MATERIALS AND METHODS

Drosophila stocks

Fly stocks were maintained at 25C or 18C on a standard media.

The following fly strains were used: wild-type (Oregon-R). The *ey*J5.71 and *ey*C7.20 (near null allele) were provided by W. J. Gehring (Basel). The *dac* null mutant was *dac*4 (Mardon *et al.*, 1994). The *so-lacZ* flies (Cheyette *et al.*, 1994) were provided by L. Zipursky (California). MB-*GAL4* enhancer-trap lines (201Y, 238Y, c739; Yang *et al.*, 1995), *GAL4-OK107* enhancer trap (Connolly *et al.*, 1996), $elav^{C155}$-*GAL4* (Lin *et al.*, 1994), UAS-*lacZ* (Brand and Perrimon, 1993), UAS-*tau-lacZ* (Callahan and Thomas, 1994), UAS-*fas II* [PEST (-)](Lin *et al.*, 1994), *UAS-GFP* (provided by E. Hafen, Zurich) and *UAS-mCD8::GFP* (Lee and Luo, 1999). P[*dnc-lacZ*] flies, which express *lacZ* driven by the *dunce* (*dnc*) enhancer (Qiu and Davis, 1993), were provided by R. Davis (Houston). $fas\ II^{e86}$ (hypomorphic, 50%), $fas\ II^{e76}$ (hypomorphic, 10%) and $fas\ II^{eB112}$ (null allele) are described in Grenningloh *et al.*, 1991. Stocks used for ectopic *fas II* expression are *UAS-fas II[PEST(-)]/CyO*, *UAS-mCD8::GFP/FM7c; UAS-fas II[PEST(-)]/CyO*, *elav-GAL4, UAS-mCD8::GFP/CyO*.

MARCM mosaic analysis

Following stocks were used: $GAL4^{e155}$, *hs-FLP;* FRT^{G13}, *UAS-mCD8::GFP/CyO* and FRT^{G13}, *tubP-GAL80/CyO* for layer development analyses, and *hs-FLP, tubP-GAL80, FRT19A; 201Y/SM1*, and $fas\ II^{eB112}$, *FRT19A/FM7c; UAS-GFP-T2/SM1* for *fas II* mutant analyses. Mosaic clones were generated as described (Lee and Luo, 1999). Eggs (0-1.5 hrs old) collection was done for 1.5 hrs on standard food at 25C. A single 60-min heat shock at 37.5C was applied at 27 - 28 hrs after egg laying for induction of mitotic

recombination in the first instar stage, and 75 – 76 hrs after egg laying for that in the early third instar stage. Clones were examined at the wandering larval stage.

Immunocytochemistry and *in situ* hybridization

Immunostaining of brains was done as described previously (Hirth *et al.*, 1995; Tettamanti *et al.*, 1997; Nagao *et al.*, 2000). The following primary antibodies were used: goat FITC-conjugated anti-horseradish peroxidase (HRP) diluted 1:300 (Jackson ImmunoResearch); Alexa Fluor-conjugated phalloidin diluted 1:40 (Molecular Probes); rabbit anti-β-GAL diluted 1:1000 (Chemicon International); rabbit anti-EY diluted 1:300 (gift from U. Walldorf); mouse anti-DAC (mABdac2-3; Mardon *et al.*, 1994) diluted 1:250; mouse anti-EYA (mAB10H6; Bonini, *et al.*, 1993) diluted 1:250; mouse anti-FAS II (mAB1D4; Grenningloh *et al.*, 1991) diluted 1:5; rabbit anti-DIF (Cantera *et al.*, 1999) diluted 1:250; rabbit anti-Synaptotagmin (Littleton *et al.*, 1993) diluted 1: 1500 and rat anti-mCD8α diluted 1:100 (Caltag). FITC-, Cy3-, or Cy5-conjugated secondary antibodies (Jackson ImmunoResearch) were used at dilution of 1:400. Fluorescent *in situ* hybrydization was according to Goto and Hayashi (1997).

Laser scanning confocal microscopy

Confocal images were captured by using Zeiss LSM410 or LSM510. Optical sections were made from 1 to 4 μm thick. Images were processed digitally and then arranged with Adobe PhotoShop.