, S. *et al.* Structure and expression of the gene responsible for the triplet repeat disorder, dentatorubral and pallidoluysian atrophy (DRPLA). *Nat. Genet.* **8,** 177–182 (1994).
63. Koide, R. *et al.* Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat. Genet.* **6,** 9–13 (1994).
64. Adachi, N. *et al.* Dentatorubral-pallidoluysian atrophy (DRPLA) presenting with psychosis. *J. Neuropsychiatry Clin. Neurosci.* **13,** 258–260 (2001).
65. Licht, D. J. & Lynch, D. R. Juvenile dentatorubral-pallidoluysian atrophy: new clinical features. *Pediatr. Neurol.* **26,** 51–54 (2002).
66. Schilling, G. *et al.* Distinct behavioral and neuropathological abnormalities in transgenic mouse models of HD and DRPLA. *Neurobiol. Dis.* **8,** 405–418 (2001).
67. Yanagisawa, H. *et al.* Protein binding of a DRPLA family through arginine-glutamic acid dipeptide repeats is enhanced by extended polyglutamine. *Hum. Mol. Genet.* **9,** 1433–1442 (2000).
68. Scharrer, B. & Scharrer, E. Neurosecretion VI. A comparison between the intercerebralis-cardiacum-allatum system of the insects and hypothalamo-hypophyseal system of the vertebrates. *Biol. Bull.* **87,** 242–251 (1944).
69. Caers, J. *et al.* More than two decades of research on insect neuropeptide GPCRs: an overview. *Front. Endocrinol.* **3,** 151 (2012).
70. Hartmann, B., Reichert, H. & Walldorf, U. Interaction of gap genes in the *Drosophila* head: tailless regulates expression of empty spiracles in early embryoinic patterning and brain development. *Mech. Dev.* **109,** 161–172 (2001).

Acknowledgements

We thank the Bloomington Stock Center, the Berkeley Drosophila Genome Project, the Developmental Studies Hybridoma Bank and H. Bellen, S. Bogdan, L. and Y.-N. Jan, J. Merriam, G. Miesenbock, J. Neul, S. Pletcher, J. Reinitz, G. Roman, P. Shaw and C.-C. Tsai for fly strains, plasmids, cDNA and/or antibodies. We thank H. Jafar-Nejad and T. Lee for help with tissue culture. Harvard Medical School provided all TriP stocks. We also thank H. Bellen, S. Cunha, N. Haelterman, C. Herman, S. Rosenberg, K. Venken, H. Zoghbi and members of the Dierick lab for critical comments on the manuscript. S.M.D. is supported by the Burroughs Wellcome Fund—the Houston Laboratory and Population Science Training Program in Gene–Environment Interaction of the University of Texas Health Science Center at Houston (BWF Grant No. 1008200). This work was funded by the Naman Family Fund for Junior Faculty at BCM and by a grant from the National Science Foundation (IOS-1119473) to H.A.D.

Author contributions

S.M.D., A.L.T., K.J.N. and H.A.D. designed the study. S.M.D., A.L.T., K.J.N. and L.H. performed the experiments and analysed the data. S.M.D., A.L.T., K.J.N. and H.A.D. wrote the paper.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Davis, S. M. *et al.* Tailless and Atrophin control *Drosophila* aggression by regulating neuropeptide signalling in the *pars intercerebralis*. *Nat. Commun.* 5:3177 doi: 10.1038/ncomms4177 (2014).