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RESEARCH ARTICLE

Insulin signalling in mushroom body neurons regulates feeding behaviour in *Drosophila* larvae

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SUMMARY

Whereas the pivotal role of insulin signalling in cell division, growth and differentiation is well documented, its role in the regulation of neuronal function and behaviour has recently become the focus of intense investigation. The simple organization of the *Drosophila* larval brain and the availability of genetic tools to impair the function of insulin receptor signalling in a spatially specific manner makes *Drosophila* an attractive model to investigate the role of the insulin pathway in specific behaviours. Here, we show that impairment of insulin signalling in the mushroom body neurons, a structure involved in associative learning, impairs feeding behaviour in the *Drosophila* larva.

Key words: *Drosophila*, feeding, insulin, growth, fat body.

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INTRODUCTION

The fruit fly *Drosophila*, like all other organisms, continually adjusts food intake according to nutrient availability and metabolism. Key peptides with counterparts in mammalian models involved in the regulation of feeding have been identified in *Drosophila*, further validating the use of this model organism (reviewed by Nässel and Winther, 2010).

Foraging third instar larvae carrying mutations in the Ran-binding protein M gene (*RanBPM*) are smaller than wild-type controls at the same developmental stage, display a marked reduction in the number of mitotically active cells in the central nervous system, feed less and do not display the food-seeking behaviour characteristic of this stage. *RanBPM* is highly expressed in the cytoplasm of the mushroom body neurons and is also expressed to a lesser degree in a large number of central nervous system neurons (Scantlebury et al., 2010). Targeted expression of *RanBPM* in the mushroom body rescues the feeding behaviour and growth phenotypes of *RanBPM* mutant larvae. RanBPM has been implicated in various signalling pathways as a putative scaffolding protein (e.g. Bai et al., 2003; Mikolajczyk et al., 2003; Menon et al., 2004; Brunkhorst et al., 2005; Hafizi et al., 2005; Haase et al., 2008; Atabakhsh et al., 2009; Gong et al., 2009; Kim et al., 2009; Chang et al., 2010) (reviewed by Murrin and Talbot, 2007). *RanBPM* expression in PC12 cells promotes TrkB-dependent activation of the serine–threonine kinase Akt, a target of insulin signalling (Yin et al., 2010).

Drosophila has a single insulin receptor gene (*InR*) that mediates the function of seven insulin-like peptides (reviewed by Teleman, 2010). Ablation of insulin-producing cells generates small ‘diabetic’ flies with an elevated level of haemolymph sugar (trehalose) (Ikeya et al., 2002; Rulifson et al., 2002), recapitulating the consequence of less severe mutations in the *InR* gene. It has served as a model to study the function of insulin signalling in a variety of processes (e.g. Broughton et al., 2005; Belgacem and Martin, 2002; Belgacem and Martin, 2006; Corl et al., 2005) (reviewed by Teleman, 2010). Increased levels of insulin suppress starvation-dependent feeding behaviour (Wu et al., 2005a). Ubiquitous activation of *InR* causes larvae to feed less and to wander precociously off the food, eventually dying of starvation (Britton et al., 2002). In *Drosophila*, insulin signalling is also involved in the nutrition-dependent regulation of neural stem cell proliferation. Humoral signals secreted by the fat body, and elicited by amino acid intake, regulate feeding and trigger neuroblast proliferation *via* insulin synthesis by glia (Zinke et al., 1999; Britton and Edgar, 1998; Chell and Brand, 2010; Sousa-Nunes et al., 2011). The role of insulin in synaptic plasticity and cognitive function is well established in humans and in animal models (e.g. Dou et al., 2005; Chiu et al., 2008; Oda et al., 2011) (reviewed by Chiu and Cline, 2010; Bosco et al., 2011; Kapogiannis and Mattson, 2011). In *Drosophila*, targeted inhibition of phosphatidylinositol 3-kinase (PI3K) was recently used to reduce synapse number to model the sensory perception decline characteristic of ageing and neurodegenerative diseases (Acebes et al., 2011). Combined, these observations prompted us to ask whether mushroom body function is required for food intake in the *Drosophila* foraging larva and whether these neurons are a target of insulin signalling in the context of this behaviour.

MATERIALS AND METHODS

Drosophila strains and culture

Synchronized early third instar foraging larvae were obtained as described previously (Rodriguez Moncalvo and Campos, 2009) and grown at 25°C unless otherwise stated, on a normal light–dark cycle. Standard genetic crosses were used to target the expression of constitutively active (CA) and/or dominant negative (DN) forms of various components of insulin signalling and the temperature-sensitive form of Dynamin (*shibire*, *shi*) to the intrinsic neurons of the larval mushroom body using the GAL4 drivers *247-GAL4* (Zars et al., 2000) and *201y-GAL4* (Connolly et al., 1996). The target genes (UAS constructs) used were *UAS-InR.A1325D* (CA) [Bloomington stock center (BSC) no. 8263], *UAS InR.K1409A* (DN) (BSC no. 8253), *UAS-PI3K-dp110* (DN), *UAS-FOXO* (kindly

donated by Brian Staveley) (Kramer et al., 2003; Leevers et al., 1996), *UAS-Akt1* (serine/threonine kinase Akt) (BSC no. 8192) and *UAS-shi^{ts}* (Kitamoto et al., 2001).

Food intake assay

The food intake assay was as described elsewhere (Iijima et al., 2009) for adult flies with some modifications. Batches of 60–70 newly hatched larvae (0–60 min) were transferred to regular food plates that were scored to facilitate the burrowing of the larvae (30×13 mm plates, Fisher Scientific, Ottawa, ON, Canada) and kept at 29°C for 51 h. Sixty larvae were recovered from the food plate, washed in distilled water and transferred onto a damp 3M filter paper placed in a Petri dish (humid chamber) where they were starved for 2.5 h. At the end of this period the larvae were transferred to a drop of freshly prepared yeast paste (5 g in 15 ml) containing 3% FD&C Blue No.1 (Brilliant Blue; McCormick, Hunt Valley, MD, USA) and left for 1.5 h. The control constituted larvae of the same genotype and age, grown in the same conditions and fed yeast paste with no dye. At the end of this period, larvae were washed several times to eliminate all yeast and transferred to a 1.5 ml Eppendorf tube, homogenized and centrifuged twice (15 min at 13,000 ***g***). The supernatants were transferred to cuvettes and the absorbance was measured at 625 nm using extracts from the control larvae as a blank. All larval cultures were kept at 29°C, the optimum temperature for the function of the yeast GAL4 transcription factor. All assays were conducted at this temperature except when the temperature-sensitive allele of *shibire* (*shi^{ts}*) was used. In this case, larvae were grown at the permissive temperature for this allele (25°C) then transferred to the restrictive temperature (31°C) for the assay; controls were kept at the permissive temperature throughout growth and assay. For all genotypes a minimum of three independent experiments with 60 larvae each were conducted.

Statistical analyses

All statistical tests were conducted using the MiniTab software package (student release version 14.11.1). The statistical tests included one-way analysis of variance (ANOVA) and Tukey's pairwise comparisons. Normality tests on the residuals of the ANOVA were conducted using the Anderson–Darling test. The level of significance in all tests was $\alpha<0.05$. All measurements are shown as mean values and s.e.m.

Immunohistochemistry and imaging

The central nervous system was dissected from early foraging third instar larvae staged as above and treated as described previously (Hassan et al., 2005). The primary antibody used was rabbit anti-phospho-Histone H3 (1:1000 dilution; catalogue no. 06-570, Millipore, Billerica, MA, USA). The secondary antibody used was a Texas Red-conjugated goat anti-rabbit IgG (1:200). The specimens were viewed under a Leica confocal microscope SP5II. Brightness and contrast were adjusted using Adobe Photoshop.

RESULTS

Reduced insulin signalling in mushroom body neurons impairs larval food intake and growth

Nutrient intake during larval development regulates the expression and secretion of a subset of the *Drosophila* insulin-like peptides (Géminard et al., 2009). Insulin signalling regulates starvation-dependent food intake behaviour in the *Drosophila* larva (Britton et al., 2002; Wu et al., 2005a; Wu et al., 2005b). Our previous work indicated that the mushroom body plays a role in larval feeding behaviour (Scantlebury et al., 2010). Therefore, we asked whether proper insulin signalling is required in mushroom body neurons for feeding behaviour during larval development. To this end, we employed *Drosophila* strains carrying previously generated GAL4-inducible components of insulin signalling to inhibit or to activate this pathway in mushroom body neurons. To direct the expression of these constructs to the larval mushroom body neurons, we used two GAL4 lines, *201y-GAL4* and *247-GAL4*, identified as having the broadest expression in this structure (Pauls et al., 2010). The expression of *201y-GAL4* and *247-GAL4* is said to be mushroom body specific (Pauls et al., 2010) and as such they have been used in a number of studies addressing the function of this structure in *Drosophila* larval and adult behaviour (Honjo and Furukubo-Tokunaga, 2005; Joiner et al., 2006; Hong et al., 2008; Brembs, 2009; Honjo and Furukubo-Tokunaga, 2009; Pauls et al., 2010; Acebes et al., 2011). However, these constructs also label a small number of other neurons in the larval brain and in the case of *247-GAL4*, expression in the ventral cord glia has been detected (Pauls et al., 2010). Nevertheless, the finding that the non-mushroom body expression does not overlap between these lines indicates that they can be used as effective tools to probe the role of mushroom body neurons.

Up-regulation of insulin signalling was achieved by targeted expression of transgenic constructs carrying a CA form of the *Drosophila* insulin receptor [*UAS-InR.A1325D(CA)*] or the downstream insulin target gene *Akt* [*UAS-Akt1*]. Insulin signalling inhibition was attained by expression of a DN form of InR [*UAS-InR.K109A(DN)*] and a DN form of PI3K co-expressed with the inhibitory transcription factor dFOXO [*UAS-pI3K(DN);UAS-FOXO*]. Synchronized third instar foraging larvae were harvested, starved for 2.5 h and allowed to feed on yeast laced with blue dye for 1.5 h. The amount of food ingested was measured by the absorbance of larval extracts at 625 nm.

We found that targeted over-expression of the downstream insulin signalling target *Akt* or of the CA form of the *InR* using either GAL4 driver had no effect in larval feeding or growth [Fig. 1A; *247:UAS-InR.A1325D(CA)*, *247:UAS-Akt1*, *201y:UAS-InR.A1325D(CA)*, *201y:UAS-Akt1*]. In contrast, targeted inhibition of insulin signalling under the regulation of either the 247 or the 201y GAL4 drivers caused a significant reduction in food intake as determined by this assay [Fig. 1A, *247:UAS-InR.K109A(DN)*, *247:UAS-PI3K-dp110(DN);UAS-FOXO*, *201y:UAS-InR.K(DN)*, *201y:UAS-PI3K(DN);UAS-FOXO*].

Larvae carrying the *247:UAS-InR.K1409A(DN)* or *201y:UAS-InR.K1409A(DN)* constructs were generally smaller but the impact on growth was not uniform in the population (Fig. 2A,C). In contrast, larvae carrying *247:UAS-PI3K-dp110(DN);UAS-FOXO* or *201y:UAS-PI3K-dp110(DN);UAS-FOXO* constructs were uniformly smaller than age-matched parental controls as indicated by representative photomicrographs in Fig. 2B,D. Developmental timing was not affected by any of these genotypes as the synchronized cultures reached the third instar stage at the expected time, as seen by the characteristic morphology of the mouth hooks and the presence of everted spiracles. These observations suggested that the food intake estimated from absorbance of larval extracts could be confounded by the reduced size of the larvae. Therefore, in all experiments, prior to obtaining the larval extracts needed to measure the presence of blue dye in the gut, we determined the fraction of larvae that were completely white, i.e. did not ingest any food during the assay. Indeed, in larvae of the genotype *247:UAS-InR.K1409A(DN)* or *201y:UAS-InR.K1409A(DN)* and *247:UAS-PI3K-dp110(DN);UAS-FOXO* or *201y:UAS-PI3K-dp110(DN);UAS-FOXO*, the fraction that did not ingest any food

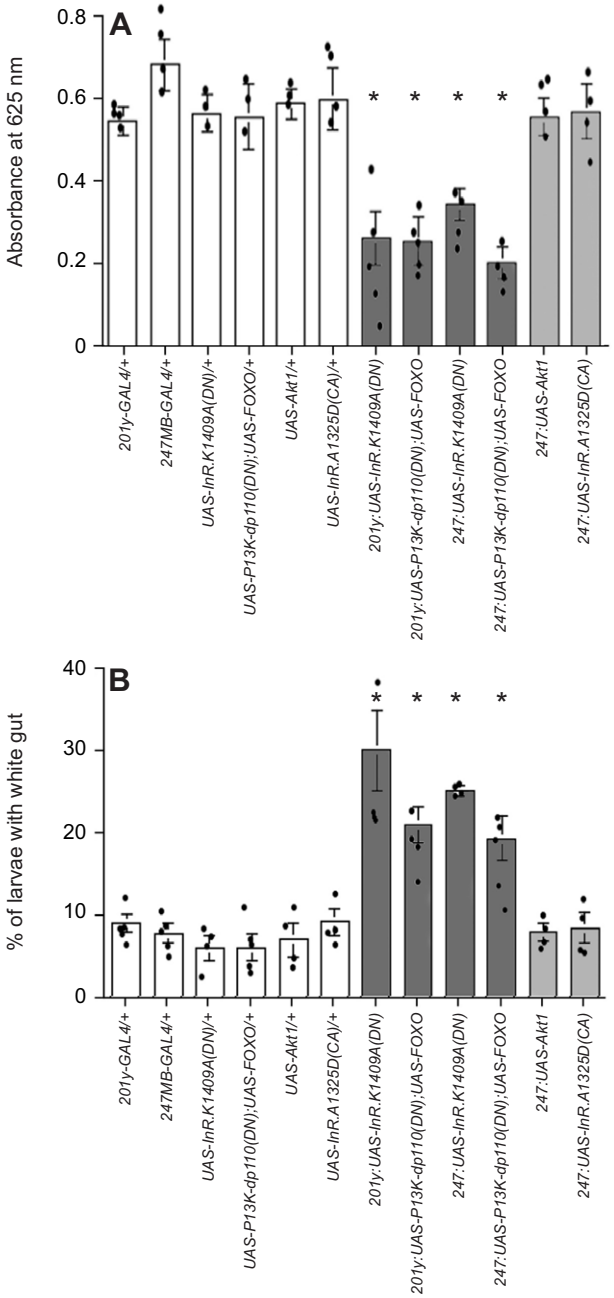

Fig. 1. Inhibition of insulin signalling in the mushroom body neurons reduces food intake. Inhibition of insulin signalling in the mushroom body neurons was achieved by co-expression of a dominant negative (DN) form of PI3K and of the inhibitory transcription factor FOXO (*UAS-PI3K-dp110;UAS-FOXO*) or by expression of a DN form of the insulin receptor (InR) [*InR.K1409A(DN)*]. Up-regulation of insulin signalling in the mushroom body neurons was attempted by expression of the constitutively active (CA) form of the InR [*InR.A1325D(CA)*] and by expression of the insulin target serine threonine kinase *Akt1* (*247:UAS-Akt1* and *201y:UAS-Akt1*). Two different GAL4 drivers broadly expressed in the mushroom body neurons were used to target the expression of these constructs (*247-GAL4* and *201y-GAL4*). (A) Food intake measured as absorbance of larval extracts. (B) Percentage of larvae devoid of any visible blue matter in the gut. For clarity in A and B, individual data points are superimposed onto the bar plots. Dark grey filled bars correspond to inhibition of insulin signalling while light grey bars correspond to activation. All measurements are shown as mean values ± s.e.m., $N \geq 3$. A: ANOVA $F_{11,41}=27.16$, $P<0.001$; B: ANOVA $F_{11,41}=16.39$, $P<0.001$. Asterisks indicate genotypes significantly different from both parental controls.

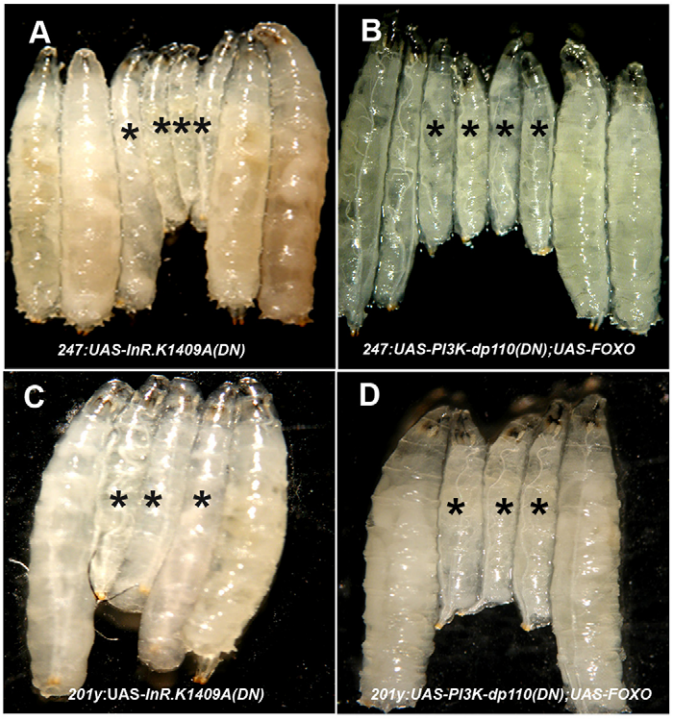

Fig. 2. Inhibition of insulin signalling in the mushroom body neurons impairs growth. Micrographs of representative third instar foraging larvae in which insulin signalling was inhibited (asterisks). The flanking larvae represent parental controls carrying one copy of GAL4 driver only (left) or one copy of the UAS construct (right). Inhibition of insulin signalling was achieved (A,C) by expression of a DN form of the InR [*247:UAS-InR.K1409A(DN)* and *201y:UAS-InR.K1409A(DN)*, respectively] and (B,D) by co-expression of a DN form of PI3K and the inhibitory transcription factor FOXO [*247:UAS-PI3K-dp110(DN);UAS-FOXO* and *201y:UAS-PI3K-dp110(DN);UAS-FOXO*]. The larvae were uniformly smaller than both parental controls when *PI3K-dp110 and FOXO* were expressed in the mushroom body (B,D) but not so when *InR.K1409A* was expressed (A,C).

during the assay was significantly and markedly higher than that of parental controls (Fig. 1B). We therefore conclude that inhibition of insulin signalling as directed by the *247*- and *201y-GAL4* constructs inhibits food intake.

Reduced insulin signalling in mushroom body neurons impairs neuroblast proliferation

In *Drosophila*, the re-entry of quiescent neuroblasts into the cell cycle at the beginning of post-embryonic development is controlled by food intake *via* a fat body-derived humoral signal, which in turn triggers insulin secretion by the glia (Britton and Edgar, 1998; Chell and Brand, 2010; Sousa-Nunes et al., 2011) (reviewed by Spéder et al., 2011). Therefore, we asked whether larvae in which insulin signalling was inhibited in the mushroom body also displayed reduced proliferation in the central nervous system as an indirect consequence of reduced food intake. Mitotically active cells were identified by immunolabelling with an antibody that recognizes the phospho-specific isoform of Histone H3 (phospho-H3) found in metaphasic chromosomes (e.g. Karcavich and Doe, 2005). We found that larvae in which insulin signalling was blocked in the mushroom body displayed an overall reduction in cell proliferation, as seen by the marked reduction in the overall level of phospho-H3 immunolabelling in the central nervous system (Fig. 3).

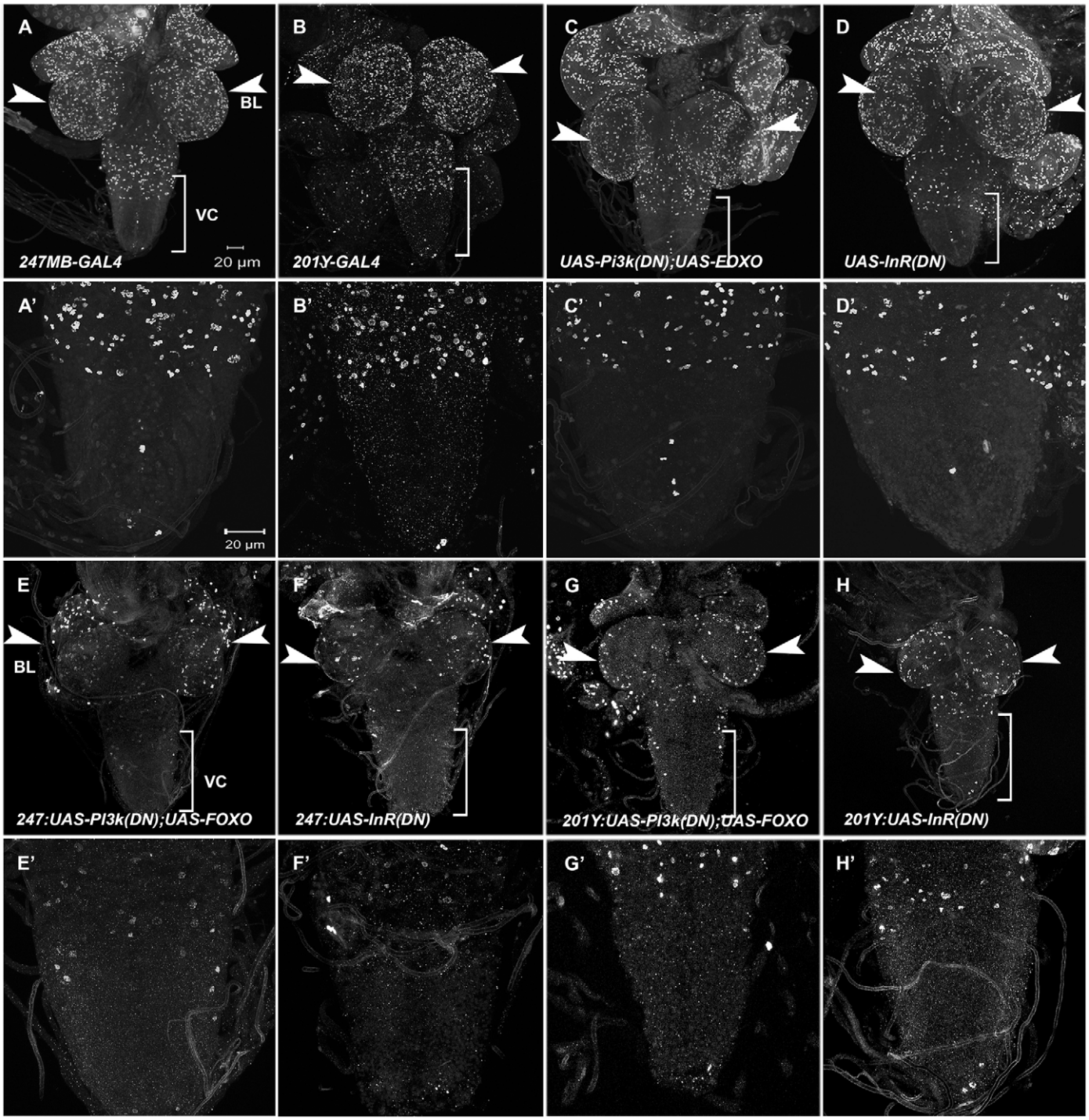

Fig. 3. Disruption of insulin signalling in the mushroom body neurons reduces neural proliferation. Confocal micrographs of larval brains immunolabelled with anti-phospho-Histone H3. Images shown are projected *Z*-stacks of 14–20 sections at 2 μm intervals (A–H) or 11–17 sections at 1 μm intervals (A′–H′). A′–H′ depict magnified views of the ventral cord indicated in the panels above by a bracket. Arrowheads point to the brain hemispheres. Insulin signalling inhibition in mushroom body neurons using either GAL driver causes an overall reduction in proliferation (compare E–H with parental controls A–D).

Reduced activity in mushroom body neurons disrupts larval feeding behaviour

Next, we addressed directly the requirement of mushroom body function for larval feeding during the early third instar stage. To this end we used, as described above, the GAL4/UAS system to suppress neuronal function (reviewed by White et al., 2001). Larvae expressing a dominant temperature-sensitive form of the Dynamin encoded by the *Drosophila shibire* gene (*UAS-shi*^{ts}) (Kitamoto, 2001) in the intrinsic mushroom body neurons were generated using standard genetic crosses and used in feeding assays as before. The advantage of using a dominant temperature-sensitive allele of the *shi* gene is that it allows the suppression of synaptic output on demand as the organism is exposed to the restrictive temperature. Therefore, in the experiments using *UAS-shi*^{t}, larvae were grown

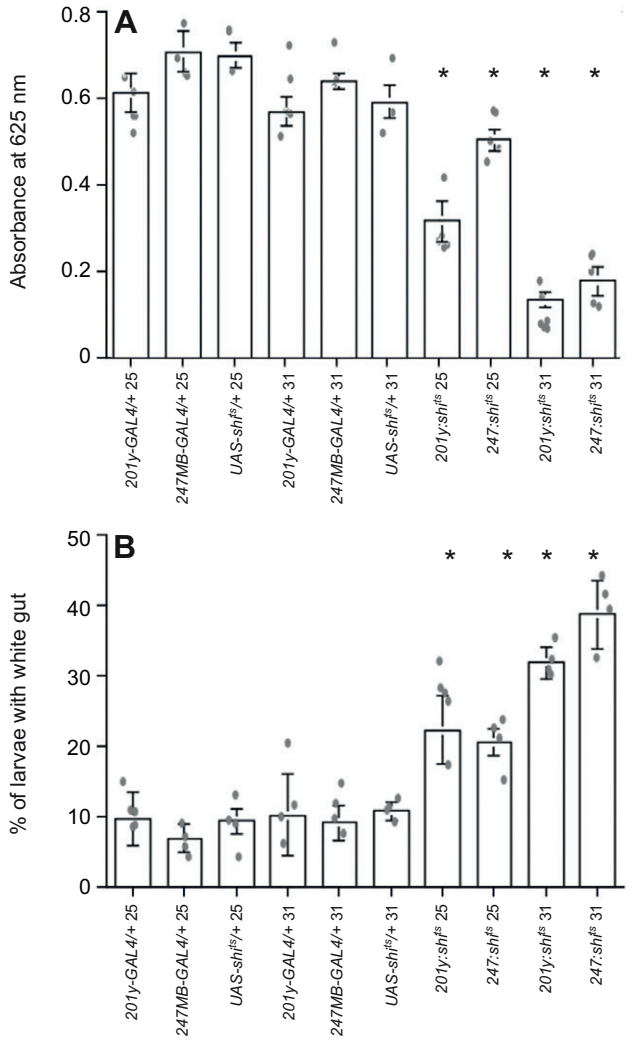

Fig. 4. Suppression of mushroom body neuronal function reduces food intake. Suppression of synaptic transmission was carried out by targeted expression of a temperature-sensitive allele of *shi* (*shi*^{ts}) using two GAL4 drivers that are broadly expressed in the mushroom body neurons (*247-GAL4* and *201y-GAL4*). All larval cultures were kept at 25°C (permissive temperature) during development until the time of the assay, at which point some were shifted to the restrictive temperature (25°C). (A) Food intake measured as absorbance of larval extracts. (B) Percentage of larvae devoid of any visible blue matter in the gut. For clarity, in both graphs individual data points are superimposed onto the bar plots. All measurements are shown as means ± s.e.m., $N \geq 4$. Asterisks indicate genotypes significantly different from both parental controls. A: ANOVA $F_{9,33}=61.73$, $P<0.001$; B: ANOVA $F_{9,31}=34.83$, $P<0.001$.

at 25°C (permissive temperature) and then kept at 31°C (restrictive temperature) during the assay, thereby bypassing the potential developmental effects of reduced food intake.

Expression of *shi*^{ts} under the regulation of either of the GAL4 drivers at the permissive temperature caused a small but significant reduction of food intake relative to that of parental controls (Fig. 4A; *247:shi*^{ts} and *201y:shi*^{ts} compared with *201y-GAL4* or *247-GAL4* and *UAS-shi*^{ts} at 25°C). This reduction was much more extreme when the assays were conducted at the restrictive temperature for the *shi*^{ts} allele (Fig. 4; *247:shi*^{ts} and *201y:shi*^{ts} compared with *201y-GAL4* or *247-GAL4* and *UAS-shi*^{ts} at 31°C). We did not detect any disruption in the growth of either *247:shi*^{ts} or *201y:shi*^{ts} larvae. Moreover, the fraction of *247:shi*^{ts} and *201y:shi*^{ts} larvae that did not ingest food in assays conducted at the restrictive temperature, as seen by the absence of blue in the gut, was significantly higher than in wild-type controls (Fig. 4B). Taken together, these observations indicate that the function of mushroom body neurons is required for feeding during foraging in the third instar stage and possibly for the early food intake that occurs soon after the larva hatches.

DISCUSSION

The impact of insulin signalling on neuronal function and circuit development is the subject of intense investigation (reviewed by Chiu and Cline, 2010). In *Drosophila*, insulin signalling has been implicated in ethanol sensitivity (Corl et al., 2005), sexually dimorphic locomotor activity (Belgacem and Martin, 2002; Belgacem and Martin, 2006) and age-related locomotor impairment (Jones et al., 2009), and in a *Drosophila* model of β-amyloid-induced neurotoxicity (Chiang et al., 2010).

Here, we report that inhibition but not activation of insulin signalling in mushroom body neurons throughout larval development disrupts starvation-induced food intake and growth of foraging third instar larvae. Suppression of mushroom body synaptic transmission restricted to the time of the assay by targeted expression of the temperature-sensitive allele of *shi* mimics in part the effect of insulin signalling inhibition. The inability to increase food intake upon activation of insulin signalling in mushroom body neurons may be explained by a failure to reach the threshold required to see such an effect.

The experiments described here do not address whether inhibition of insulin signalling disrupts the development and/or maintenance of mushroom body neurons. Targeted expression of a DN form of the *PI3K* gene under the regulation of *247-* and *201y-GAL4* has been used in *Drosophila* adults to induce synapse loss in the mushroom body in order to evaluate the role of this structure in olfactory perception (Acebes et al., 2011). These authors report that while there is no reduction in mushroom body cell number or an effect on odorant perception in *247-* or *201y-GAL4;UAS-PI3K* (DN) flies, clear morphological defects were detected in their pedunculus and calyx neurites. We did not detect overt disruption in the morphology of the mushroom body in larvae in which insulin signalling was inhibited by targeted expression of *PI3K(DN)* and *FOXO* using the same GAL4 drivers as Acebes and colleagues (Acebes et al., 2011) and as evaluated by the expression of membrane-bound GFP (data not shown). The *247-* and *201y-GAL4* are expressed in both embryonic and larval-born mushroom body neurons that persist to adulthood (Pauls et al., 2010). Which type of neurons, embryonic and/or larval-born, were affected in adults was not distinguished by the analysis of Acebes and colleagues (Acebes et al., 2011). It is conceivable that a more prolonged inhibition of insulin signalling is required for the appearance of these morphological disruptions.

The role of the mushroom body in the establishment and retrieval of olfactory memory in *Drosophila* adults and other insects is well documented (Heisenberg et al., 1985; de Belle and Heisenberg, 1994) (reviewed by Margulies et al., 2005; Busto et al., 2010). Mushroom body function contributes to the regulation of motor activity in the adult fly (Heisenberg et al., 1985; Martin et al., 1998) and has been implicated in the processing of contextual information required for the fine-tuning of walking activity (Serway et al., 2009). Therefore, while this structure has not been directly implicated in the control of food intake, the observations reported to date suggest

that it may serve a number of task-relevant behaviours. Of note is the requirement for mushroom body synaptic output for the retrieval of both aversive and appetitive memory in the *Drosophila* foraging larva (Honjo and Furukubo-Tokunaga, 2005; Honjo and Furukubo-Tokunaga, 2009).

In an attempt to identify brain centres that regulate food intake in the *Drosophila* adult, Al-Anzi and colleagues screened a collection of GAL4 lines for those that would alter fat deposition when directing the expression of proteins that either superactivate or silence neuronal function (Al-Anzi et al., 2009). The lines identified were broadly expressed in the central nervous system and included the mushroom body neurons to differing extents. These observations support the notion that distinct populations of neurons, perhaps including the mushroom body neurons, control food intake and metabolism, perhaps by responding to humoral factors.

The *Drosophila* short neuropeptide F, the fly orthologue of the mammalian neuropeptide Y, is expressed in the larval and adult ventral cord and in most of the mushroom body neurons (Johard et al., 2008). In adult flies, ubiquitous knockdown of short neuropeptide F reduces food intake while broad overexpression promotes feeding (Lee et al., 2004). Whether a lack of short neuropeptide F function in the mushroom body contributes to the reduced feeding phenotype resulting from ubiquitous knockdown has not been determined. The receptor for a second fly orthologue of the mammalian neuropeptide Y, the *Drosophila* neuropeptide F, is required in the dopaminergic neurons that innervate the mushroom body medial lobe and peduncles (so-called MP-mushroom body neurons) for appetitive memory in adult flies. These observations support a model in which dopamine signalling controls mushroom body output (Krashes et al., 2009). These authors propose that the promotion of appetitive memory by neuropeptide F is mediated by the suppression of dopaminergic inhibition of mushroom body neurons. Their results indicate that starvation causes an increase in neuropeptide F, which in turn alleviates the inhibition of the mushroom body mediated by the MP-mushroom body neurons, thus leading to the expression of the learned behaviour.

In fed mice, neuropeptide Y-expressing neurons are suppressed by high levels of circulating insulin and perhaps brain-derived insulin. A recent study indicates that at least part of this mechanism has been conserved through evolution (Root et al., 2011). The authors report that in the adult fly, insulin signalling functions as a satiety signal by inhibiting the expression of *Drosophila* short neuropeptide F receptor 1 in olfactory receptor neurons, which in turn reduces presynaptic facilitation and starvation-dependent odour-driven food searching. In contrast, starvation or targeted inhibition of insulin signalling in olfactory receptor neurons up-regulates *Drosophila* short neuropeptide F receptor 1 expression and induces presynaptic facilitation and odour-driven food searching (Root et al., 2011).

Starvation-induced food intake in the *Drosophila* larvae is suppressed by overexpression of insulin (Wu et al., 2005a). Similarly, ubiquitous activation of InR causes larvae to feed less and to wander prematurely off the food, eventually dying of starvation (Britton et al., 2002). Our results indicate that inhibition of insulin signalling in the mushroom body neurons, but not activation, suppresses food intake and reduces growth and central nervous system proliferation. The last effect is an indirect consequence of reduced feeding, which impairs the glial-dependent activation of neuroblast proliferation, as demonstrated in recent publications (Chell and Brand, 2010; Sousa-Nunes et al., 2011). These observations cannot be reconciled with a simple model in which mushroom body function is the target of insulin as a satiety signal and do not exclude other brain regions from playing a role in this process. The identity of the central networks modulated by nutritional status and/or insulin signalling, their relative contribution and interaction remain to be elucidated. Our observations indicate that the mushroom body is a plausible candidate for a sensor of humoral factors that relay the nutritional status of the organism and for future investigations aimed at understanding the neural circuitry underlying the regulation of food intake and the role of insulin in this process.

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