Chem. Senses 26: 223–230, 2001

Foraging Behaviour in *Drosophila* Larvae: Mushroom Body Ablation

Kathleen A. Osborne[1,2,3], J. Steven de Belle[1] and Marla B. Sokolowski[2]

[1]Department of Biological Sciences, University of Nevada, Las Vegas, NV 89154, USA and
[2]Department of Zoology, University of Toronto, Mississauga, Ontario, Canada L5L 1C6

[3]Present address: National Institute of Mental Health, Laboratory of Molecular Biology, Bethesda, MD 20892, USA

Correspondence should be sent to: Marla B. Sokolowski, Department of Zoology, University of Toronto, 3359 Mississauga Rd, Mississauga, Ontario, Canada L5L 1C6. e-mail:msokolow@credit.erin.utoronto.ca

Abstract

Drosophila larvae and adults exhibit a naturally occurring genetically based behavioural polymorphism in locomotor activity while foraging. Larvae of the rover morph exhibit longer foraging trails than sitters and forage between food patches, while sitters have shorter foraging trails and forage within patches. This behaviour is influenced by levels of cGMP-dependent protein kinase (PKG) encoded by the *foraging* (*for*) gene. Rover larvae have higher expression levels and higher PKG activities than do sitters. Here we discuss the importance of the *for* gene for studies of the mechanistic and evolutionary significance of individual differences in behaviour. We also show how structure–function analysis can be used to investigate a role for mushroom bodies in larval behaviour both in the presence and in the absence of food. Hydroxyurea fed to newly hatched larvae prevents the development of all post-embryonically derived mushroom body (MB) neuropil. This method was used to ablate MBs in rover and sitter genetic variants of foraging to test whether these structures mediate expression of the foraging behavioural polymorphism. We found that locomotor activity levels during foraging of both the rover and sitter larval morphs were not significantly influenced by MB ablation. Alternative hypotheses that may explain how variation in foraging behaviour is generated are discussed.

Introduction

The single gene mutant approach developed by Benzer (Benzer, 1967) has been used by *Drosophila* behaviour geneticists to identify genes involved in a variety of phenotypes, including courtship (Hall, 1994), rhythms (Hall, 1998), learning (Dubnau and Tully 1998) and olfaction (described in companion papers). More recently natural variants have been employed to identify and isolate genes involved in normal individual differences in behaviour [reviewed by Sokolowski (Sokolowski, 1998)]. Here we discuss research on *foraging*, a naturally polymorphic gene, and present some new data on rover and sitter adult responses to yeast and on mushroom body ablation and larval behaviour.

Drosophila larval foraging behaviour

In *Drosophila melanogaster*, foraging behaviour occurs as a naturally occurring behavioural dimorphism. In 1980, M.B. Sokolowski first noticed that foraging third instar *D. melanogaster* larvae taken directly from the wild can be separated into two distinct groups based on the locomotory component of their foraging behaviour. She called larvae that travel a long distance while foraging in the presence of food (a yeast and water paste) 'rovers' and those that moved significantly less on the same substrate 'sitters' (Figure 1A). Rover/sitter differences are only expressed in the presence of food; their path lengths do not differ in non-nutritive (agar) environments (Pereira and Sokolowski, 1993). When more than one food patch is present in the environment rover larvae have a much higher probability of leaving the food. Rovers move from patch to patch whereas sitters move to the nearest patch and remain feeding on it (Figure 1B).

The adult fly

These naturally derived strains of rover and sitter animals also differ in two food-based adult behaviours. The first is post-feeding locomotion. In the assay designed by Nagle and Bell (Nagle and Bell, 1987), a single fly is placed on a drop of sucrose solution in the centre of a circular arena and allowed to feed. The distance the fly travels from the drop of sugar during the first 30 s post-feeding is scored. In this assay rover adults travel in a relatively straight line away from the drop of sucrose after feeding, while sitter adults circle close to it (Pereira and Sokolowski, 1993). These different responses to the same assay conditions are

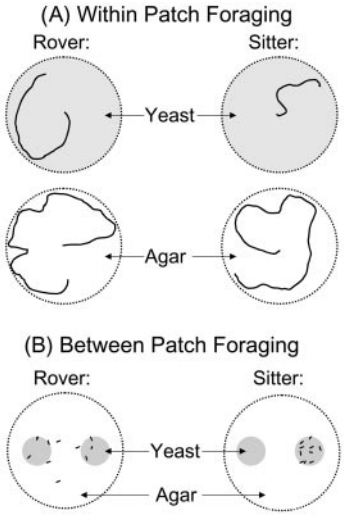

Figure 1 **(A)** Schematic of typical larval foraging path lengths on a yeast paste (food) and on agar (no-food) during a 5 min test interval (see Figure 4 legend for further details about protocols for the behavioural assays). **(B)** Schematic of larval locomotion when the distribution of food is patchy. Rover larvae move from patch to patch whereas sitter larvae move to the nearest patch and remain feeding on it.

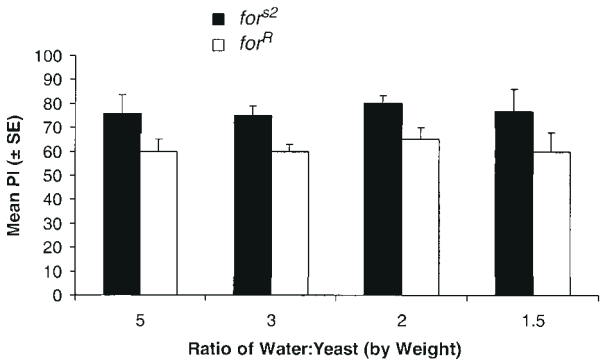

Figure 2 Attraction to a yeast odour is measured in a T-maze. One hundred 2- to 5-day-old flies were introduced into the maze and given 120 s to choose between two odours (a given concentration of yeast or dH_2O). A detailed protocol is described elsewhere (Shaver *et al.*, 1998). A performance index PI = (no. of flies in yeast odour tube/total number of flies) × 100 is plotted here on the *y* axis. The *x* axis shows increasing concentrations of yeast used in each choice test. Ten replicates of each yeast concentration choice test was done for each of the rover (for^R) and sitter (for^{s2}) strains. Two-way analysis of variance (ANOVA) showed significant differences between the response of the strains ($P < 0.003$), no effect of yeast concentration ($P = 0.8$) and no strain × yeast concentration interaction ($P = 0.9$) (E. Benchimol and M.B. Sokolowski, unpublished data). In all concentrations for^{s2} sitter flies moved to the yeast odour more frequently than did for^R rover flies. Yeast odours signal the presence of feeding, oviposition and mating sites for flies.

interpreted as the adults making qualitatively different decisions about the quality of food (Tortorici and Bell, 1988; Bell, 1990; Pereira and Sokolowski, 1993). Remaining close to the drop of sucrose (called 'intensive search behaviour') indicates that the animal evaluates the food source highly, while moving in a straight line away from the drop ('ranging') is indicative of search behaviour for an alternative food source (Tortorici and Bell, 1988; Bell, 1990). The apparent difference in the quality rating given to the same sucrose solution by the rover and sitter naturally occurring foraging morphs may reflect a difference in food search strategies. If so, this difference in food quality assessment may provide insight into the environment in which each behavioural morph would thrive. It was predicted that rovers would be more successful than sitters in locating food in a patchy environment, while sitters would be better able to exploit a more homogeneous food environment than their rover counterparts by utilizing less energy in search behaviour. Indeed, Sokolowski *et al.* showed that both rover and sitter alleles are selected for under different environmental conditions (Sokolowski *et al.*, 1997). When raised at high densities for many generations, the frequency of the rover allele (for^R) increases, while under low-density conditions the sitter allele (for^s) is preferentially selected. Examination of the foraging behaviour in *D. melanogaster* has identified one way in which natural selection can act on individual differences in behaviour.

The second adult behaviour studied shows that rovers and sitters differ in their ability to locate food (yeast) using both the T-maze (Tully and Quinn, 1985) and the olfactory trap assays (Woodard *et al.*, 1989). The T-maze is constructed such that flies placed at a choice point are exposed to odorants from both arms of the assay. A given concentration of yeast is placed in an odour cup and air is pumped over the cup so that yeast odour fills one arm of the maze. Water is placed in a second odour cup so that humidified air fills the other arm of the maze. Flies are scored for their attraction to yeast by moving into the appropriate arm of the maze. The olfactory trap assay is similar to the T-maze in that flies must again move to the yeast source, this time placed in a small trap. One significant difference between the T-maze and the olfactory trap assay is that the T-maze test is performed over several minutes, while the olfactory trap assay is conducted over several days. Under both test conditions, sitter adults locate the yeast at a significantly higher rate than do rover adults [olfactory trap (Shaver *et al.*, 1998); T-maze (Figure 2)]. These results can be interpreted in a number of ways. For example, (i) rover adults may have a reduced olfactory acuity to yeast compared with their sitter counterparts—a difference not observed in the larval life stage (Shaver *et al.*, 1998); or (ii) sitter adults which exhibit higher levels of intensive search may be generally more 'motivated' to search, resulting in a more rapid movement towards yeast odours than their rover counterparts. Like larvae, rover and sitter adults do not differ in their locomotion in the absence of food (Pereira and Sokolowski, 1993). Neither the rover nor the sitter morph is a sick animal as determined from general fitness assays that include development rate, longevity, fecundity,

muscle movement and pupation height (Sokolowski *et al.*, 1984; Graf and Sokolowski, 1989; Sokolowski, 1980; Sokolowski and Hansell, 1992). The fact that rovers and sitters are equally healthy, combined with the realization that both morphs are found in appreciable quantities in nature (30% sitters, 70% rovers in Ontario orchards), allows each to be considered wild type.

Genetic analysis of foraging behaviour

Extensive genetic analysis localized the single major gene responsible for foraging behaviour (*foraging*; *for*) to the left arm of chomosome 2 (Sokolowski, 1980; de Belle and Sokolowski, 1987, 1989; de Belle *et al.*, 1989). In order to further map and ultimately clone *for*, de Belle *et al.* (de Belle *et al.*, 1989, 1993) employed ethyl methanosulphonate (EMS) and gamma radiation to generate sitters derived from rover mutants. Each of the resulting mutant lines was also marked with recessive lethality. de Belle *et al.* (de Belle *et al.*, 1989) developed this technique, termed lethal tagging, to enable genes responsible for continuously varying traits to be mapped. Continuously varying traits are characterized by a mean behavioural score and some measure of variance. Larval foraging behaviour is a good example of a continuously varying trait. While foraging third instar larvae may be genetically identical, these larvae will rarely move exactly the same distance in the assay. As a result, populations of animals of a given genotype must be tested and their means compared statistically. Continuously varying traits are also more susceptible to subtle changes in both the external and genetic environments than discontinuous traits (e.g. lethality), and therefore test conditions must remain rigorously constant between and within strains to be compared. Lethal tagging allowed de Belle *et al.* (de Belle *et al.*, 1989, 1993) to indirectly localize the *foraging* gene by mapping a discontinuously varying trait (lethality) which was closely linked to the *foraging* (*for*) gene.

Cloning of the *foraging* gene

The paper by Osborne *et al.* (Osborne *et al.*, 1997) was the first to identify a molecular basis for a naturally occurring behavioural polymorphism in any organism. *dg2*, one of the two cGMP-dependent protein kinases (PKG) found in *D. melanogaster* (Kalderon and Rubin, 1989), was found to be synonymous with *for* (Osborne *et al.*, 1997). The importance of this work was highlighted in a review of this paper by Pennisi (Pennisi, 1997). The evidence that *for* is synonymous with *dg2* was:

1. The level of transcription of *dg2* was reduced in sitter animals compared with their rover counterparts. This result was also reflected in the level of *dg2* protein determined by Western blots, and the level of PKG kinase activity measured in the rover and sitter behavioural morphs.
2. Disruption of *dg2* by insertional mutagenesis reduced the level of *dg2* expression and changed larval foraging behaviour from rover to sitter.
3. Transgenic rescue, regarded by many as the most convincing evidence that a single gene is responsible for a specific phenotype, was used to demonstrate that an increase in the abundance of *dg2* was responsible for rover behaviour. The increase in the level of PKG in sitter behaving lines, through the addition of a transgenic *dg2*–T2 transcript under the control of the heat shock promoter, changed larval foraging behaviour from sitter to rover. A correlated increase in PKG activity in the larval CNS was also found.

It was not surprising to find that PKG, a member of the cGMP signal transduction pathway, is involved in foraging behaviour. Signal transduction occurs when a cell translates an external cue, such as an odour, photon of light or hormone in the blood stream, to an internal signal that then elicits a cellular response. The case of learning and memory in *Drosophila* is arguably the most successful examination to date of the role that components of a signal transduction pathway play in the generation of behaviour. By examining a series of mutants in the cAMP signal transduction system for their effect on learning and memory {including cAMP phosphodiesterase [PDE; *dunce; dnc* (Nighorn *et al.*, 1991)]; particulate adenylyl cyclase [AC; *rutabaga; rut* (Levin *et al.*, 1992)]; the catalytic domain of cAMP-dependent protein kinase [PKA; *DCO* (Skoulakis *et al.*, 1993)]; cAMP response element binding protein [CREB (Frank and Greenberg, 1994)]}, the cAMP signal transduction system has eloquently been implicated in the generation of learning and memory. Also, the elements of the cAMP signal transduction system identified as being important in learning and memory are found in enhanced levels in the adult mushroom bodies (Skoulakis *et al.*, 1993), structures independently implicated in olfactory learning by structure–function analysis (Heisenberg *et al.*, 1985).

In the past five years many aspects of the cGMP signal transduction pathway have come to light [reviewed recently (Wang and Robinson, 1997; Lohmann *et al.*, 1997; Ruth, 1999)]. As can be seen in Figure 3, PKG is not the only compound to mediate a cellular response to an increased level of cGMP. Although the cGMP signal transduction pathway is involved in regulating systems throughout the body (such as vision, olfaction, taste), this does not a priori implicate PKG in these areas. Osborne *et al.* (Osborne *et al.*, 1997) demonstrated a function for PKG in the modulation of foraging behaviour in *D. melanogaster*. [See Sokolowski and Riedl for a review of PKG and foraging behaviour (Sokolowski and Riedl, 1999).]

There are two important questions to be asked. The first is how do rovers and sitters differ in their *for* DNA sequences? The higher levels of PKG expression in the rover compared with the sitter morph suggests that they likely differ in their regulatory regions. The T2 transcript of

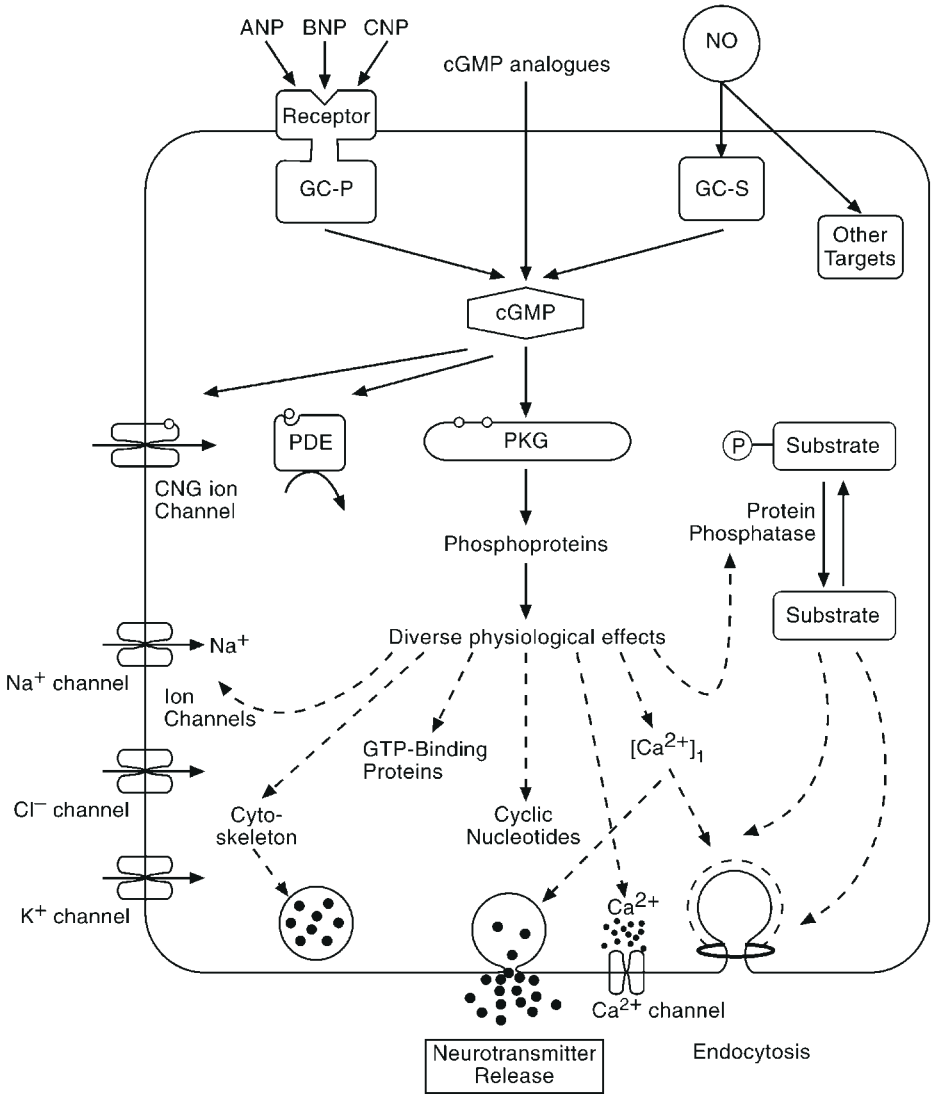

Figure 3 Schematic of cGMP signal transduction pathway in the nervous system [modified from (Wang and Robinson, 1997)]. For convenience, a single cell is shown with all neuronal cGMP signalling pathways known to function in the nervous system. ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; CNP = C-type natriuretic peptide; NO = nitric oxide; GC-S = soluble guanylyl cyclase; GC-P = particulate guanylyl cyclase; PKG = cGMP-dependent protein kinase; PDE = phosphodiesterase; CNG ion channel = cyclic nucleotide gated ion channel.

for/*dg2* was used to successfully rescue sitter larval behaviour to that of rover (Osborne *et al.*, 1997). The sequences of the T2 transcript from natural rover and sitter morphs did not differ, suggesting that rovers and sitter do not differ in the exons encoding T2 (M.B. Sokolowski, unpublished data). Investigation of rover and sitter sequence variation in the regulatory regions of *for* is currently under investigation. The second question is one concerning RNA and protein expression. Specifically, when during development and where in the organisms must *for* be expressed to produce rover as compared with sitter behaviour? This question requires analyses of patterns of gene expression in combination with targeted gene expression. We are interested in determining whether transcript-specific functions can be applied to the natural rover and sitter behavioural variants and to the behavioural and developmental phenotypes exhibited by *for* mutants. This work is also underway. A complementary approach is structure–function analysis. This addresses the requirement of certain structures (e.g. mushroom bodies) for the performance of behaviour. The remainder of this paper addresses the question of whether the mushroom bodies are involved in the generation of foraging-specific locomotion.

Structure–function mapping

The paired mushroom bodies (MBs) in the insect brain are most closely associated with sensory input (Strausfeld, 1976; Schürmann, 1987; Strausfeld *et al.*, 1998). In *Drosophila* adults, MBs are third-order chemosensory neuropils with prominent links to the antennal lobes (ALs)

via several antenno-cerebral fiber tracts (Stocker *et al.*, 1990, 1994; Heisenberg, 1994). Although direct connections from the visual system to the MBs are described in several insect groups (Strausfeld *et al.*, 1998), only a single neuron providing this link has been reported in *Drosophila* (Technau, 1983). Surgical interference, chemical ablation and genetic studies have suggested that MBs either influence or are required for a variety of different behavioural functions [reviewed by several groups (Erber *et al.*, 1987; Heisenberg, 1998; de Belle and Kanzaki, 1999)]. In *Drosophila*, MBs are necessary for normal olfactory learning and memory (Heisenberg *et al.*, 1985; de Belle and Heisenberg, 1994, 1996) [reviewed by Heisenberg (Heisenberg, 1989, 1994)]; they are implicated in aspects of courtship behaviour (Ferveur *et al.*, 1995; O'Dell *et al.*, 1995; Neckameyer, 1998; Joiner and Griffith, 2000); and they have modifying effects on both walking activity (Martin *et al.*, 1998) (R. Strauss and J.S. de Belle, unpublished observations) and visual learning (Liu *et al.*, 1999). Genes mediating odour learning and memory are also reported to have preferential expression in the MBs (Crittenden *et al.*, 1998), and MB-targeted transgene expression studies have further linked signalling pathways underlying memory formation to these structures (Connolly *et al.*, 1996; Zars *et al.*, 2000).

Drosophila MBs consist mainly of ~2500 intrinsic neurons known as Kenyon cells (KCs) arranged in parallel arrays (Technau and Heisenberg, 1982) [reviewed by Heisenberg (Heisenberg, 1989, 1994)]. KCs are derived from four neuroblasts in each hemisphere (Ito and Hotta, 1992; Prokop and Technau, 1994) that begin proliferating in stage 13 embryos (Tettamanti *et al.*, 1997) and continue uninterrupted until adult eclosion (Truman and Bate, 1988; Ito and Hotta, 1992). Concentric layers of KC arrays in the MB pedunculus project into different lobular structures that are added sequentially. First to appear is the γ lobe in the embryo, followed by the α′/β′ lobes in the larva and finally the α/β lobes in the pupa (Yang *et al.*, 1995; Ito *et al.*, 1997; Armstrong *et al.*, 1998; Lee *et al.*, 1999). During metamorphosis, a large proportion of KC projections in the α′/β′ lobes are retracted and then regrown, while neurogenesis contributes new KCs to the α/β lobes (Technau and Heisenberg, 1982; Armstrong *et al.*, 1998; Lee *et al.*, 1999). Although the meaning of this drastic reorganization of the CNS is unknown, it suggests that KCs perform functions specific to each stage of development, perhaps reflecting the contrasting sensory environments in which larvae and adults live.

Several genes are known to influence foraging behaviour in *Drosophila* (Sokolowski and Riedl, 1999). These include *foraging* [*for* (de Belle *et al.*, 1989, 1993; Osborne *et al.*, 1997; Sokolowski, 1998)], *Chaser* [*Csr* (Pereira *et al.*, 1995)], *no-bridge* and *ellipsoid body open* [*nob* and *ebo* (Varnam *et al.*, 1996)] and probably *Shaker* [*Sh* (Renger *et al.*, 1999)]. The well-characterized *for* gene is naturally polymorphic (Sokolowski *et al.*, 1997), with the rover allele dominant over the sitter allele [for^R and for^s (de Belle and Sokolowski, 1987; de Belle *et al.*, 1989b, 1993)].

Although neural mechanisms underlying differences in foraging behaviour are poorly understood, they likely involve variation in either sensory perception and/or higher brain-determined 'evaluation' responses to the food environment and/or some distribution of these functions throughout the organism [i.e. sensory, central brain, motor, gut (Sokolowski and Riedl, 1999)]. Possible neuroanatomical foci for processes involved in foraging behaviour differences have been described previously. First, mutations in two genes (*nob* and *ebo*) that are required for proper development of the central complex (CC) were shown to suppress rover path length in for^R larvae (Varnam *et al.*, 1996). Secondly, a mutant *for* allele tagged with a P[Gal4]-enhancer trap insertion also identified elevated levels of gene activity in the MBs (Osborne *et al.*, 1997) [J. Ewer, personal communication; Fly Trap (K. Kaiser), http://panic.molgen.gla.ac.uk/flytrap/html/enhancer/index.html].

Based on these observations and others suggesting a MB role in the regulation of motor behaviour (above), hydroxyurea (HU) ablation experiments were performed. HU ablation of MB neuroblasts in brains of newly hatched larvae prevents the birth of all KCs from this time onward (Ito and Hotta, 1992; Prokop and Technau, 1994). Only the embryonically derived neurons in the γ lobe persist through to later stages of development (Ito *et al.*, 1997; Armstrong *et al.*, 1998; Lee *et al.*, 1999). An additional fifth neuroblast with a similar temporal pattern of proliferation gives rise to AL local and projection interneurons that are also ablated in HU-treated animals (de Belle and Heisenberg, 1994; Stocker *et al.*, 1997). This convenient method of neuroanatomical interference is administered orally and can be targeted to defined windows of development. It has been especially useful for inferring structure–function relationships in the brains of flies (see above) and throughout the nervous systems of other insects (Sweeney *et al.*, 2000).

We found that MB ablation with HU did not significantly affect general locomotor activity (Figure 4A) or locomotor activity levels expressed by rover and sitter larvae during foraging (Figure 4B). These data (i) show that HU does not have obvious general negative effects on larvae; and (ii) allow us to reject the hypothesis that MBs are involved in larval locomotion and foraging behaviours. However, it is important to note that ablation does not block proliferation of KCs projecting to the γ lobe born prior to larval hatching (Armstrong *et al.*, 1998). Consequently, we cannot completely rule out a MB role in foraging behaviour, since our HU-ablated larvae would have had intact γ lobes. These structures are not detected in paraffin sections with fluorescence microscopy but can be observed in whole mounted brains with the aid of transgenic cell markers and a confocal microscope [compare figure 1 of de Belle and Heisenberg (de Belle and Heisenberg, 1994) with figure 3 of Armstrong *et al.* (Armstrong *et al.*, 1998)]. It would be

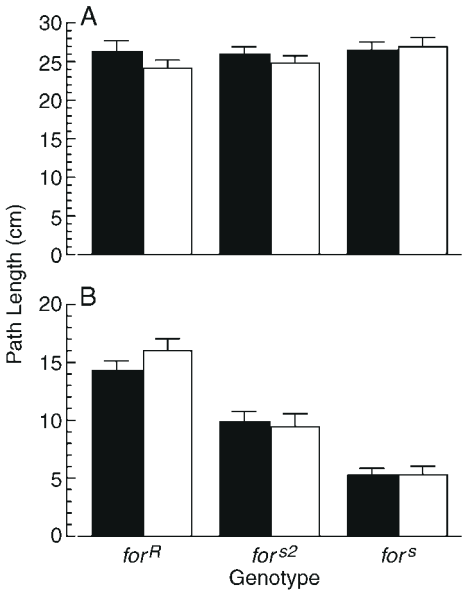

Figure 4 The results show that larval locomotor behaviour is not affected by MB ablation. Bars are mean ± SE larval path length; dark histograms are controls and open histograms are HU-treated larvae; $20 \leq n \leq 30$ larvae/bar. **(A)** Agar (non-nutrient substrate). A two-way ANOVA testing the effects of genotype, HU-treatment and interaction was not significant [$F(5,154) = 1.34$, $P = 0.25$]. **(B)** Yeast (foraging substrate). A two-way ANOVA detected a significant effect of genotype [$F(2,154) = 83.44$, $P < 0.0001$] only. HU treatment [$F(1,154) = 0.50$, $P = 0.50$] and interaction [$F(2,154) = 0.96$, $P = 0.39$] were not significant. A Student–Neuman–Keuls test showed that all three genotypes were significantly different ($P \leq 0.05$). The for^R, for^s (naturally derived rover and sitter strains respectively) and for^{s2} [a γ-radiation-induced mutation generated on a rover for^R genetic background (de Belle *et al.*, 1989, 1993; Pereira *et al.*, 1993)] were HU ablated as done elsewhere (de Belle and Heisenberg, 1994; Sweeney *et al.*, 2000). Briefly, larvae were collected between 0 and 1 h post-hatch and incubated in an HU- and heat-killed yeast suspension (50mg/ml) for 4 h. They were then washed in distilled water and transferred to normal medium and standard conditions. Control larvae were treated similarly except that HU was omitted. Third instar larvae (96 ± 1.5 h after larval hatching) were tested for general larval locomotion on a smooth agar surface (Sokolowski and Hansell, 1992) or locomotion during foraging on a thin homogenous yeast suspension layer (distilled water and baker's yeast in a 2:1 ratio by weight). A randomly sampled larva was placed in a covered Petri dish (8.5 cm ∅) containing 20 ml of 1.6% agar or a yeast suspension allowed to move freely for 5 min. The length of the visible trail left by each larva (path length) was measured and recorded with a digitizer/electronic graphics calculator. Each tested larva was transferred to single food vials and allowed to mature under standard conditions so that their heads could be sectioned as in (Heisenberg and Böhl, 1979). HU-treated animals were only included in the analysis for the behavioural data when MB ablation appeared complete at the level of the light microscope.

interesting to investigate whether variation in foraging behaviour is dependent on the MB γ lobe by testing transgenic larvae that have toxin gene expression driven by a γ-lobe-specific Gal4 enhancer [reviewed by Sweeney *et al.* (Sweeney *et al.*, 2000)].

Among alternative regions of the brain that may be considered as potential mediators of foraging-related differences in behaviour, the CC and CC precursor cells are most notable. CC involvement in the regulation of adult motor behaviour is well documented (Strauss *et al.*, 1992; Strauss and Heisenberg, 1993). In addition, CC mutant-analyses suggest that the CC precursor also has a role in motor activity related to feeding (Varnam *et al.*, 1996). Some mutants [*central complex deranged* (*ccd*), *central body defect* (*cbd*), *central brain deranged* (*ceb*) and *central complex* (*cex*)] had general defects in larval locomotion (on a non-nutritive agar surface). More interestingly, two of the mutants (*ebo* and *nob*) displayed foraging-specific reductions in locomotor activity (on a nutritive yeast surface). General locomotor activity of *ebo* and *nob* in the absence of food was normal. These CC mutations suppressed rover path length in for^R larvae but only in the presence of food [*nob;* for^R and ebo^2*;* for^R had sitter phenotypes (Varnam *et al.*, 1996)]. Feeding-related motor behaviour in this previous study was not examined in adults because CC mutants have general walking behaviour deficits that would have confounded data interpretations (Strauss *et al.*, 1992; Strauss and Heisenberg, 1993).

Despite our findings that MBs appear not to influence larval foraging, a parallel study of feeding-related behaviour in adults would be beneficial. Several recent reports describe MB influences on motor behaviour in *Drosophila* adults. For example, MB-less males actively court immature males longer (Neckemeyer, 1998); they sustain higher levels of walking activity over extended periods of time [13 h (Martin *et al.*, 1998)]; when entrained by a 12:12 LD light regime they are subsequently more active under constant dark conditions; and they have slightly longer circadian rhythms than their unablated brothers (C. Helfrich-Förster and J.S. de Belle, unpublished results). In all of these examples there is no general loss or reduction of behaviour after MB ablation, as is observed with some of the CC mutants (Strauss and Heisenberg, 1993). On the contrary, activity increases in the absence of MB neuropil. Neuroanatomically, these observations are somewhat puzzling because *Drosophila* MBs do not appear to have intimate connections with motor centres or descending neurons in the brain (Ito *et al.*, 1998). Nonetheless, if MBs do have an influence on feeding-related motor activity in adults but not in larvae, it would be reasonable to suspect α/β lobe KC fibers to be involved as they are born during the pupal stage of development (Armstrong *et al.*, 1997; Lee *et al.*, 1999). We anticipate that spatial and temporal control of transgene expression will be a critical tool for the assignment of foraging-specific (and other) functions to brain regions in *Drosophila*.

Acknowledgements

Thanks to C. and M. Riedl for help with the figures and to E. Benchimol for the unpublished data. Research was funded by a Research grant from the Medical Research Council (MRC) of Canada to M.B.S.

References

Armstrong, J.D., de Belle, J.S., Wang, Z. and **Kaiser, K.** (1998) *Metamorphosis of the mushroom bodies; large-scale rearrangements of the neural substrates for associative learning and memory in* Drosophila. Learn. Mem., 5, 102–114.

Bell, W.J. (1990) *Searching behavior patterns in insects.* Annu. Rev. Entomol., 35, 447–467.

Benzer, S. (1967) *Behavioral mutants of* Drosophila *isolated by countercurrent distribution*. Proc. Natl Acad. Sci. USA, 58, 1112–1119.

Connolly, J.B., Roberts, I.J.H., Armstrong, J.D., Kaiser K., Forte, M., Tully, T. and **O'Kane, C.J.** (1996) *Associative learning disrupted by impaired G(S) signaling in* Drosophila *mushroom bodies*. Science, 274, 2104–2107.

Crittenden, J.R., Skoulakis, E.M.C., Han, K.-A., Kalderon, D. and **Davis, R.L.** (1998) *Tripartite mushroom body architecture revealed by antigenic markers.* Learn. Mem., 5, 38–51.

de Belle, J.S. and **Heisenberg, M.** (1994) *Associative odor learning in* Drosophila *abolished by chemical ablation of mushroom bodies.* Science, 263, 692–695.

de Belle, J.S. and **Heisenberg, M.** (1996) *Expression of* Drosophila *mushroom body mutations in alternative genetic backgrounds: a case study of the* mushroom body miniature *gene (*mbm*).* Proc. Natl Acad. Sci. USA, 93, 9875–9880.

de Belle, J.S. and **Kanzaki, R.** (1999) *Protocerebral olfactory processing.* In Hansson, B.S. (ed.), Insect Olfaction. Springer, Stuttgart, pp. 243–281.

de Belle, J.S. and **Sokolowski, M.B.** (1987) *Heredity of rover/sitter: alternative foraging strategies of* Drosophila melanogaster *larvae.* Heredity, 59, 73–83.

de Belle, J.S. and **Sokolowski, M.B.** (1989) *Rover/sitter foraging behavior in* Drosophila melanogaster*: genetic localization to chromosome-*αL *using compound autosomes*. J. Insect Behav., 2, 291–299.

de Belle, J.S., Hilliker, A.J. and **Sokolowski, M.B.** (1989) *Genetic localization of* foraging *(*for*): a major gene for larval behavior in* Drosophila melanogaster. Genetics, 123, 157–163.

de Belle, J.S., Sokolowski, M.B. and **Hilliker, A.J.** (1993) *Genetic analysis of the* foraging *microregion of* Drosophila melanogaster. Genome, 36, 94–101.

Dubnau, J. and **T. Tully** (1998) *Gene discovery in* Drosophila: *new insights for learning and memory.* Annu. Rev. Neurosci., 21, 407–444.

Erber, J., Homberg, U. and **Groneberg, W.** (1987) *Functional roles of the mushroom bodies in insects.* In Gupta, A.P. (ed.), Arthropod Brain: Its Evolution, Development, Structure, and Functions. Wiley, New York, pp. 485–511.

Ferveur, J.-F., Störtkuhl, K.F., Stocker, R.F. and **Greenspan, R.J.** (1995) *Genetic feminization of brain structures and changed sexual orientation in male* Drosophila*.* Science, 267, 902–905.

Frank, D.A. and **Greenberg, M.E.** (1994) CREB*: a mediator of long-term memory from mollusks to mammals*. Cell, 79, 5–8.

Graf, S. and **Sokolowski, M.B.** (1989) *The effect of development, food-patch quality and starvation on* Drosophila melanogaster *larval foraging behavior.* J. Insect Behav., 2, 301–313.

Hall, J.C. (1994) *The mating of a fly.* Science, 264, 1702–1714.

Hall, J. C. (1998) *Molecular nuerogenetics of biological rhythms.* J. Neurogenet., 12, 115–181.

Heisenberg, M. (1989) *Genetic approach to learning and memory (mnemogenetics) in* Drosophila melanogaster. In Rahmann, H. (ed.), Fundamentals of Memory Formation: Neuronal Plasticity and Brain Function. Fischer, Stuttgart, pp. 3–45.

Heisenberg, M. (1994) *Central brain function in insects: genetic studies on the mushroom bodies and central complex in* Drosophila*.* In Schildberger, K. and Elsner, N. (eds), Neural Basis of Behavioural Adaptations. Fischer, Stuttgart, pp. 61–79.

Heisenberg, M. (1998) *What do the mushroom bodies do for the insect brain? An introduction*. Learn. Mem., 5, 1–10.

Heisenberg, M. and **Böhl, K.** (1979) *Isolation of anatomical brain mutants of* Drosophila *by histological means.* Z. Naturforsch., 34c, 143–147.

Heisenberg, M., Borst, A., Wagner, S. and **Byers, D.** (1985) Drosophila *mushroom body mutants are deficient in olfactory learning.* J. Neurogenet., 2, 1–30.

Ito, K. and **Hotta, Y.** (1992) *Proliferation pattern of postembryonic neuroblasts in the brain of* Drosophila melanogaster. Devl Biol., 149, 134–148.

Ito, K., Awano, W., Suzuki, K., Hiromi, Y. and **Yamamoto, D.** (1997) *The* Drosophila *mushroom body is a quadruple structure of clonal units each of which contains a virtually identical set of neurones and glial cells*. Development, 124, 761–771.

Ito, K., Suzuki, K., Estes, P., Ramaswami, M., Yamamoto, D. and **Strausfeld, N.J.** (1998) *The organization of extrinsic neurons and their implications in the functional roles of the mushroom bodies in* Drosophila melanogaster *Meigen*. Learn. Mem., 5, 52–77.

Joiner, M.A. and **Griffith, L.C.** (2000) *Visual input regulates circuit configuration in courtship conditioning of* Drosophila melanogaster. Learn. Mem., 7, 32–42.

Kalderon, D. and **Rubin, G.M.** (1989) *cGMP-dependent protein kinase genes in* Drosophila. J. Biol. Chem., 264, 10738–10748.

Lee, T., Lee, A. and **Luo, L.** (1999) *Development of the* Drosophila *mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast.* Development, 126, 4065–4076.

Levin, L.R., Han, P.L., Hwang, P.M., Feinstein, P.G., Havis, R.L. and **Reed, R.R.** (1992) *The* Drosophila *learning and memory gene* rutabaga *encodes a* Ca^{2+}*/calmodulin-responsive adenylyl cyclase.* Cell, 68, 479–489.

Liu, L., Wolf, R., Ernst, R. and **Heisenberg, M.** (1999) *Context generalization in* Drosophila *visual learning requires the mushroom bodies.* Nature, 400, 753–756.

Lohmann, S.M., Vaandrager, A.B., Smolenski, A., Walter, U. and **DeJonge, H.R.** (1997) *Distinct and specific functions of cGMP-dependent protein kinases*. Trends Biochem. Sci., 22, 307–312.

Martin, J.-R., Ernst, R. and **Heisenberg, M.** (1998) *Mushroom bodies suppress locomotor activity in* Drosophila melanogaster. Learn. Mem., 5, 179–191.

Nagle, K.J and **Bell, W.J.** (1987) *Genetic control of the search tactic of* Drosophila melanogaster*: an ethometric analysis of rover/sitter traits in adult flies*. Behav. Genet., 17, 385–408.

Neckameyer, W.S. (1998) *Dopamine and mushrooom bodies in* Drosophila*: experience-dependent and -independent aspects of sexual behavior*. Learn. Mem., 5, 156–165.

Nighorn, A., Healy, M.J. and **Davis, R.L.** (1991) *The cyclic AMP phosphodiesterase encoded by the* Drosophila dunce *gene is concentrated in the mushroom body neuropil.* Neuron, 6, 455–467.

O'Dell, K.M.C., Armstrong, J.D., Yang, M.Y. and **Kaiser, K.** (1995) *Functional dissection of the* Drosophila *mushroom bodies by selective*

feminization of genetically defined subcompartments. Neuron, 15, 55–61.

Osborne, K.A., Robichon, A., Burgess, E., Butland, S., Shaw, R.A., Coulthard, A., Pereira, H.S., Greenspan, R.J. and **Sokolowski, M.B.** (1997) *Natural behavior polymorphism due to a cGMP-dependent protein kinase of* Drosophila. Science, 277, 834–836.

Pennisi E. (1997) *What makes fruit flies roam?* Science, 277, 763–764.

Pereira, H.S. and **Sokolowski, M.B.** (1993) *Mutations in the larval* foraging *gene affect adult locomotory behavior after feeding in* Drosophila melanogaster. Proc. Natl Acad. Sci. USA, 90, 5044–5046.

Pereira, H.S., Macdonald, D.E., Hilliker, A.J. and **Sokolowski, M.B.** (1995) *Chaser (*Csr*), a new gene affecting larval foraging behaviour in* Drosophila melanogaster. Genetics, 140, 263–270.

Prokop, A. and **Technau, G.M.** (1994) *Normal function of the mushroom body defect gene of* Drosophila *is required for the regulation of the number and proliferation of neuroblasts*. Devl Biol., 161, 321–337.

Renger, J.J., Yao, W.-D., Sokolowski, M.B. and **Wu, C.-F.** (1999) *Neuronal polymorphism among natural alleles of a cGMP-dependent kinase gene,* foraging *in* Drosophila. J. Neurosci., 19, 1–8.

Ruth, P. (1999) *Cyclic GMP-dependent protein kinases: understanding* in vivo *functions by gene targeting*. Pharmacol. Ther., 82, 355–372.

Schürmann, F.-W. (1987) *The architecture of the mushroom bodies and related neuropils in the insect brain.* In Gupta, A.P. (ed.), Arthropod Brain: Its Evolution, Development, Structure, and Functions. Wiley, New York, pp. 231–264.

Shaver, S.A., Varnam, C.J., Hilliker, A.J. and **Sokolowski, M.B.** (1998) *The* foraging *gene affects adult but not larval olfactory-related behavior in* Drosophila melanogaster. Behav. Brain Res.**,** 95, 23–29.

Skoulakis, E.M.C., Kalderon, D. and **Davis, R.L.** (1993) *Preferential expression in mushroom bodies of the catalytic subunit of protein kinase A and its role in learning and memory*. Neuron**,** 11, 197–208.

Sokolowski, M. B. (1980) *Foraging strategies of* Drosophila melanogaster*: a chromosomal analysis.* Behav. Genet., 10, 291–302.

Sokolowski, M. B. (1998) *Genes for normal behavioral variation: recent clues from flies and worms*. Neuron, 21, 1–4.

Sokolowski, M. B. and **Hansell, K.P.** (1992) *The* foraging *locus: behavioral tests for normal muscle movement in rover and sitter* Drosophila melanogaster *larvae*. Genetica, 85, 205–209.

Sokolowski, M.B. and **Riedl, C.** (1999) *Behaviour-genetic and molecular analysis of naturally occurring variation in* Drosophila *larval foraging behaviour.* In Gerlai, R. and Crusio, W. (eds), Molecular-genetic Techniques for Brain and Behaviour. Elsevier, London, pp. 517–532.

Sokolowski, M.B., Kent, C. and **Wong, J**. (1984) Drosophila *larval foraging behaviour: developmental stages.* Anim. Behav, 32, 645–651.

Sokolowski, M.B., Pereira, H.S. and **Hughes, K.** (1997) *Evolution of foraging behavior in* Drosophila *by density-dependent selection.* Proc. Natl Acad. Sci. USA, 94, 7373–7377.

Stocker, R.F. (1994) *The organization of the chemosensory system in* Drosophila melanogaster*: a review*. Cell Tissue Res., 275, 3–26.

Stocker, R.F., Heimbeck, G., Gendre, N. and **de Belle, J.S.** (1997) *Neuroblast ablation in* Drosophila *P[GAL4] lines reveals origins of antennal target interneurons.* J. Neurobiol., 32, 443–456.

Stocker, R.F., Leinhard, M.C., Borst, A. and **Fischbach, K.-F.** (1990) *Neuronal architecture of the antennal lobe in* Drosophila melanogaster. Cell Tissue Res., 262, 9–34.

Strausfeld, N.J. (1976) Atlas of an Insect Brain. Springer, Heidelberg.

Strausfeld, N.J., Hansen, L., Li, Y., Gomez, R.S. and **Ito, K.** (1998) *Evolution, discovery, and interpretations of arthropod mushroom bodies.* Learn. Mem., 5, 11–37.

Strauss, R. and **Heisenberg, M.** (1993) *A higher control center of locomotor behavior in the* Drosophila *brain*. J. Neurosci., 13, 1852–1861.

Strauss, R., Hanesch, U., Kinkelin, M., Wolf, R. and **Heisenberg, M.** (1992) no-bridge *of* Drosophila melanogaster*: portrait of a structural brain mutant of the central complex.* J. Neurogenet., 8, 125–155.

Sweeney, T.S., Hidalgo, A., de Belle, J.S. and **Keshishian, H.** (2000) *Functional ablation*. In Sullivan, B., Ashburner, M. and Hawley, S. (eds), *Drosophila* Protocols. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 449–477.

Technau, G.M. (1983) *Die entwicklung der Corpora Pedunculata von* Drosophila melanogaster. Doctoral thesis, University of Würzburg.

Technau, G.M. and **Heisenberg, M.** (1982) *Neural reorganisation during metamorphosis of the corpora pedunculata in* Drosophila melanogaster. Nature, 295, 405–407.

Tettamanti, M., Armstrong, J.D., Endo, K., Furokubo-Tokunaga, K., Kaiser, K. and **Reichert, H.** (1997) *Analysis of mushroom body development by enhancer-trap expression patterns in the* Drosophila *brain*. Devl Genes Evol., 207, 242–252.

Tortorici, C. and **Bell, W.J.** (1988) *Search orientation in adult* Drosophila melanogaster*: responses of rovers and sitters to resource dispersion in a food patch.* J. Insect Behav., 1, 209–224.

Truman, J.W. and **Bate, M.** (1988) *Spatial and temporal patterns of neurogenesis in the central nervous system of* Drosophila melanogaster. Devl Biol., 125, 145–157.

Tully, T. and **Quinn, W.G**. (1985) *Classical conditioning and retention in normal and mutant* Drosophila melanogaster. J. Comp. Physiol., 157, 263–277.

Varnam, C.J., Strauss, R., de Belle, J.S. and **Sokolowski, M.B.** (1996) *Larval behavior of central complex mutants in* Drosophila melanogaster*: interactions between* no bridge, foraging *and* Chaser. J. Neurogenet., 11, 99–115.

Wang, X. and **Robinson, P.J.** (1997) *Cyclic GMP-dependent protein kinase and cellular signalling in the nervous system*. J. Neurochem., 68, 443–456.

Woodard, C., Huang, T., Sun, H., Helfand, S.L. and **Carlson, J.** (1989) *Genetic analysis of olfactory behavior in* Drosophila*: a new screen yields the* ota *mutants.* Genetics, 123, 315–326.

Yang, M.Y., Armstrong, J.D., Vilinsky, I., Strausfeld, N.J. and **Kaiser, K.** (1995) *Subdivision of the* Drosophila *mushroom bodies by enhancer-trap expression patterns.* Neuron, 15, 45–54.

Zars, T., Fischer, M., Schulz, R. and **Heisenberg, M.** (2000) *Localization of a short-term memory in* Drosophila. Science, 288, 672–5.

Accepted October 31, 2000