Acknowledgments: The authors would like to thank Dr. R. Bijlsma for his helpful advice on this study and for providing of technical grade malathion, and also would like to thank to TUBİTAK BAYG for the PhD scholarship granted to Burcu Koçak Memmi.

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Microchaetae density is not greatly influenced by the overexpression of *akt.*

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Introduction

The notum of *Drosophila melanogaster* develops from a field of neuronal precursor tissue with the number of individual bristles being closely correlated to the number of sensory neurons formed (Jan and Jan, 1994). The density of the microchaetae on the dorsal notum is sensitive to both canonical and non-canonical Notch signalling at different stages of development (Tata and Hartley, 1995; Ramain *et al.,* 2001). Our laboratory has recently reported upon the role of the Huntingtin interacting protein-1 (Hip1) in neurogenesis (Moores *et al.,* 2008); we have found that analysis of microchaetae density provides a sensitive assay with which to approach subtle aspects of cell signalling. Two recent reports have investigated the activity of the *akt* kinase in *Drosophila* models of Huntington disease (Liévens *et al.,* 2008; Branco *et al.,* 2008). As part of our investigations into *Drosophila* homologues of genes with links to Huntington disease, we have explored the consequences of the overexpression of *akt* upon microchaetae density.

Drosophila melanogaster Strains and Culture

The *y w; apGal4/CyO (y w; P{GawB}ap^{md544}/CyO)* and the *y w; pnrGal4/TM3,UASy^{+} Ser (y w; P{GawB}pnr^{md237}/TM3, P{UAS-y.C}MC2, Ser^{1})* (Calleja *et al.,* 1996) driver lines plus the *w; UASlacZ^{4-1-2}* (Brand and Perrimon, 1993) and the *w; UASGFP* (Yeh *et al*., 1995) expression control lines were obtained from the Bloomington Drosophila Stock Center. The *w; UASAkt $^{1.1}$/CyO* line (*UASakt*) was described in Staveley *et al.* (1998). Males carrying the *UASlacZ*, *UASGFP* and *UASakt* responsive genes were each crossed to *apGal4* and *pnrGal4* virgin females and raised upon standard cornmeal-yeast-molasses-agar medium at 25ºC. Critical class males and females were

selected based upon the absence of *Cy* for *apGal4* crosses and *Ser* for *pnrGal4* crosses, aged in fresh medium for three to five days, then collected and stored as frozen at -70°C in 1.5 mL microcentrifuge tubes.

Biometric Analysis of Dorsal Notum Microchaetae

Whole flies are mounted on aluminium scanning electron microscope studs with the dorsal notum facing upward, desiccated overnight and gold coated using either a S150 Gold Sputter Coater or an EMSK550 Gold Sputterer. Samples are photographed using a Hitachi 570 SEM or a FEI Quanta 400 ESEM. Micrographs are analyzed using the ImageJ digital image analysis software (Abramoff *et al.,* 2004). The dorsal notum microchaetae were counted and total dorsal notum area (μm^2) was calculated for each image and then used to calculate the bristle density, expressed as number of microchaetae per 100 μm^2. Bristle density values were exported into GraphPad Prism 4 and mean ± standard error of the mean plotted for each individual genotype. Groups were subjected to one way ANOVA analysis with Neuman-Keuls post-tests to determine significance between pairs.

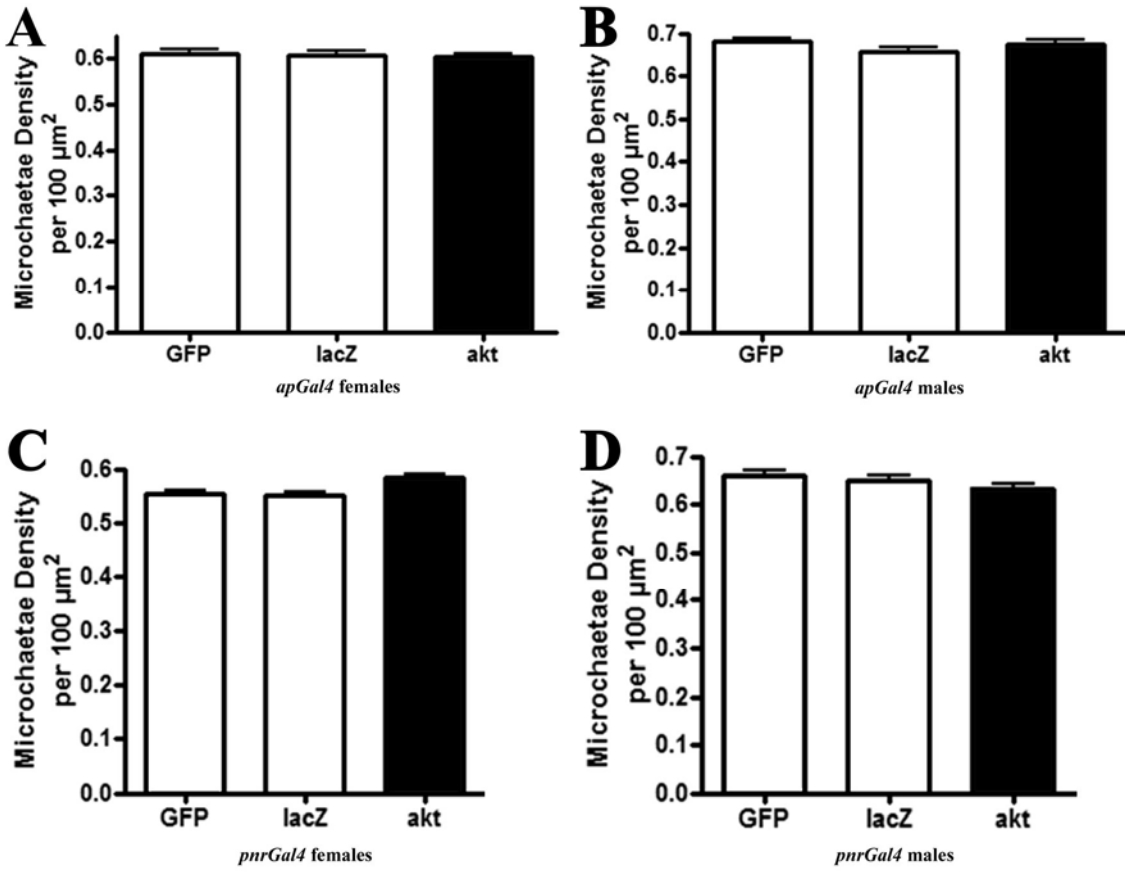

Figure 1. Directed expression of *akt* in the dorsal notum has little to no effect upon microchaetae density. Graphic representations of microchaetae density (values represent mean ± SEM). The genotypes are as follows: A) *w/w;UASGFP/apGal4(GFP)*; *w/w;UASlacZ/apGal4 (lacZ)*; *w/w;UASakt/apGal4(akt)* females; B) *w;UASGFP/apGal4(GFP)*; *w;UASlacZ/apGal4 (lacZ)*; *w;UASakt/apGal4(akt)* males; C) *w/w;UASGFP/+; pnrGal4/+(GFP)*; *w/w;UASlacZ/+; pnrGal4/+(lacZ)*; *w/w;UASakt/+*; *pnrGal4/+(akt)* females; D) *w;UASGFP/+; pnrGal4/+(GFP)*; *w;UASlacZ/+; pnrGal4/+(lacZ)*; *w;UASakt/+*; *pnrGal4/+(akt)* males. n = 30 for each value.

Results and Discussion

The directed expression of *akt* throughout the dorsal notum was driven by the *apterous-Gal4* (*apGal4*) and by the *pannier-Gal4* (*pnrGal4*) transgenes. Microchaetae density was determined for thirty electron micrographs of males and of females for each genotype. There was no statistically significant difference based on one-way ANOVA in microchaetae density in response to *akt* expression by *apGal4* compared to controls for either females (Figure 1A) or males (Figure 1B). However, there was a slight but statistically significant increase in the microchaetae density with *akt* expression driven by *pnrGal4* compared to both the *lacZ* and *GFP* controls in female (Figure 1C) but not male (Figure 1D) flies ($p < 0.01$ by Neuman-Keuls post-test). No statistical difference in microchaetae density was observed as a result of *GFP* and *lacZ* expression directed by *apGal4* or *pnrGal4* in either males or females. No difference in the gross morphology, position, or number of macrochaetae in response to *akt* expression under either transgene was noted.

The insulin signalling pathway provides a mechanism whereby cell size, cell number, and cell death are regulated (reviewed in Burgering, 2008). To evaluate a potential role for insulin signalling in mechanosensory bristle formation, an inducible *akt* transgene was expressed in the developing dorsal notum. We found that *akt* causes a slight increase in the bristle density in one instance (*UASakt; pnrGal4* females) transgene but not in the others. The middle of the dorsal notum, where *pnrGal4* expression is heightened, may be more sensitive to increased expression of *akt* than the lateral dorsal notum. However, it is clear that increased expression of *akt* does little to influence microchaetae density. As such, we believe that detailed analysis of microchaetae density may provide the opportunity to investigate the effects of *akt* upon the subtle alteration of neuronal cell signalling.

Acknowledgements: We thank Colleen Connors, Stephanie Mooney, and Shawn Hussey for help with data collection. BES is supported by the National Sciences and Engineering Council of Canada (NSERC) Discovery Grant and NSERC Research Tools and Instruments Programs. JMM was supported by a Canada Graduate Scholarship (CGS-M), and JNM was supported by NSERC Industrial Postgraduate Scholarships (IPS-M & IPS-D).

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