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LARVAL BEHAVIOR OF *DROSOPHILA* CENTRAL COMPLEX MUTANTS: INTERACTIONS BETWEEN *NO BRIDGE*, *FORAGING*, AND *CHASER*

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The central complex (CC) is a prominent component of the adult insect brain. In *Drosophila melanogaster*, mutations which alter CC structure also impair adult locomotion. This has led to the suggestion that the CC functions as a higher organizer of adult locomotor patterns (Strauss and Heisenberg, 1993). In the present study, we describe altered larval behavior resulting from mutations in six CC structural genes. Differences from the control strain were found for larvae from each CC mutant strain in at least one of three assays. *central body defect*^{1} (*cbd*^{1}), *central complex deranged*^{1} (*ccd*^{1}), *central brain deranged*^{1} (*ceb*^{1}) and *central complex*^{1} (*cex*^{1}) larvae all had general defects in locomotion (on a non-nutritive agar surface). Both *ellipsoid body open*^{2} (*ebo*^{2}) and *no bridge*^{1} (*nob*^{1}) had larval foraging behavior defects (on a nutritive yeast surface). Only *cex*^{1} larvae required significantly longer time in a roll over assay of muscle tone. Genetic analysis suggested that *nob*^{1} interacts additively with two other genes influencing larval foraging behavior, *foraging* (*for*) and *Chaser* (*Csr*). *for* also had an influence on adult foraging, whereas here we found that *Csr* did not. We did not include adult foraging behavior tests of the CC mutants due to general locomotion defects in these flies (Strauss and Heisenberg, 1993).

Keywords: Brain mutants; protocerebral bridge; foraging; walking

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INTRODUCTION

The central complex (CC) is a large median neuropil in the insect brain. It consists of four separate but interconnected substructures and has a highly repetitive inner order. Detailed anatomical studies of the CC have been made in *Schistocerca* (Williams, 1975), *Musca* (Strausfeld, 1976) and *Drosophila* (Hanesch et al., 1989). No large fiber tracts connect it directly to either sensory or motor neuropils, but rather, input and output are distributed diffusely throughout the central brain (Strausfeld, 1976). Of these, small fiber sensory input is the most easily recognized. For example, connections have been reported from the antennal lobes in ants (Goll, 1967) the optic lobes in crickets (Honegger and Schürmann, 1975) and the ocellar neuropil in grasshoppers (Goodman and Williams, 1976). Various CC functions have been suggested (reviews: Homberg, 1987; Heisenberg, 1994). Based on patterns of ipsi- and contra-lateral projections within and between substructure neuropils, the CC appears well suited to overlay inputs from both brain hemispheres and coordinate synchronized bilateral outputs (Mobbs, 1985; Hanesch et al., 1989).

CC function has been investigated using genetic dissection in *D. melanogaster*. Recessive ethylmethane sulfonate (EMS)-induced *X*-linked mutations altering CC gross anatomy were isolated using a histological screen for brain structure defects (Heisenberg and Böhl, 1979). The *no bridge*^{1} (*nob*^{1}) mutant has a disruption of the protocerebral bridge (PB) and has been well characterized in both flying and walking behavior paradigms (Strauss et al., 1992). In one study, *nob*^{1} flies were shown to be deficient in contra-lateral brain hemisphere habituation following ipsi-lateral gustatory stimulation, whereas both brain hemispheres in the *Berlin* control strain were habituated concurrently (Bouhouche et al., 1993).

Analysis of adult locomotion in 15 independent CC mutant strains suggested that the central complex is a regulator and organizer of walking behavior (Strauss and Heisenberg, 1993). Impaired walking and reduced speeds were observed for all mutants in at least one of three walking tests. Three mutant strains [*central body defect*^{1} (*cbd*^{1}), *central complex*^{1} (*cex*^{1}) and *nob*^{1}] were examined in detail. In *nob*^{1}, a decrease in total walking speed was associated with shortened stride length. The timing of swing phases and the duration of leg swings were unaffected by the mutation. Using mosaic analyses, characters such as walking activity and reduced step length were associated with the CC in *nob*^{1}, whereas in *cex*^{1}, the ven-

tral ganglion was associated with the temporal succession of swing phases (gait) and the duration of leg swings (Strauss and Heisenberg, 1993). Other studies have also indicated that some CC mutations have additional defects in other areas of the central nervous system (Ilius et al., 1994; de Belle and Heisenberg, 1996). Strauss and Heisenberg (1993) have suggested that the CC organizes patterns of insect locomotion while other tissues generate these patterns.

Further evidence that the CC functions as a walking pattern organizer comes from comparisons among insect species with dissimilar timing of CC differentiation (Hanström, 1928; Hanesch et al., 1989; Strauss and Heisenberg, 1993). Hemimetabolous species have legs and develop an adult-like CC in premature larval stages. For example, *Tenebrio molitor* (mealworm and flour beetle) first instar larvae have legs and a distinct fan shaped body. Other CC sub-structures are added in the fifth, sixth and seventh instars at which time the late larval CC is homologous to that of the adult (Wegerhoff and Breidbach, 1992). On the other hand, larvae of holometabolous species such as the Diptera are legless and CC differentiation does not occur until pupation. In *D. melanogaster* larvae, a CC precursor can be distinguished as a subset of fibers in the interhemispheric commissure (Hanesch, 1987; Hanesch et al., 1989). This correlation between the presence of legs and the timing of CC differentiation has led to the hypothesis that legless larval locomotion does not use an adult-type locomotor organizer (the CC) (Hanesch et al., 1989).

Although the adult CC is absent in fly larvae, mutations which disrupt CC structure and walking in the adult may have a similar effect on larval locomotion, perhaps through disruption of the CC precursor (Hanesch, 1987; Strauss et al., 1992). In the present study, we examined the influence of six CC mutations on *D. melanogaster* larval behavior. Locomotion was measured on both non-nutritive and nutritive (foraging) substrates. We also used the time taken for a larva to right itself completely after having been rolled over as a behavioral measure of muscle usage and tone (Ball et al., 1985).

In *D. melanogaster*, larvae can be classified as "rovers" or "sitters" on the basis of the distance they move while foraging on a thin layer of yeast during a 5 min test period (Sokolowski, 1980). This naturally occurring genetic polymorphism is influenced by larval age and other environmental factors (Graf and Sokolowski, 1989). Neural mechanisms underlying this foraging polymorphism are unknown. However, they likely involve differences in

either sensory perception or higher brain-determined "evaluation" responses to the food environment (Pereira and Sokolowski, 1993). Rover and sitter larvae do not differ in size or rate of development, nor in behavioral tests measuring muscle strength or general locomotion in a non-nutritive environment (Graf and Sokolowski, 1989; Sokolowski and Hansell, 1992).

Two genes are known to influence larval foraging behavior. *foraging* (*for*, 2–10, 24A3–5) is naturally polymorphic, with the rover allele (for^R) dominant to the sitter allele (for^s) (de Belle and Sokolowski, 1987; de Belle et al., 1989; 1993). *Chaser* (*Csr*, 3-?, 95F7–96A1) is a second gene identified in a mutagenesis screen for modifiers of *for* (Pereira et al., 1995). *Csr* mutations are dominant and suppress the sitter phenotype in homozygous for^s larvae.

Adult foraging behavior is also known to be influenced by *for*. After consuming a drop of sucrose, for^R flies walk significantly further than for^s flies (Pereira and Sokolowski, 1993). Spontaneous locomotor activity in the absence of sucrose is not affected by different *for* alleles. This showed that *for* has a specific effect on foraging behavior in adult flies, as well as in larvae (Pereira and Sokolowski, 1993).

In the present study, we found that all six CC mutant strains tested differed significantly from the wild type control in at least one test of larval locomotion. The nob^1 mutation affected larval foraging specifically and showed additive interactions with alleles of both *for* and *Csr*. Unlike in larvae, we did not find an influence of *Csr* on adult foraging behavior.

MATERIALS AND METHODS

Strains and Genetic Background

We used *Canton Special* (*CS*) (maintained in Würzburg since 1978) as a wild-type control strain. Six independent recessive *X*-linked single gene mutant strains associated with CC structural defects are listed in Table I. These were originally isolated in mass histology screens of male offspring of EMS-treated wild-type *Berlin* males mated to *C(1)DX, y w f* females (Heisenberg & Böhl, 1979). Adult brain anatomy defects in CC mutants are illustrated in Strauss & Heisenberg (1993). Additional mutations and chromosomes are described in Lindsley and Zimm (1992), unless stated otherwise.

TABLE I CC genes and the visible genetic markers used for outcrossing them to *CS*

CC Mutant Genes and Alleles					*Visible Genetic Markers*		
New	*Previous*		*Mapping*	*References**	*Genes*	*Mapping*	
central body defect					*dusky wavy*		
cbd^{1}	cbd^{KS96}	*1*-41	11A2-7	*c, d, f, i*	*dy wy*	*1*-36.2 to 40.7	10E2 to 11E
central complex deranged					*crossveinless cut*		
ccd^{1}	ccd^{KS135}	*1*-15		*c, d, f, i*	*cv ct*	*1*-13.7 to 20	5B to 7B1-2
central brain deranged					*singed*		
ceb^{1}	ceb^{849}	*1*-23	7F1-8A5	*b, d, i*	*sn*	*1*-21.0	7D1-2
central complex d					*usky wavy*		
cex^{1}	cex^{KS181}	*1*-43.6	11A7-B9	*i*		*1*-36.2 to 40.7	10E2 to 11E
ellipsoid body open					*yellow white*		
ebo^{1}	ebo^{KS263}	*1*-3.8	3D4	*b, c, d, f, g, i*	*y w*	*1*-0.0 to 1.5	*1*B1 to 3C2
no bridge					*crossveinless*		
nob^{1}	nob^{KS49}	*1*-12	4F5-12	*a, c, d, e, f, h, i*	*cv*	*1*-13.7	5B

**a*, Bouhouche et al., 1993; *b*, de Belle and Heisenberg, 1996; *c*, Hanesch, 1987; *d*, Heisenberg, 1989; *e*, Heisenberg, 1994; *f*, Heisenberg et al., 1985; *g*, Illius et al., 1994; *h*, Strauss; et al., 1992; *i*, Strauss and Heisenberg, 1993.

For more than a decade, artificial selection (used occasionally to maintain defective brain anatomy phenotypes), selection pressure under normal culture conditions and drift have likely all contributed to divergent genetic variability in the original CC mutant strains. To minimize potential polygenic influences on brain anatomy and behavior, we replaced the original genetic backgrounds of all CC mutants with that of *CS* (de Belle and Heisenberg, 1996). Chromosome balancer strains used were *In(1)FM7a, y^{31d} sc^{8} w^{a} v^{of} B* (*FM7a*), *al Bl/In(2LR)O, Cy dp^{lvl} pr cn^{2}* (*al Bl/CyO*) and *Sb H^{32}g/In(3LR)TM6b, Hu e Tb* (*Sb H/TM6b*). *FM7a/Y^{CS}; 2^{CS}; 3^{CS}; 4^{CS}* (*FM7a; CS*) was constructed from *FM7a* and *CS* by chromosome substitution using *al Bl/CyO* and *Sb H/TM6b*. Visible recessive genetic markers mapping close to each CC gene were chosen (see Table I), separated from various multiply marked *X*-chromosomes and placed in the *CS* genetic background by repeated genetic recombination. For example, *crossveinless* (*cv, 1*-13.7, 5B) was used for outcrossing *nob^{1}* (*1*-12, 4F5-12). Each CC mutation was then placed in the *CS* background by further cycles of recombination with an outcrossed marked strain [*e.g., cv(CS)*] and selection of unmarked male progeny [*e.g., cv^{+} nob^{1}/Y*]. Unmarked chromosomes [*e.g., cv^{+} nob^{1} (CS)*] were then isolated using *FM7a; CS*. This crossing scheme allowed for the "passive" exchange of Chromosome-*4* alleles and placed each CC mutation in a genetic background consisting of from 93% to 98% *CS* alleles. For convenience, the *(CS)* notation for outcrossed mutant CC alleles will not be indicated hereafter.

Three alleles of *foraging* (*for^{R}*, *for^{s}* and *for^{s2}*) and one allele of *Chaser* [*Csr^{3}* (formerly *Csr-3*)] were also used in this study (de Belle et al., 1989; 1993; Pereira and Sokolowski, 1993; Pereira et al., 1995). The *for^{s}* strain is marked with an allele of *ebony* (*e^{11}*) and was used as the sitter control in larval behavior tests (see below). While *e^{11}* does not influence larval behavior (Sokolowski, 1980), we used the unmarked *for^{s2}* strain to control for possible pleiotropic effects of *e^{11}* in adult behavior tests (Pereira and Sokolowski, 1993) (see below).

We constructed two homozygous combinations of *nob^{1}* and *for* alleles by chromosome substitution using *FM7a; CyO/Sco* (*nob^{1}; for^{R}* and *nob^{1}; for^{s}*). The *Csr^{3}* allele was then introduced by crossing *for^{s}; Csr^{3}* males with both *nob^{1}; for^{R}* and *nob^{1}; for^{s}* females, generating four additional genotypes for testing (*nob^{+}/nob^{1}; for^{R}/for^{s}; Csr^{3}/Csr^{+}*, *nob^{1}/Y; for^{R}/for^{s}; Csr^{3}/Csr^{+}*, *nob^{+}/nob^{1}; for^{s}/for^{s}; Csr^{3}/Csr^{+}* and *nob^{1}/Y; for^{s}/for^{s}; Csr^{3}/Csr^{+}*).

The deficiency *Df(1)HC244* (3E8; 4F11–12) uncovers *nob* adult brain and locomotor phenotypes (Strauss et al., 1992). We generated nob^1/*Df(1)HC244; for*^{R} flies by crossing nob^1/*Y; for*^{R} with *Df(1)HC244/FM7a* to determine whether larval foraging is associated with *nob* specifically (see below). nob^1 structural defects in adult brains of all newly constructed strains were confirmed with autofluorescence microscopy (Heisenberg and Böhl, 1979).

Flies were maintained on 45 ml of *Drosophila* medium (dead yeast, sucrose, agar, propionic acid) at 24 ± 1° with a 12L:12D photocycle (standard conditions). Larval behavior tests were performed in the early third instar [96 ± 1.5 hr after larval hatching (ALH)]. Groups of 100 first instar larvae (1.5 ± 1.5 hr ALH) were reared in Petri dishes (8.5 cm ∅) containing 35 ml of medium under standard conditions (de Belle and Sokolowski, 1987).

Larval Behavior

General larval locomotion was examined on a smooth agar surface (Sokolowski and Hansell, 1992). A randomly sampled larva was placed in a Petri dish (8.5 cm ∅) containing 20 ml of 1.6% agar and 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.

Locomotion during foraging was examined as described in Pereira et al. (1995). Briefly, black Plexiglas plates (25 cm × 37 cm × 0.5 cm) with six engraved circular arenas (8.5 cm ∅ × 0.5 mm deep) were used. Arenas were filled evenly with a homogenous yeast suspension (distilled water and bakers' yeast in a 2:1 ratio by weight). A randomly sampled larva was placed in the center of each arena, covered with a Petri dish lid and allowed to move freely for 5 min. Path length was measured and recorded as above.

The roll over behavior test was adapted from Ball et al. (1985). A randomly sampled larva was placed in an agar-filled Petri dish as above. After 60 s of acclimatization, the larva was gently rolled over with a soft paint brush until its ventral surface was facing upward. The amount of time required for the larva to completely right itself was recorded. If this did not occur within 3 min, observation was discontinued and roll over time was scored as 180 s.

Adult Behavior

The locomotor component of adult foraging behavior was quantified using a modification of procedures described in Pereira and Sokolowski (1993). Male flies (4- to 6-day-old) were fed water only for 18 ± 0.5 h prior to testing. Individuals were placed on a 0.2 µl drop of 0.25 M sucrose in the center of a white arena (1 m^2 × 30 cm high), illuminated from above with a 40 W bulb. After the sucrose drop was consumed, the maximum distance from the center of the arena walked by the fly within 30 s was recorded.

RESULTS

Larval Behavior

In all but two cases, we observed reduced locomotor behavior in CC mutant larvae compared with the *CS* control (Fig. 1). On agar (Fig. 1*A*), path lengths of both nob^1 and ebo^2 larvae were not different from that of *CS* while those of the remaining four CC mutant strains were shorter. On yeast (Fig. 1*B*), all six CC mutant strains had shorter path lengths than the "rover-like" *CS* control. This suggests that nob^1 and ebo^2 larvae have foraging-related defects while cbd^1, ccd^1, ceb^1 and cex^1 have general locomotor impairments.

In the roll over test (Fig. 2), only cex^1 was slower than the *CS* control while ceb^1 was slightly faster. This finding indicates that larval muscle function is likely not affected in five of the six CC mutants. The anatomical focus of roll-over difficulties in cex^1 larvae is unknown.

We performed a deficiency analysis to test whether nob^1 larval foraging differences were attributed to the nob^1 mutation (Fig. 3). In a for^R background, both nob^1 (nob^1; for^R) and $nob^1/Df(1)HC244$ ($nob^1/0$; for^R), as well as the for^s control (+; for^s) had sitter path lengths which were significantly shorter than the rover path lengths of nob^1/nob^+ (nob^1/+; for^R) and the for^R control (+; for^R). This shows that nob^1 fails to complement the deficiency *Df(1)HC244* for larval foraging behavior.

We compared path lengths for eight allelic combinations of *nob*, *for* and *Csr* to determine if interactions among the three genes have an effect on larval locomotion (Fig. 4). Path lengths were not different on agar, showing that these genes do not influence general larval locomotion (Fig. 4*A*). Interestingly, we did observe path length differences for larvae tested on

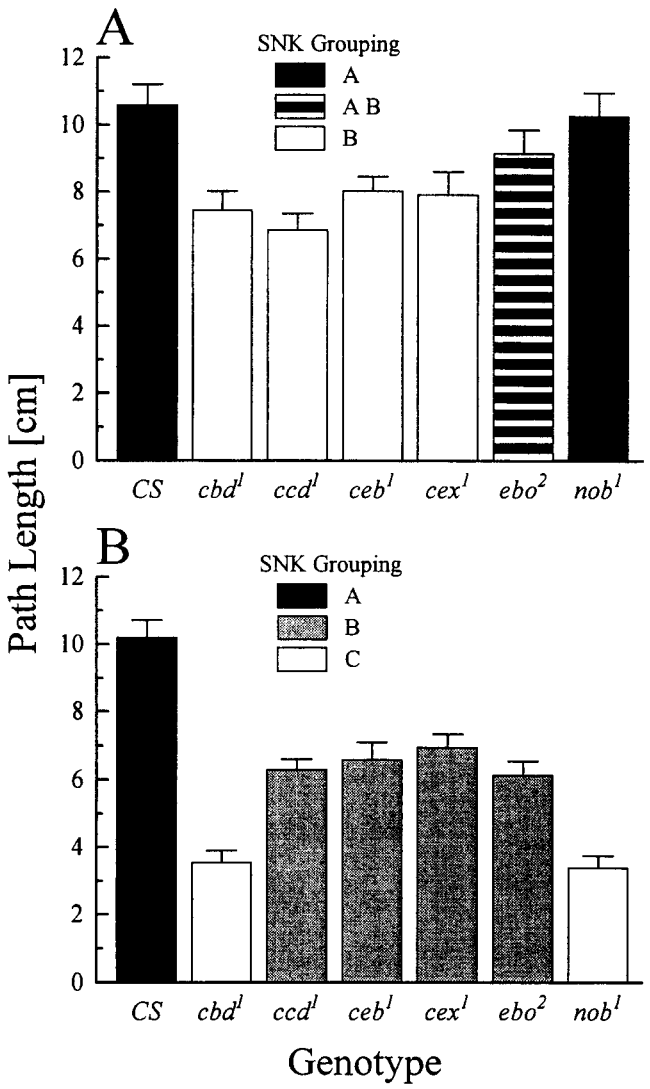

FIGURE 1 Locomotor behavior of CC mutant larvae. Bars are mean ± SE path length. In all figures, bars of the same shading, in whole or in part, were not significantly different. (*A*) On agar (non-foraging surface), differences were significant [analysis of variance (ANOVA) $F_{[6,133]} = 6.31$, $P < 0.0001$, $n = 20$/bar]. A Student-Numan-Keuls test (SNK) identified two partly overlapping groups ($P \leq 0.05$) (Zar, 1984; SAS Institute, 1985). (*B*) On yeast (foraging surface); differences were significant (ANOVA, $F_{[6,168]} = 31.06$, $P < 0.0001$, $n = 25$/bar) and fell into three groups (SNK, $P \leq 0.05$).

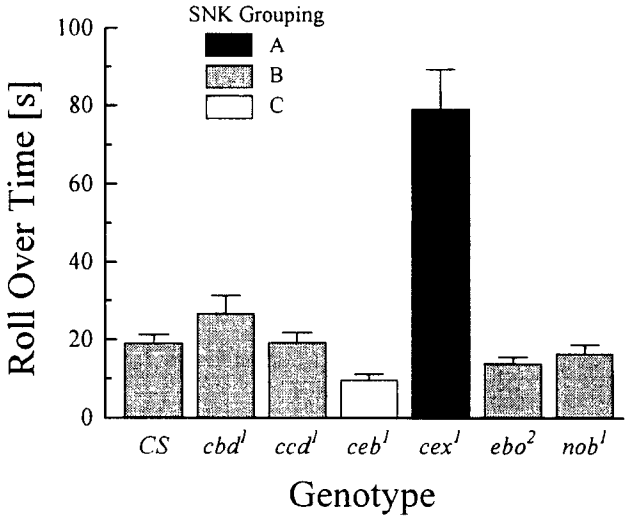

FIGURE 2 Roll over time of CC mutant larvae. Bars are mean ± SE. Statistical comparisons were made using log transformed data to correct for deviations from normality (Zar, 1984). Differences were significant (ANOVA, $F_{[6,91]} = 21.4$, $P < 0.0001$, $n = 14$/bar). A SNK test identified three groups ($P \leq 0.05$).

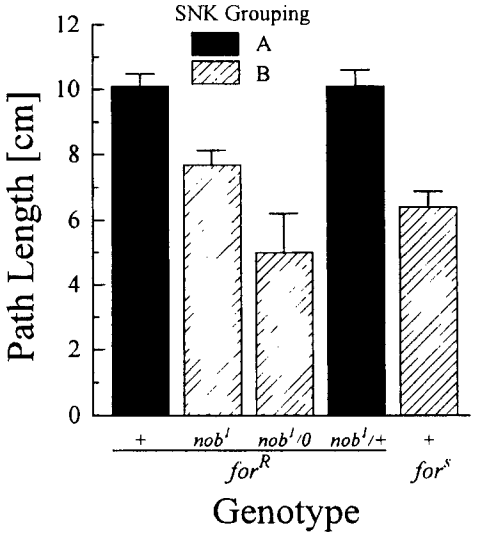

FIGURE 3 Deficiency analysis of the nob^1 larval foraging path length. Bars are mean ± SE. Differences were significant (ANOVA, $F_{[5,143]} = 12.77$, $P < 0.0001$, $9 \leq n \leq 34$) and fell into two groups (SNK, $P \leq 0.05$).

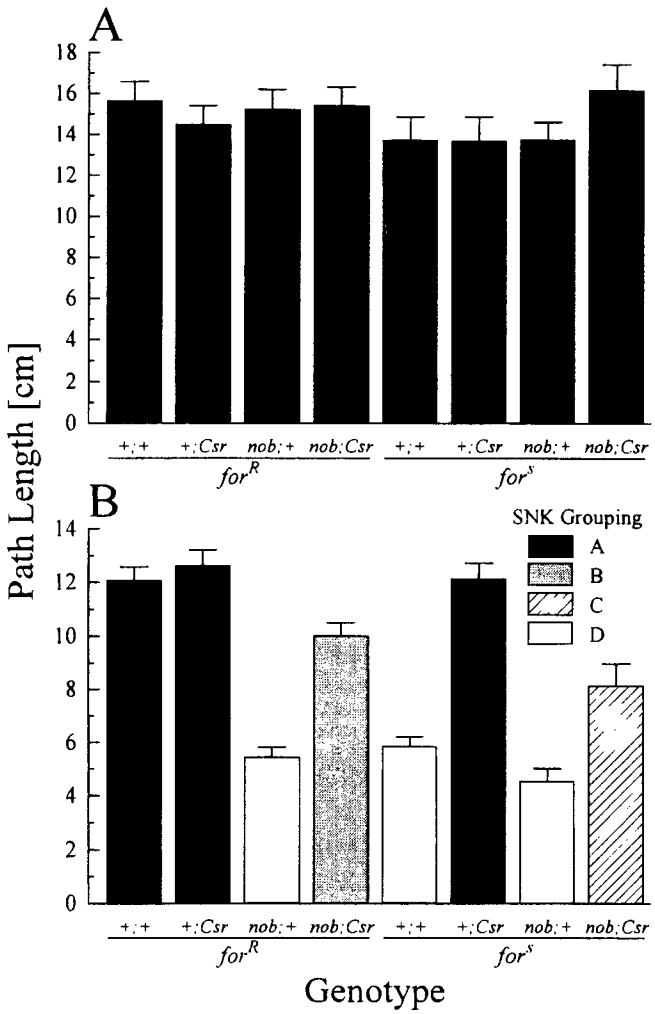

FIGURE 4 Locomotor behavior of *nob, for* and *Csr* larvae. Bars are mean ± SE path length. (*A*) On agar, differences were not significant (ANOVA, $F_{[7,156]} = 0.84$, $P = 0.55$, $13 \leq n \leq 25$). (*B*) On yeast, differences were significant (ANOVA, $F_{[7,220]} = 43.80$, $P < 0.001$, $21 \leq n \leq 34$). A SNK test identified four groups ($P \leq 0.05$).

yeast (Fig. 4*B*). Genotypes fell into four groups. The rover group consisted of the for^R control (+ ; for^R; +), as well as nob^+/nob^1; for^R/for^s; Csr^3/Csr^+ (+; for^R; *Csr*) and nob^+/nob^1; for^s/for^s; Csr^3/Csr^+ (+; for^s; *Csr*). These observations are consistent with previous characterization of *Csr* (Pereira et al.,

1995). The sitter group consisted of the for^s control (+; for^s; +), as well as nob^1; for^R (*nob*; for^R; +) and nob^1; for^s (*nob*; for^s; +). Two intermediate groups were comprised of one strain each, both bearing mutant nob^1 and Csr^3 alleles but different *for* alleles. Foraging path lengths of nob^1/Y; for^R/for^s; Csr^3/Csr^+ (*nob*; for^R; *Csr*) were significantly longer than those of nob^1/Y; for^s/for^s; Csr^3/Csr^+ (*nob*; for^s; *Csr*). These results demonstrate the additive effects of nob^1 (in decreasing "rover-like" path length) and Csr^3 (in increasing "sitter-like" pathlength) on *for*.

Adult Behavior

We tested four allelic combinations of *for* and *Csr* to examine interaction effects on adult foraging behavior (Fig. 5). *nob* was not included in this analysis because it is known to have a general influence on walking in flies (Strauss et al., 1992). Genotypes fell into two statistically different groups. Consistent with Pereira and Sokolowski (1993), for^R flies (for^R; +) walked further from the recently ingested sucrose drop than did for^{s2} (for^{s2}; +). However, unlike in larval foraging tests (Fig. 4*B*), the mutant Csr^3 allele did not increase walking distance in combination with either *for* allele (for^R; *Csr* and for^s; *Csr*).

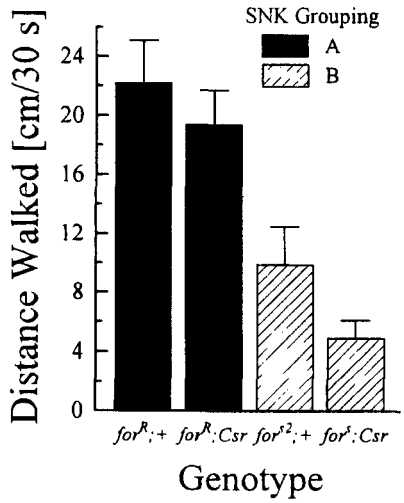

FIGURE 5 Locomotion after feeding of *for* and *Csr* adult flies. Bars are mean ± SE distance walked after feeding. Differences were significant (ANOVA, $F_{[3,46]} = 13.52$, $P < 0.0001$, $7 \leq n \leq 17$) and fell into two groups (SNK, $P \leq 0.05$).

DISCUSSION

The hypothesis that the relatively limited behavioral repertoire of legless larvae does not require an elaborate organizer of locomotion was put forth by Hanesch et al. (1989). Thus, we might expect mutations affecting the development and function of an adult-specific structure such as the CC to have no influence on larval behavior. However, larvae of six CC mutant strains showed reduced locomotor behavior in our study. For cbd^1, ccd^1, ceb^1 and cex^1 the reduction was of a general nature (independent of substrate nutrient quality), whereas, for ebo^2 and nob^1, it was foraging-specific. Locomotion on a non-nutritive surface and muscle function tests were both normal for ebo^2 and nob^1 as well, indicating that neither mutant is defective in a non-neuronal or general respect. Furthermore, these behavioral phenotypes were not a function of polygenic variability because all mutants shared a common *CS* "rover" genetic background. A "motivation deficit", as the reduced foraging path length may be termed, can be a hallmark of a higher control center (Kien and Altman, 1992). Task-dependent deficits in adult walking speed have been described for some CC mutants (Strauss and Heisenberg, 1993). The observation that ebo^2 and nob^1 larvae also have task-dependent locomotor defects may indicate that decision-making processes during foraging occur in higher brain centers rather than in the periphery.

Convincing anatomical evidence favors the CC precursor as a focus for the behavioral deficits common to different mutant third instar larvae. Electron microscopy studies by Hanesch (1987) identified strongly reduced fiber numbers in the CC precursor bundles of cbd^1, ccd^1, ebo^2 and nob^1 white pupae (ceb^1 and cex^1 were not included in her study). In ccd^1 and nob^1, the reduction was restricted to the CC precursor bundle which is well defined by a glial boundary layer. In cbd^1 and ebo^2, she found an additional reduction in the number of fibers within the interhemispheric commissure but lying outside of the CC precursor boundary (Hanesch, 1987). Quantitatively, Strauss et al. (1992) found that fiber number in nob^1 white pupae CC precursor bundles was reduced to about 1300, compared with the *Berlin* wild-type control which had approximately 4600. Both of the above studies have dealt with mutations in their "original" genetic backgrounds (mainly *Berlin* and *C(1)DX, y w f*). It is worth noting that for all of these mutations, adult brain anatomy phenotypes have become more severe after outcrossing to *CS* (de Belle and Heisenberg, 1996; J. S. de Belle, unpublished data).

Multiple structural defects can be recognized in most brain anatomy mutants (de Belle and Heisenberg, 1996). In addition to CC deformities, both cbd^1 and ccd^1 often have deranged optic lobes (Strauss and Heisenberg, 1993; J. S. de Belle, unpublished data). ceb^1 flies have severely mutant mushroom bodies (MBs) and are deficient in olfactory learning (Heisenberg, 1989; Strauss and Heisenberg, 1993; de Belle and Heisenberg, 1996; J. S. de Belle, unpublished data). Two of the three *ebo* alleles not examined here also have MB phenotypes (de Belle & Heisenberg, 1996)—conceivably ebo^2 has a similar mild defect which was not visible under the light microscope. Although brain anatomy phenotypes are not always CC-specific in CC mutant adults, we found no evidence for an influence of additional structures on the behavior of CC mutant larvae.

Mosaic analysis can often localize the focus of mutant behavior (Hotta and Benzer, 1972). Although we have not used this method to study locomotor defects in mutant larvae, results from similar experiments with adults may be of some predictive value. In cbd^1 and cex^1 flies, mosaic analyses of walking identified mutant effects in the brain and ventral ganglion (Strauss and Heisenberg, 1993). In particular, cex^1 showed severe gait problems which were attributed to a mutant focus in the ventral ganglion. Interestingly, we found that cex^1 larvae had considerable difficulty in the roll over test, a phenotype not likely related to higher brain malfunction. Mosaic studies have not been performed on ccd^1 and ceb^1. A study of ebo^2 showed that some parameters of tethered flight were coupled with defects in both the brain and the ventral ganglion (Ilius et al., 1994). Finally, mosaic analyses of nob^1 identified a focus in the brain for aberrant walking (showing full correlation with the state of the protocerebral bridge) (Strauss et al., 1992; Strauss and Heisenberg, 1993; R. Strauss, unpublished data). It is highly improbable that six strains with common CC anatomy and adult locomotor defects would also have common pleiotropic effects on larval locomotion and foraging. We therefore extend the hypothesis of Hanesch et al. (1989) and suggest that the suite of genes supporting proper development and function of the differentiated adult CC is also involved in a larval behavior organizer.

Comparatively specific mutant anatomical and behavioral phenotypes have been described for nob^1 (see above). nob^1 larvae were especially interesting in our study since they showed a strong sitter phenotype—a short path length on yeast—despite the rover backgrounds of both *CS* and for^R. Deficiency analysis of hemizygous nob^1 larvae in a for^R background

confirmed that nob^1 itself influences larval foraging behavior. We showed that *nob* also interacts with other larval foraging-related genes. Larvae bearing both nob^1 and Csr^3 and either for^R or for^s had intermediate foraging path lengths, suggesting that nob^1 and Csr^3 have independent and opposite effects on the foraging phenotype. Note the absolute differences in path length between experiments, demonstrating the sensitivity of larval behavior to genetic background and daily environmental variation [for example, compare nob^1 (in a *CS* "rover" background) in Figure 1*A* with $nob^1\ for^R$ in Figures 3 and 4*B*].

Our adult foraging results show that, unlike *for*, *Csr* has an effect on larval foraging behavior only. Further examination of *nob*, *for*, and *Csr* interaction will be aided by molecular analysis of these genes.

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