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**Pupal expression pattern of an Ocellar specific *P-GAL4* enhancer trap strain of *Drosophila melanogaster*.**

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*Drosophila* adult brain is a highly complex system, consisting of millions of neurons, organized into specific functional modules with intricate and highly specific internal and external connectivity. The genetic pathways which regulate the developmental program of the construction of this remarkable structure have scarcely been understood. A *P-GAL4* enhancer trap screen (Brand and Perrimon, 1993) was done as an attempt to identify genes with an expression in the brain during development (Shyamala and Chopra, 1999). We here report the expression pattern of an ocellar specific strain, in the brain, during its pupal development.

The F1 embryos of the cross between *P-GAL4* strain SG19.1 and the UAS *Lac-Z* strain were raised at 22°C. Formation of white pupa was taken as zero hour, and pupae were staged accordingly as the number of hours After Puparium Formation (APF). The staged pupae were dissected out, and the brain along with ventral ganglion was stained for  $\beta$ -galactosidase activity using the standard protocol (VijayRaghavan *et al.*, 1986).

The insertion of reporter gene in the strain SG19.1 is in the third chromosome, and the homozygous individuals are viable. At 0 hr APF (Figure 1a) the expression of reporter gene is seen in a pair of cells in the brain located in the Interhemispheric junction (IJ) (Hanesch *et al.*, 1989), a pair of doublets in the Suboesophageal ganglion (SOG) (Truman, 1990), (Figure 1a) along with two cells present towards the posterior abdominal neuromeres of Ventral ganglion. At 14 hr stage the expression gets restricted to the cells at IJ and SOG with a marked enhancement in the expression levels (Figure 1b-c). Expression in the ventral ganglion cells is withdrawn. A pattern similar to that of 14 hr stage is observed through 24, 37, 48, 72 till 82 hr APF (Figures 1d and 1e: 37 and 48 hr APF, respectively). In 99 hr APF and in the pharate brain (Figure 1f), the reporter gene expression is consistently restricted to only the pair of cells situated in the cortical region present anterior to the superior medial Protocerebrum (SMP) (Ref: Flybrain). These cells are equivalent to the cells seen in the Interhemispheric junction of brain during the earlier pupal stages.

Earlier studies have shown that the adult head sections of this strain have reporter gene expression in the three Ocellar neurons present on the forehead, ocellar nerve, and in the cells situated in the frontal brain (Shyamala and Chopra, 1999). The ocellar specific expression of the reporter gene during pupal stages strongly suggests that the native gene at the site of *P-GAL4* insertion may have an important role in the development and differentiation of the ocellar neurons.

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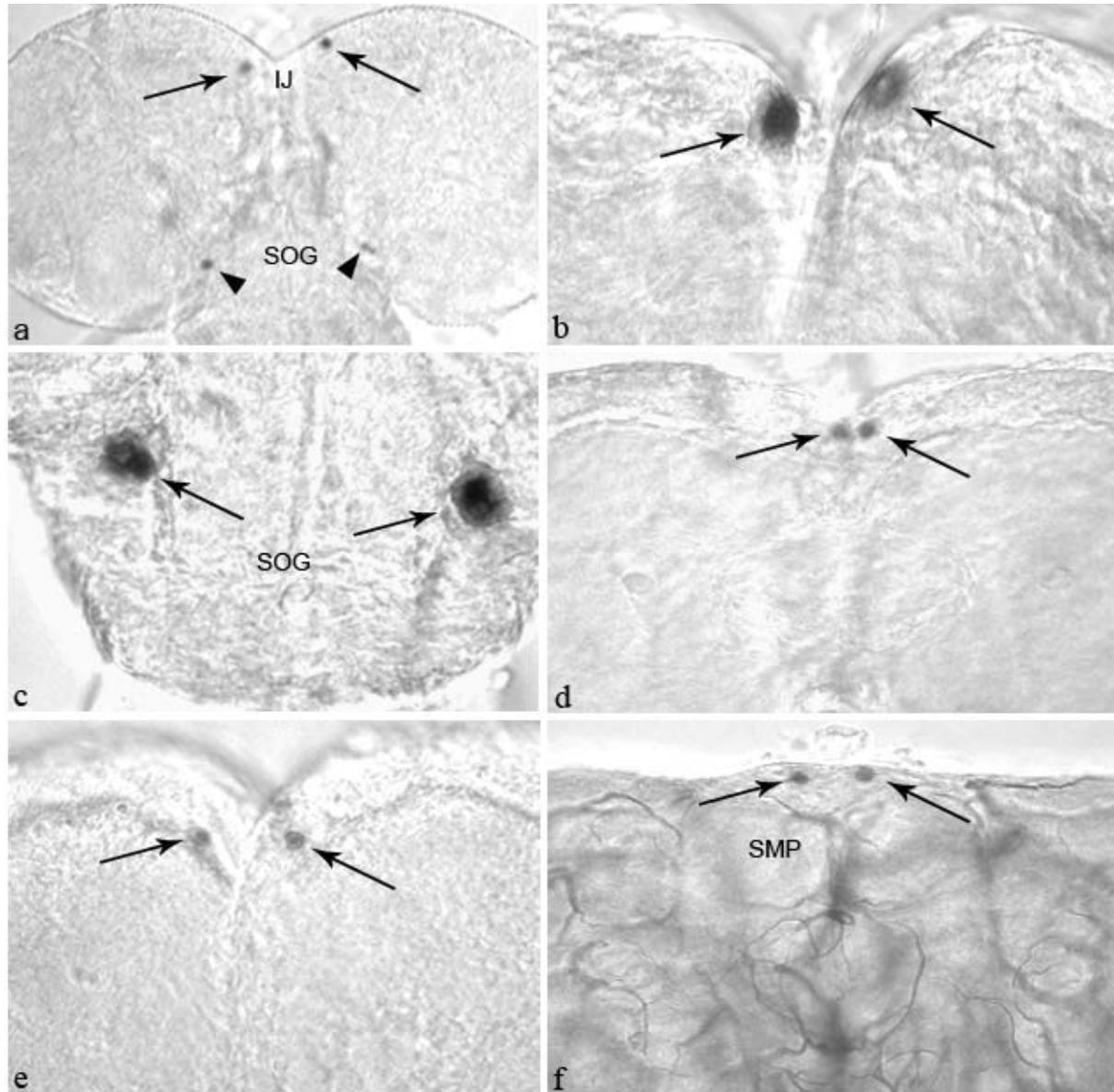


Figure 1. Brain of *P-Gal4* insertion strain SG 19.1 during pupal stages. Arrowheads and arrows indicate cells with reporter gene expression. a) 0 hr APF in dorsal view (DV), cells with expression are seen in IJ and in SOG. b and c) 14 hr APF stage in DV. b) stained cells can be seen in IJ region. c) paired cells seen in the SOG with reporter expression. d) 37 hr APF stage in DV, showing cells at IJ. e) 48 hr APF stage in DV, two cells with reporter expression can be seen in IJ. f) pharate brain in frontal view, showing a pair of cells anterior to the SMP with reporter gene expression.

References: Brand, A.H., and P. Norbert 1993, *Development* 118: 401-415; Hanesch, U., K.F. Fischback, and M. Heisenberg 1989, *Cell Tissue Res*, 257: 343-366; Shyamala, B.V., and A. Chopra 1999, *J. Genet.* 78: 87-97; Truman, J.W., 1990, *J. Neurobiol.* 21: 1072-1084; Vijayraghavan, K., M.A. Crosby, P.H. Mathers, and E.M. Meyerowitz 1986, *EMBO J.* 5: 3321-3326; Flybrain <http://flybrain.neurobio.arizona.edu>.