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ORIGINAL ARTICLE

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Analysis of neural elements in head-mutant *Drosophila embryos* suggests segmental origin of the optic lobes

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Abstract We describe the development of 20 sensory organs in the embryonic *Drosophila* head, which give rise to 7 sensory nerves of the peripheral nervous system (PNS), and 4 ganglia of the stomatogastric nervous system (SNS). Using these neural elements and the optic lobes as well as expression domains of the segment polarity gene *engrailed* in the wild-type head of *Drosophila* embryos as markers we examined the phenotype of different mutants which lack various and distinct portions of the embryonic head. In the mutants, distinct neural elements and *engrailed* expression domains, serving as segmental markers, are deleted. These mutants also affect the optic lobes to various degrees. Our results suggest that the optic lobes are of segmental origin and that they derive from the ocular segment anteriorly adjacent to the antennal segment of the developing head.

Key words *Drosophila* · Head development · Segmentation mutants · Nervous system · Optic lobe

Introduction

Since the influential reviews of Siewing (1963) and Rempel (1975), it has been widely accepted that the insect head consists of an unsegmented anterior end, termed the acron, and six segments which fused with the acron in the course of head tagma evolution: a labral, antennal, intercalary and three gnathal segments.

In this view the acron has been defined as the eye-bearing part of the head including a fraction of the protocerebrum (mushroom bodies, optic lobes). Consequently, the optic lobes have been considered diagnosic structures of the acron in the embryo (Rempel 1975; Jürgens and Hartenstein 1993). This assignment of optic structures to the acron is ultimately based on a comparison of the heads of annelids and insects which are thought to derive from annelid-like ancestors (Rempel 1975). The tip of the annelid head, the prostomium, lacks an obvious segmentation (see, however, Fischer 1985 and Dorresteijn et al. 1993, who present evidence that a segment is incorporated in the prostomium during embryogenesis). Since reliable morphological segment markers are also missing in the ocular region of the embryonic insect head, this region has been deemed homologous to the annelid prostomium.

The labral segment has been defined as comprising the clypeus and labrum (which are fused in *Drosophila*) and the remaining part of the protocerebrum, in particular neurosecretory cells of the pars intercerebralis, the protocerebral bridge, the central body and the accessory lobes or "Nebenlappen" (Rempel 1975). Essentially three arguments led to the postulation of a labral segment in the first place. First, as in other segments, a pair of coeloms occurs transiently in the labrum of many species. Secondly, the labrum initially often appears as a bilobed structure suggesting its evolutionary origin from fused appendages. And thirdly, in crustaceans which like the insects belong to the Mandibulata, two pairs of ganglia are formed in front of the antennal segment which have been assigned to the acron (archicerebrum) and a first head segment (labral segment; Siewing 1963). However, Scholtz (1994) suggests that both pairs of preantennal ganglia belong to either the same acron or segment because he finds that *engrailed (en)* expressing cells in these ganglia derive from the same first stripe of *en* expression.

The labral segment has been thought to be followed by the antennal segment which includes the deutocerebrum, then by the intercalary segment which includes the tritocerebrum and, finally, by the three segments of the gnathocephalon which include the suboesophageal ganglion. A conspicuous feature of the theory of head segmentation summarized above is that a labral segment is interspersed between an eye-bearing acron and the antennal segment (hypothesis I in Fig. 1A).

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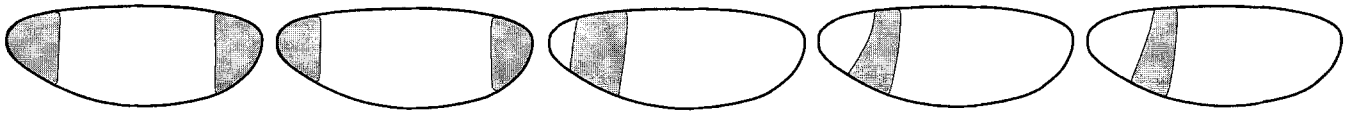
A

hypothesis I			hypothesis II
segment/head region	diagnostic structure in *Drosophila* embryo		segment/head region
acron	optic lobe	clypeolabrum	labral
labral	clypeolabrum	optic lobe	ocular
antennal	deutocerebrum/dorsal organ		antennal
intercalary	tritocerebrum		intercalary
gnathocephalon	subesophageal ganglion/gnathal buds		gnathocephalon

B



Tsl
anterior: 83-100%
posterior: 0-17%

Tll
anterior: 80-100%
posterior: 0-20%

otd
70-90%

ems
dorsal: 70-76%
ventral: 74-89%

btd
65-77%

Fig. 1A Two theories of head segmentation (*I* and *II*) and structures which are diagnostic for different segments/head regions. **B** Transitory distributions of transcripts *(btd, ems, otd)* or protein (Tsl, T11) during blastoderm stage of the *Drosophila* embryo after Finkelstein and Perrimon (1990), Pignoni et al. (1990, 1992), Walldorf and Gehring (1992), Wimmer et al. (1993) and Martin et al. (1994). Since Tor is expressed in the entire blastoderm but is active only in a range determined by Tsl the distribution of the latter is shown. The expression patterns change during development and a segmental pattern is observed in later stages. However, fate-mapping data and the deletion of markers in confined head-regions of *tor, otd, ems* and *btd* suggest that the blastodermal expression (activity) is responsible for the phenotypes described. Anterior to the left, ventral side down *(btd* buttonhead, *ems* empty spiracles, *otd* orthodenticle, *Tll* Tailless, *tor* torso, *Tsl* Torso-like *%, % egg length (0% posterior pole))*

Recent observations challenge this traditional view. Segmental expression patterns of several segment polarity genes in the embryonic head of *Drosophila* (Lee et al. 1992; Ouelette et al. 1992; Schmidt-Ott and Technau 1992; Tabata et al. 1992), as well as the segment polarity gene expression patterns of *engrailed (en)* in other insects (Schmidt-Ott et al. 1994a) and crustaceans (Scholtz 1994) suggest an ocular segment which includes the optic lobes and which is anteriorly adjacent to the antennal segment as outlined in hypothesis II (Fig. 1A). An essential difference of the two hypotheses is their answer to the question: where is anterior in the insect head?

In order to answer this question, we made use of *Drosophila* lack-of-function mutations in genes which affect head development such as *torso (tor),* a key element of the maternal terminal organizer system required for zygotic expression of the terminal gap-gene *tailless (tll),* and the head gap-genes *orthodenticle (otd), empty spiracles (ems)* and *buttonhead (btd)* (for review see Jürgens and Hartenstein 1993). These genes are initially expressed in single broad stripes in the blastodermal head anlage (Fig. 1B). Their expression domains in wild-type embryos correlate with the deletion of adjacent segments in the respective mutants (Jürgens et al. 1986), i.e. in the absence of *tor* activity the anteriormost structures are deleted, in *otd* mutants both the ocular and antennal segments are absent, in *ems* mutants the posterior ocular, antennal and intercalary segments are missing, and in *btd* mutants the antennal, intercalary and mandibular segments fail to develop (Cohen and Jürgens 1990; Schmidt-Ott et al. 1994b).

In the present study, we describe in detail the development of neural elements in the wild-type head of the *Drosophila* embryo and we examine the neural head phenotypes caused by the lack of *tor, otd, ems, btd* and *tll* activities. For a schematic summary of the phenotypes in the peripheral (PNS) and stomatogastric nervous systems (SNS), and for documentation of the PNS and SNS phenotypes in *btd* and *ems* we refer to Schmidt-Ott et al. (1994b). Based on concomittant deletions of neural elements including the optic lobes and segmental *en* expression domains in the mutants, neural elements can be assigned to different segments. Our results suggest that, in the *Drosophila* embryo, the optic lobes derive from a segment anteriorly adjacent to the antennal segment.

Materials and methods

Strains of *D. melanogaster* were kept under standard conditions; wild-type (Oregon R) and mutant embryos were obtained as described previously (Wieschaus et al. 1984). The neural elements were analysed in embryos lacking *torso (tor)* activity (collected from homozygous tor^{PM} *females;* Sprenger et al. 1989; Casanova and Struhl 1993) and in embryos homozygous for the mutations $Df(3R)tll^{8}$ uncovering *tailless (tll;* Strecker et al. 1988), *Df(1)KA14* uncovering *orthodenticle (otd;* Finkelstein and Perrimon 1990), *empty spiracles*^{9Q64} *(ems;* Dalton et al. 1989; Walldorf and Gehring 1992), and *buttonhead*^{XG} *(btd;* Wimmer et al. 1993). Elements of the stomatogastric (SNS) and peripheral nervous systems (PNS) were labelled by monoclonal 22C10 antibody staining (Fujita et al. 1982) and *en* expression by monoclonal 4D9 antibody staining (Patel et al. 1989). The development of the optic lobes was traced in the various mutant backgrounds by anti-β-galactosidase antiserum staining of embryos carrying an enhancer trap insertion at the *sine oculis* gene locus with *lacZ* expression in

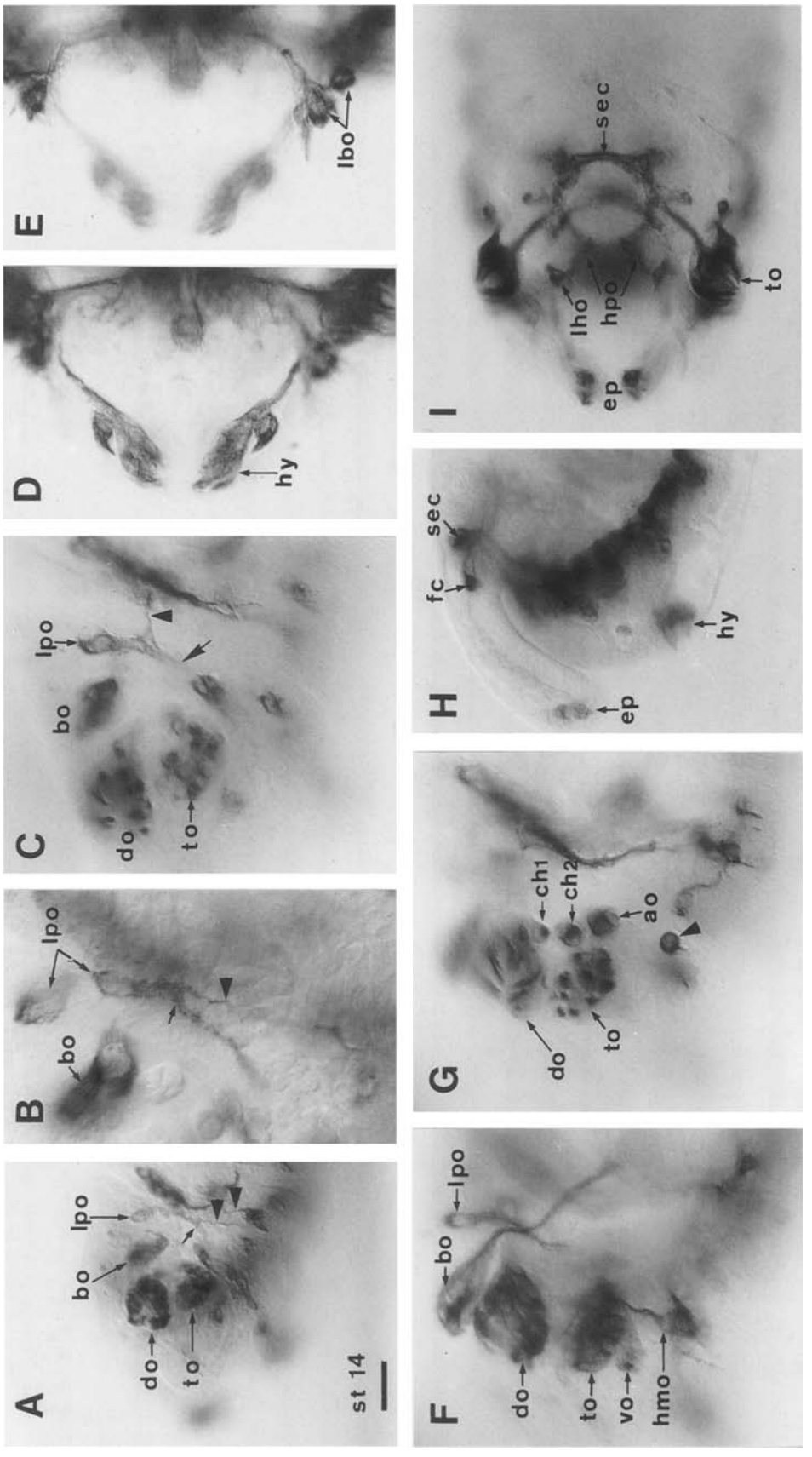


Fig. 2A–I Wild-type head at stage 14 stained with 22C10 antibody. The *triangles* in **A** and **B** point to the first and second fascicle of the prothoracic nerve. The fasciculation of the anterior fascicle with the lateropharyngeal nerve is indicated by an *arrow*. In **C** the *arrow* points to the lateropharyngeal nerve and the *triangle* to the transient "bridge" between the lateropharyngeal nerve and the prothoracic nerve. The *triangle* in **G** points to a chordotonal organ which fasciculates either with axons of the hypomaxillary organ *(hmo)* or the labial organ *(lbo)*. Anterior to the left, lateral (**A–C, F–H**), ventral (**D, E**) or dorsal (**I**) views (*ao* associated organ, *bo* Bolwig organ, *ch1* chordotonal organ ventroposterior to the dorsal organ, *ch2* two chordotonal organs dorsoposterior to the terminal organ, *do* dorsal organ, *ep* epiphysis, *fc* frontal connective, *hmo* hypomaxillary organ, *hpo* hypopharyngeal organ, *hy* hypophysis, *lho* latero-hypomaxillary organ, *lpo* lateropharyngeal organ, *lbo* labial organ, *sec* supraoesophageal commissure, *st* stage, *to* terminal organ, *vo* ventral organ; *bar* 20 µm **A**, 8 µm – **B**, 13 µm – **C–I**)

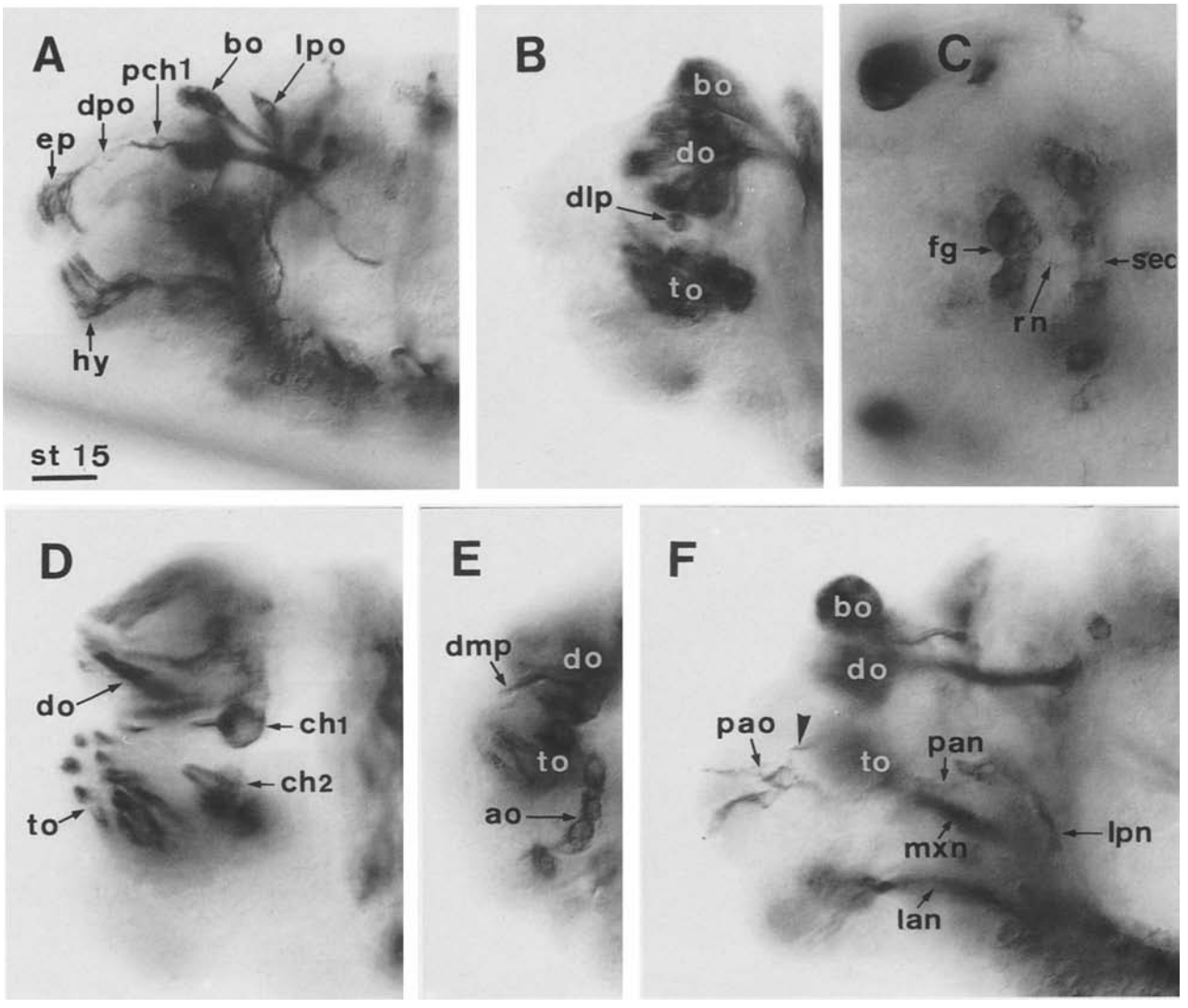


Fig. 3A–F Wild-type head at stage 15 stained with 22C10 antibody. The *arrowhead* in **F** points to the axon of the dorsolateral papilla which projects to the pao. Anterior to the left, lateral (**A, B, D–F**) and dorsal (**C**) views (see also legend of Fig. 2; *dlp* dorsolateral papilla, *dmp* dorsomedial papilla, *dpo* dorsopharyngeal organ, *fg* frontal ganglion, *lan* labial nerve, *lpn* lateropharyngeal nerve, *mxn* maxillary nerve, *pan* papilla nerve, *pao* papilla organ, *pch1* pharyngeal chordotonal organ, *rn* recurrent nerve; *bar* 20 μm – **A**, 13 μm – **B, C, E, F,** 8 μm – **D**)

the optic lobes (Bier et al. 1989; Green et al. 1993; Cheyette et al. 1994). For antibody stainings we used the Vectastain-ABC-Kit Elite (Camon). For staining procedures and examination of whole mount preparations see Schmidt-Ott and Technau (1992) and Schmucker et al. (1992). Stages of embryos refer to the staging by Campos-Ortega and Hartenstein (1985).

Results

The neural elements of the embryonic *Drosophila* head are grouped in three nervous systems: the central nervous system (CNS), the peripheral nervous system (PNS) and the stomatogastric nervous system (SNS; Horridge 1965; Penzlin 1985). The CNS of the head consists of the supraoesophageal and the suboesophageal ganglion. The optic lobes are part of the supraoesophageal ganglion but, contrary to the remainder of this ganglion, they arise by epithelial invaginations in holometabolous insects (Haget 1977). The arrangement of distinct neural elements throughout the embryo and the location of their progenitors along the entire longitudinal axis of the blastoderm make them suitable tools for determining the activity range of gap-like head genes in the respective mutants where adjacent segments are deleted. For identifying neural elements of the PNS and SNS, we used the monoclonal antibody 22C10 which detects an antigen in the membrane of neurons (Fujita et al. 1982). In order to study which parts of the CNS are affected in different head-mutant embryos, we used the enhancer trap line *A6-2-45* (Bier et al. 1989) with *lacZ* expression in the developing optic lobes and the monoclonal antibody 4D9 against the *en* protein (En; Patel et al. 1989). En staining also served for identifying head segments.

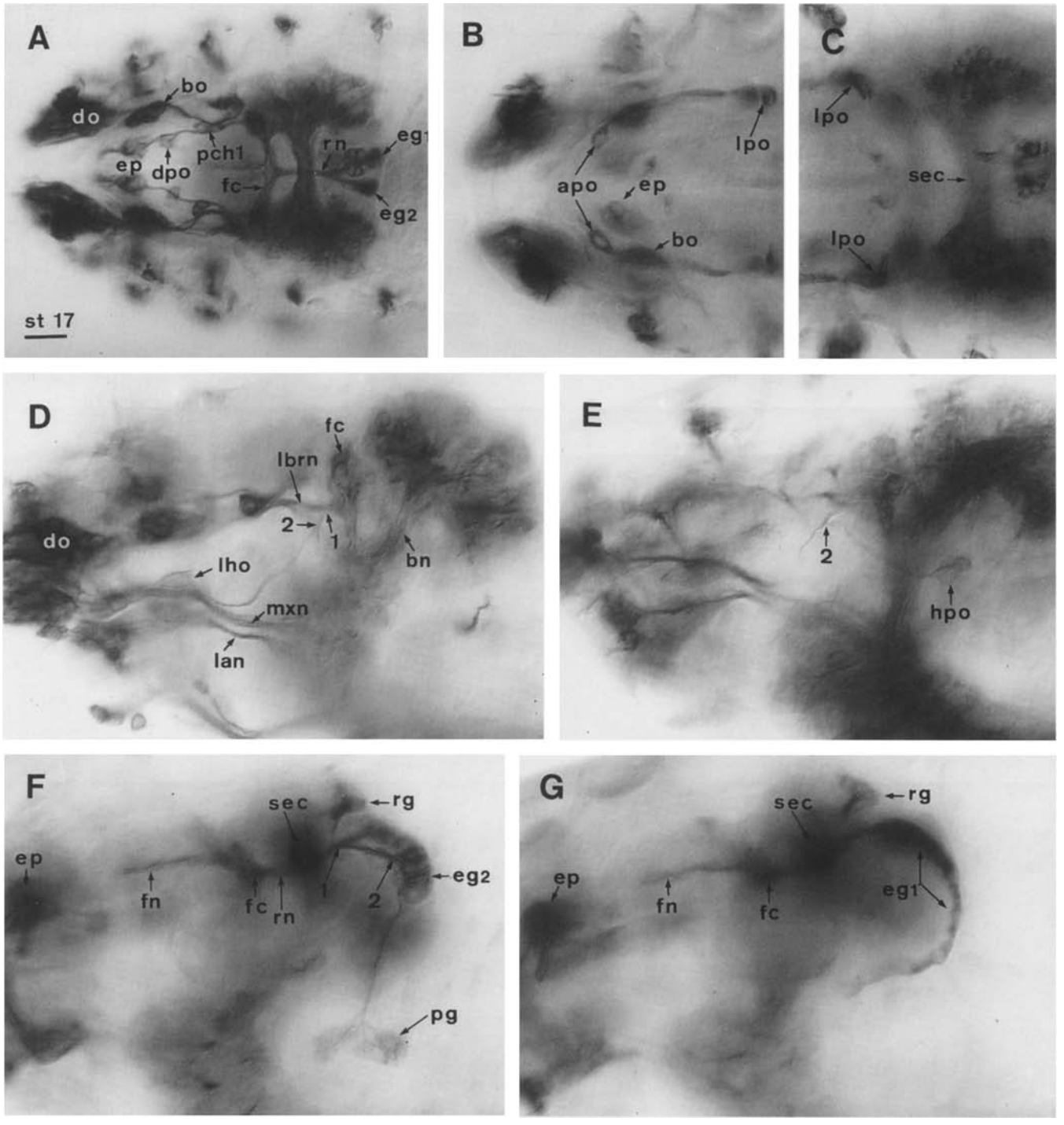
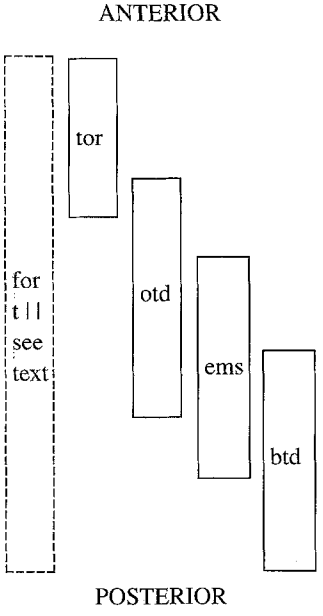


Fig. 4A–G Wild-type head at stage 17 stained with 22C10 antibody. Anterior to the left, dorsal (**A–C**) and lateral (**D–G**) views. In **D** and **E** "*2*" indicates the fasciculation of the projections of the lho and the hpo, "*1*" the fasciculation of their common fascicle with the labral nerve. (See also legends to Figs. 2 and 3; *eg1* oesophageal ganglion 1, *eg2* oesophageal ganglion 2, *fn* frontal nerve, *lbrn* labral nerve, *pg* proventricular ganglion, *rg* part of ring gland; *bar,* 20 μm – **A**, 13 μm – **B–G**)

Neural elements of the embryonic head

Using 22C10 antibodies we identified a total of 20 distinct sensory organs in the head and determined the time when they can be first detected (Figs. 2–4). Some of the organs described below can be seen in stage 13/14, others not before stage 15, and some become hidden by larger sensory organs at later stages. As will be shown, the axons of these sensory organs fasciculate to form a total of 7 sensory nerves of the PNS which project into distinct locations of the embryonic brain. We refer to

Table 1 Deletions of neural markers and *en (engrailed)* and *wg (wingless)* expression domains in the embryonic head. Wg has been analysed only in *btd, ems* and *otd* (Cohen and Jürgens 1990). The markers are listed with respect to the anteroposterior sequence of their progenitors in the early embryo. Segmental identities are indicated on the *right side.* The assumption of a labral segment beeing interspersed between an eye-bearing acron and the antennal segment would contradict the assumptions that *otd* and *ems* mutants have a gap-like head-phenotype and that terminal structures are deleted in *tor* mutants. Epidermal *en* and *wg* expression domains are written in *capitals,* those in the central nervous system with *small letters.* En Cl is located in the clypeolabrum which becomes epipharynx at later stages, Wg hb, En hs and En shs (ocular segment) lie in the protocerebrum, of Wg AS, En AS and En ant (antennal segment) En ant lies in the deutocerebrum, Wg ic and En ic (intercalary segment) lie in the tritocerebrum, and Wg MD, En MD and En md in the mandibular lobe and anterior subesophageal ganglion. *en* expression in the dorsal brain hemispheres (cf. Fig. 6F) has been omitted because this expression is weak and a reliable analysis in all mutants was not possible. For a detailed description of the dynamic En and Wg pattern in the course of head development we refer to Baker (1988) and Schmidt-Ott and Technau (1992). *AN* antennal segment, *btd* buttonhead, *ch1* chordotonal organ ventroposterior to the dorsal organ, *ems* empty spiracles, *En ant* En antennal spot, *En AS* En antennal stripe, *En CL* En clypeolabral spot, *En hs* En head spot, *En MD/md* En mandibular stripe, *En shs* Engrailed secondary head spot, *IC* intercalary segment, *LR* labral segment, *MD* mandibular segment, *OC* ocular segment, *otd* orthodenticle, *tll* tailless, *tor* torso, *Wg AS* Wg antennal stripe, *Wg hb* Wg head blob, *Wg MD* Wg mandibular stripe



Structure	Expression	Segment
SNS Epiphysis Dorsopharyngeal organ Pharyngeal chordotonal organ	En CL	LR
Supraesophageal commissure		?
Laterohypopharyngeal organ (?) Optic lobe epithelial invagination	Wg hb	OC
Bolwig's organ Dorsomedial papilla Hypopharyngeal organ	En hs & shs	
Dorsal organ ch1	Wg AS En AS & ant (ant not in all ems embryos)	AN
Dorsolateral papilla Associated organ	Wg ic En ic	IC
Papilla organ Lateropharyngeal organ Anterior pharyngeal organ (?)	Wg MD En MD & md	MD

them as the labral, Bolwig, antennal, papilla, laterophary-ngeal, maxillary and labial nerves.

The labral nerve is established at stage 13/14 and projects into the frontal connective which connects the suboesophageal ganglion with the SNS (see below). It receives axons from the epiphysis (ep), the dorsopharyngeal organ (dpo) and the pharyngeal chordotonal organ (pch1) (Figs. 2H, I, 3A, 4A). In addition, the labral nerve collects the axons of two other sensory organs, the latero-hypopharyngeal organ and the hypopharyngeal organ. At late stage 14, both organs lie closely together (Fig. 2I). However, in the course of embryonic development the latero-hypopharyngeal organ becomes located in the immediate vicinity of the maxillary nerve (Fig. 4D), whereas the hypopharyngeal organ – with two rod-like structures – lies close to the posterior pharynx (Fig. 4E).

The Bolwig nerve is established by stage 13 and projects into the optic lobes (Fig. 3A). It collects the axons of the 12 sensory cells of the Bolwig organ, the light sensory organ of the larva (Figs. 2A, F, 3A, 4A; see also Schmucker et al. 1992).

The antennal nerve is established by stage 13 and projects into the brain neuropile (Fig. 3F). It receives axons of the dorsal organ including a chordotonal organ (ch1; Figs. 2F, 3D) and the dorsomedial papilla (Figs. 3D, E). The ch1 is visible by stage 14 (Fig. 2G); the dorsomedial papilla is best distinguished at stage 15.

The papilla nerve is established by stage 15 and projects into the suboesophageal ganglion at a position above the maxillary nerve (Fig. 3F). It collects the axons of the papilla organ (Fig. 3F; visible by stage 14) and of the dorsolateral papilla (Fig. 3B; visible by stage 15). By late stage 15, the papilla nerve comes in close contact (or fasciculates with) the maxillary nerve. However, both nerves remain separated in the vicinity of the suboesophageal ganglion. The dorsolateral papilla and the papilla organ can be distinguished best at stage 15, since they become hidden by the dorsal, terminal and ventral organs later in development.

The latero-pharyngeal nerve is established at stage 13. At this stage a projection grows out from the antero-dorsal suboesophageal ganglion and pioneers the latero-pharyngeal nerve. Shortly thereafter, it fasciculates with a projection of the latero-pharyngeal organ and with an axon that grows out from the first thoracic neuromere (Fig. 2A, B). Thus, a transient "bridge" forms between the latero-pharyngeal nerve and the anterior fascicle of the first thoracic neuromere (Fig. 2C). This bridge dis-

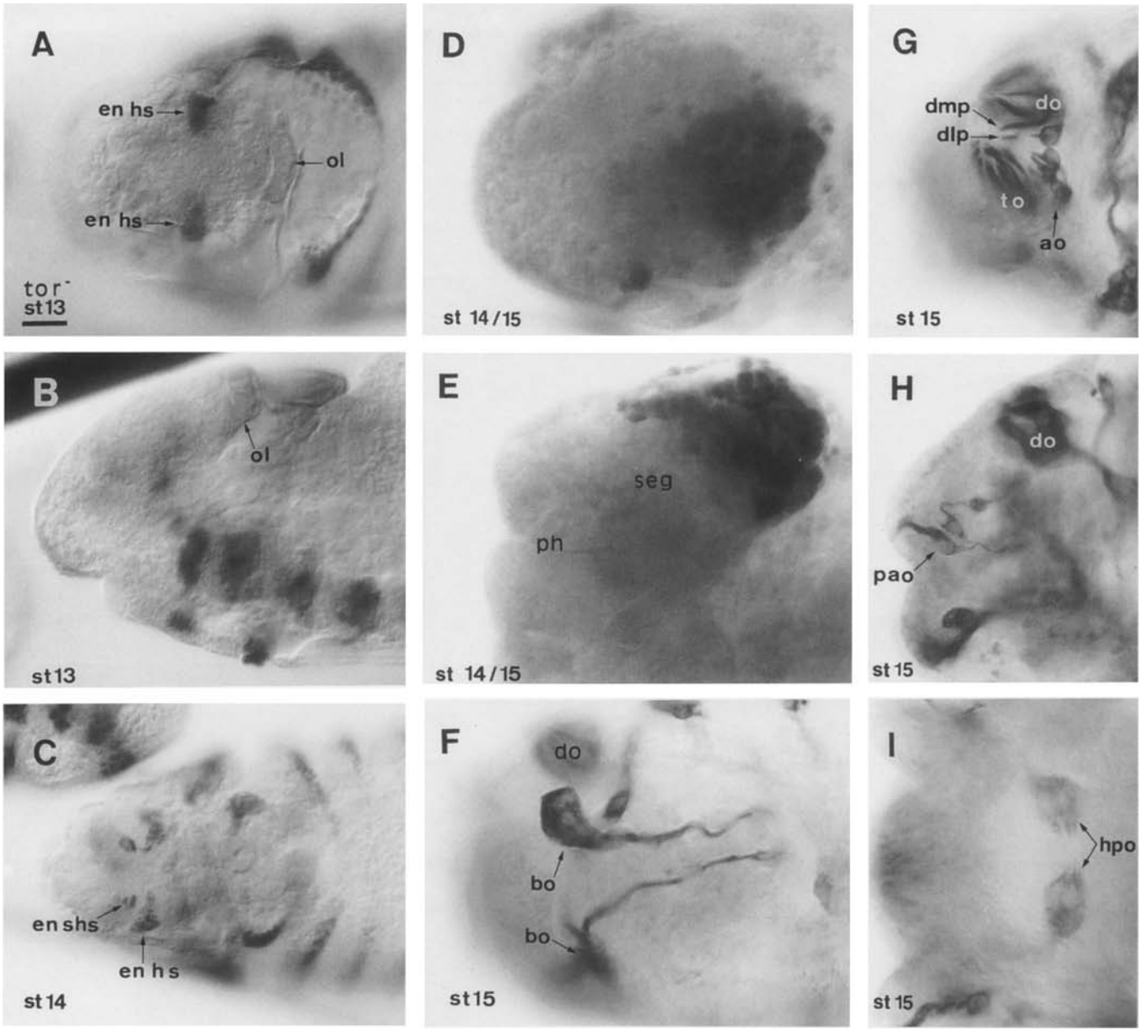


Fig. 5A–I *tor* mutant embryos stained with 4D9 antibody against the En antigen (**A–C**), anti-β-galactosidase antiserum (**D, E**) or 22C10 antibody (**F–I**). In **D** and **E** the enhancer trap line *A6-2-45* has been crossed into *tor* mutant background (Cf. Figs. 6J, 7G and 8A for the *lacZ* expression pattern in non-mutant embryos). The supraoesophageal ganglia and optic lobes are fused medially. Anterior to the left, dorsal (**A, C, D, F, I**) and lateral (**B, E, G, H**) views (See also legends to Figs. 2–4; *en hs* en head spot, *en shs* en secondary head spot, *ol* optic lobes; *bar* 20 μm – **A, B,** 25 μm – **C,** 13 μm – **D–H,** 11 μm – **I**)

appears at the end of stage 14 (Fig. 3A, F). Each laterofrontal organ forms two rod-like structures (Fig. 4C). By stage 15/16 the anterior pharyngeal organ can be identified. Its single axon fasciculates with the laterofrontal nerve. By stage 17, the anterior pharyngeal organ is found in a position dorsal to the Bolwig organ (Fig. 4B).

The maxillary nerve is established at stage 13/14 and projects into the suboesophageal ganglion (Fig. 3F). It receives fascicles from the terminal organ, two chordotonal organs (ch2), the ventral organ, the associated organ and the hypomaxillary organ (Figs. 2F, G, 3D, E). The associated organ is visible by stage 13 in a position above the dorsoposterior margin of the epidermal lobe of the maxillary segment (not shown). The cells of the hypomaxillary organ and the labial organ lie close to each other (Fig. 2F). Therefore, it is difficult to determine the number of cells that contribute to each of the two organs and whether the chordotonal organ in this region (triangle in Fig. 2G) is part of either the labial or hypomaxillary organ.

The labial nerve is established by stage 13/14 and projects into the suboesophageal ganglion below the maxillary nerve (Fig. 3F). It collects axons from the hypophysis and the labial organ (Figs. 2D, E, 3A). The pro-

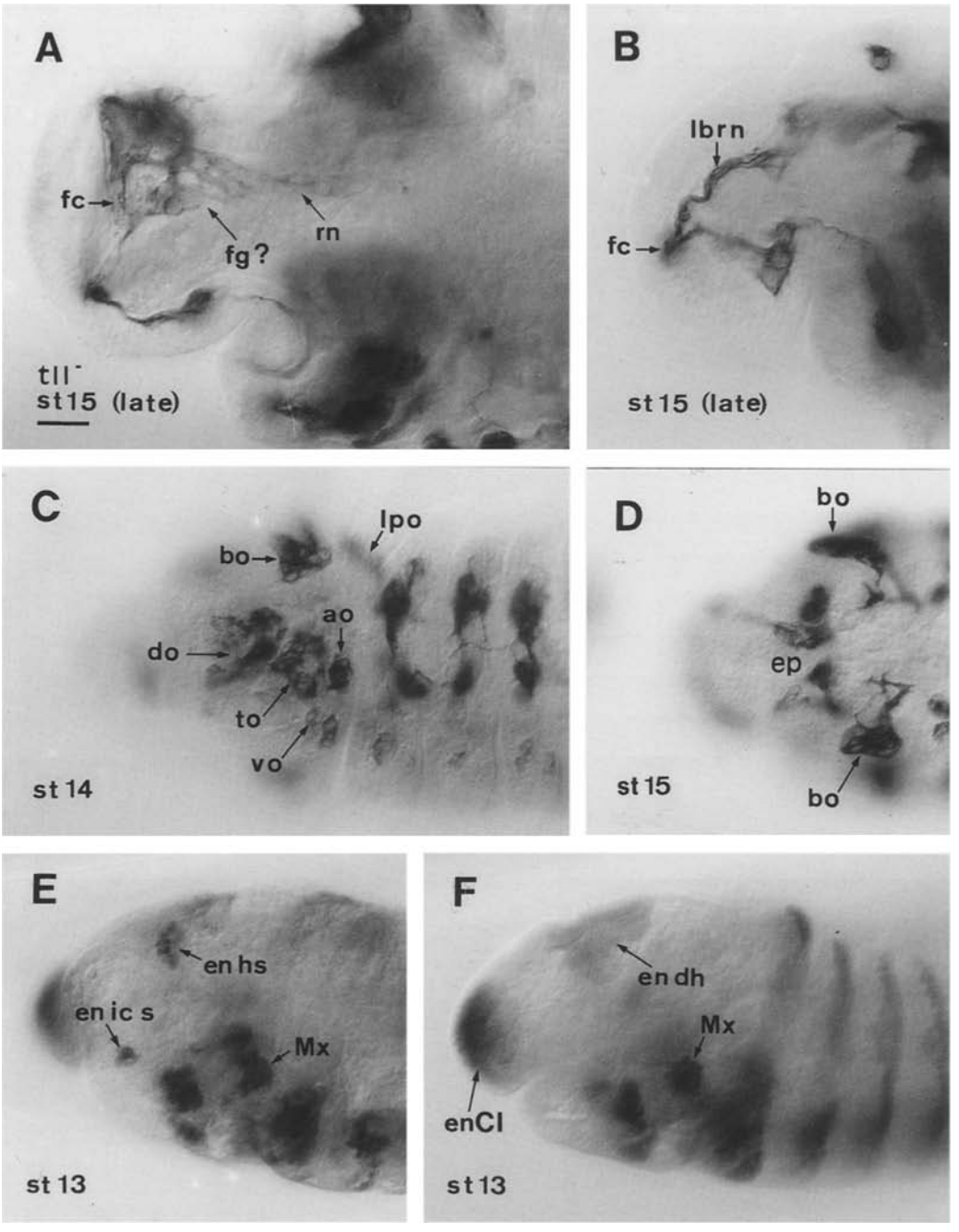


Fig. 6A–J *tll* mutant embryos stained with 22C10 antibody (**A–D**) or with 4D9 antibody (**F–H**). Expression of *lacZ* in the optic lobes of A6-2-45 in *tll* mutant (**I**) or non-mutant (**J**) embryos. In *tll* mutants, the optic lobe typically splits into three groups of cells (*triangles* in **I**) an scattered cells (*arrows* in **I**) are found medioposteriorly to the remnants of the optic lobes. Anterior to the left, dorsal (**A, D, I, J**) and lateral (**B, C, E–H**) views (See also legends to Figs. 2–5; *en ant s* en antennal spot, *en As* en antennal stripe, *en Cl* en clypeolabral spot, *en ic s* en intercalary spot, *Mx* en expression in maxillary lobe; *bar* 13 µm – **A, B, G, I, J,** 25 µm – **C, D,** 20 µm – **E, F, H**)

jection from the hypophysis to the suboesophageal ganglion is established first. Once it reaches the suboesophageal ganglion, by stage 14, axons of the labial organ fasciculate with this nerve.

In addition to the seven sensory nerves which connect the sensory organs of the PNS to the embryonic brain, the nerves of the SNS and their associated ganglia are prominent structures of the embryonic head which can be visualized by 22C10 antibody staining (González-Gaitán et al. 1994, Hartenstein et al. 1994). The frontal connective tissue of the SNS becomes visible at stage 14 between the brain hemispheres. During stage 15, an increasing number of cells of the frontal ganglion become associated with it (Fig. 3C). At this stage, the frontal

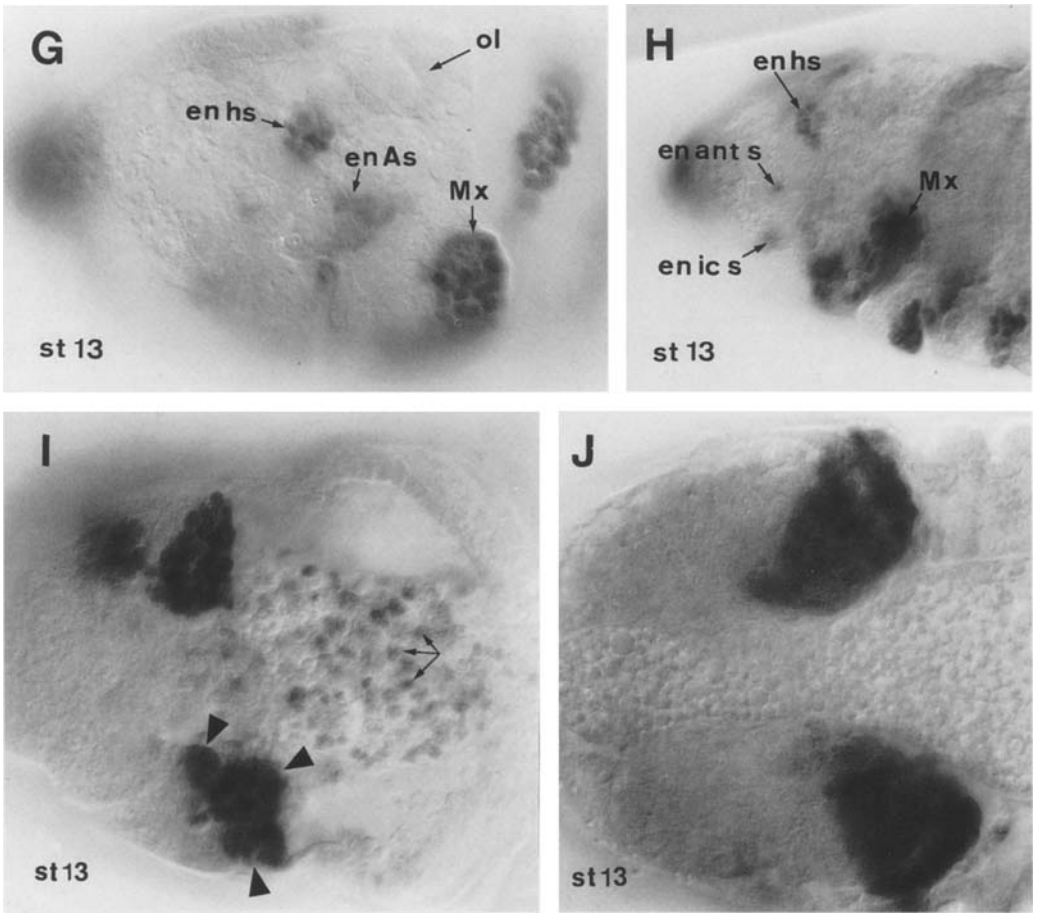


Fig. 6G–J

ganglion splits along the frontal commissure into two cell groups. Shortly afterwards, these cells no longer stain. Also at stage 15, the recurrent nerve is pioneered by two axons (not shown). The cell body of one of these axons is located ventroposterior to the supraoesophageal commissure. The other axon grows out from the frontal commissure.

The recurrent nerve passes underneath the supraoesophageal commissure and splits into two branches. One branch is distorted to the left side of the embryo and collects axons from oesophageal ganglion 2 (eg2) and the proventricular ganglion (Fig. 4A, F). The other branch is distorted to the right side and terminates in a long row of cells following the oesophagus – oesophageal ganglion 1 (eg1; Fig. 4A, G).

The frontal nerve leaves the frontal commissure anteriorly (Fig. 4A, F, G). This nerve becomes visible at stage 16. In strongly stained embryos, a dorsal part of the ring gland is also stained by 22C10 antibodies. From this organ, a nerve connects each side to the brain hemisphere (Fig. 4F, G). Since staining in the dorsal part of the ring gland is sometimes difficult to see, we did not use it as a marker in the mutant analysis (see later).

Lack of neural elements in gap-like head mutants

In order to determine the segmental origin of the optic lobes and neural elements of the PNS and SNS, we examined their presence or absence in mutants where part of the segmental En pattern is deleted (Figs. 5–9; Table 1, see legend for nomenclature of *en* expression domains in the head).

In *tor* mutant embryos the SNS, epiphysis, dorsopharyngeal organ and pch1 are missing. We noticed three cells with rod-like projections in the hypopharyngeal organ whereas in the wild-type there are only two (Fig. 5I). This can be explained if one assumes that the single sensory cell of the latero-hypopharyngeal organ has failed to separate from the hypopharyngeal organ (Figs. 2I, 4D; cf *btd*). Other sensory organs of the head develop normally (Fig. 5G, H). The brain hemispheres and the optic lobes are medially fused (Fig. 5A, B, D, E, F). The En clypeolabral spot is absent. The En secondary head spot lies anterior to the En head spot and not posterior to it as in the wild-type protocerebrum (Fig. 5C).

In *tll* mutant embryos the epiphysis, Bolwig organ, dorsal organ, terminal organ, ventral organ, associated organ, papilla organ, latero-pharyngeal organ, hypophysis and labial organ can be identified despite alterations seen in the PNS throughout the entire embryo (Fig. 6C, D). In these mutant embryos the Bolwig organ is often

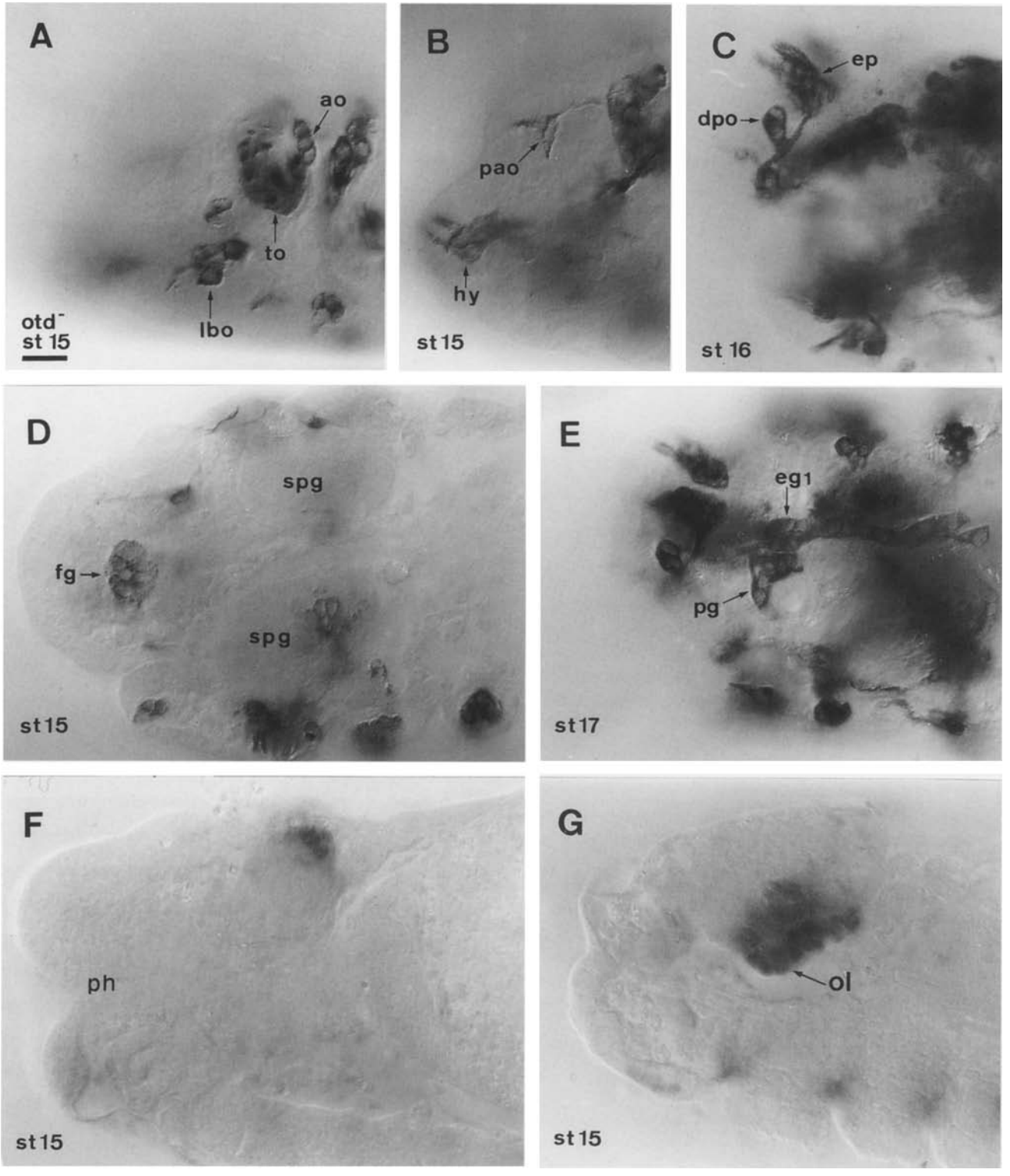
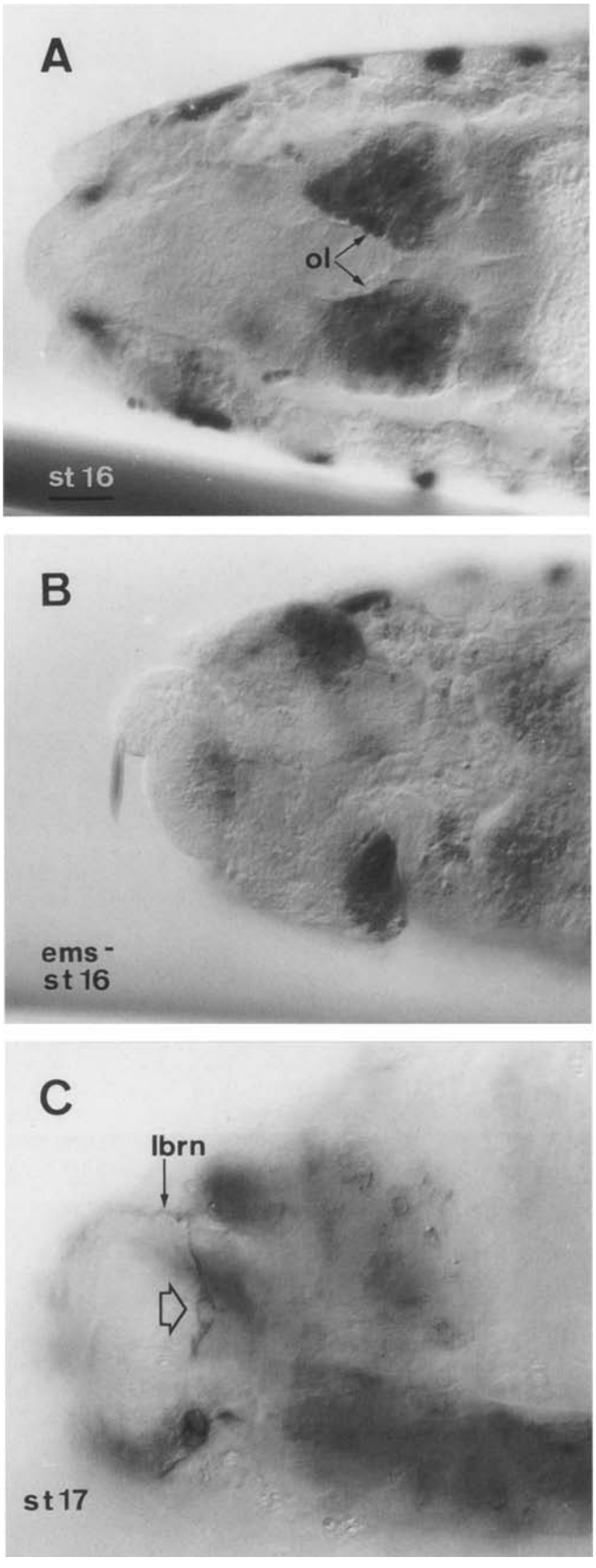


Fig. 7 *otd* mutant embryos stained with 22C10 antibody (**A–E**). Expression of *lacZ* in the optic lobes of A6-2-45 in *otd* mutant (**F**) and non-mutant (**G**) embryos. Anterior to the left, lateral (**A–C, F, G**) and dorsal views (**D, E**). (See also legends to Figs. 2–4; *ol* optic lobes, *ph* pharynx, *spg* supraoesophageal ganglion, *bar* 13 μm)

enlarged and the SNS is strongly affected as well. The frontal ganglion and frontal commissure are shifted anteriorly (Fig. 6A, B). The recurrent and frontal nerves are not fully developed. The labral nerve projects anteriorly to the mislocalized frontal commissure and, thus, inverts its normal orientation (Fig. 6B). The optic lobes are partly reduced but epithelial invaginations do form in some cases. Typically, each optic lobe is split into three groups



of cells after the formation of the epithelial invagination (Fig. 6I, J). In addition, all *en* expression domains are present in the mutant head which indicates that none of the head segments are missing (Fig. 6E-H). Since *en* expressing cells in the dorsal hemispheres of the wild-type embryo fail to segregate into the supraoesophageal ganglion of *tll* mutant embryos (Fig. 6F), their brain is reduced (Pignoni et al. 1990).

In *otd* mutant embryos the Bolwig organ, dorsal organ, hypopharyngeal organ and laterohypopharyngeal organ are missing (Fig. 7A–C). The SNS is present (Fig. 7E) but the frontal ganglion and the frontal connective are shifted towards the anterior and their contact with the supraoesophageal ganglion is disrupted (Fig. 7D). The supraoesophageal ganglion is reduced in size and no supraoesophageal commissure forms. The optic lobes are almost completely deleted (Fig. 7F, G). The En head spot, En secondary head spot, En antennal stripe and En antennal spot are missing (not shown).

In *ems* mutant embryos the Bolwig organ, dorso-medial and lateral papillae, dorsal organ and associated organ are missing. Instead of the hypopharyngeal and latero-hypopharyngeal organ we found a single cell which might correspond to the latero-hypopharyngeal organ (Fig. 8C). The oesophageal ganglion (eg1) is reduced in size. The optic lobes are also reduced in size and towards the end of embryogenesis are found in a posterior (instead of a ventral) position in each hemisphere (Figs. 7G, 8A, B). The En head spot, En secondary head spot, En antennal stripe and En intercalary spot are always missing, whereas the En antennal spot is often but not always missing or reduced on one or both sides (not shown).

In *btd* mutant embryos the dorsal organ, dorso-lateral papilla, associated organ, latero-pharyngeal organ and papilla organ are missing. The hypopharyngeal organ contains a third sensory cell which might correspond to the latero-hypopharyngeal organ (cf. *tor*). The SNS is present (Fig. 9C, D, E). The optic lobes often fuse dorsomedially by late stage 15 whilst the supraoesophageal ganglion becomes inclined bringing the optic lobes into a dorsal position (Fig. 9A, B). The En antennal stripe, En antennal spot, En intercalary spot and En expression in the mandibular segment are missing (not shown).

The finding that the strongest defects in optic lobes are observed in *otd* mutant embryos, the only mutant in which the *en* as well as *wg (wingless)* expression domain of the ocular segment are deleted, suggests that the optic lobes are part of the ocular segment (see Discussion).

Fig. 8A–C *ems*. Expression of *lac*Z in the head of *A6-2-45* in non-mutant (**A**) and *ems* mutant embryos (**B**). The mutant embryo in **C** was stained with 22C10 antibody. The *arrow* points to a single cell which might correspond to the latero-hypopharyngeal organ which projects to the labral nerve (*lbrn, ol* optic lobe; *bar* 20 μm – **A, B,** 13 μm – **C**)

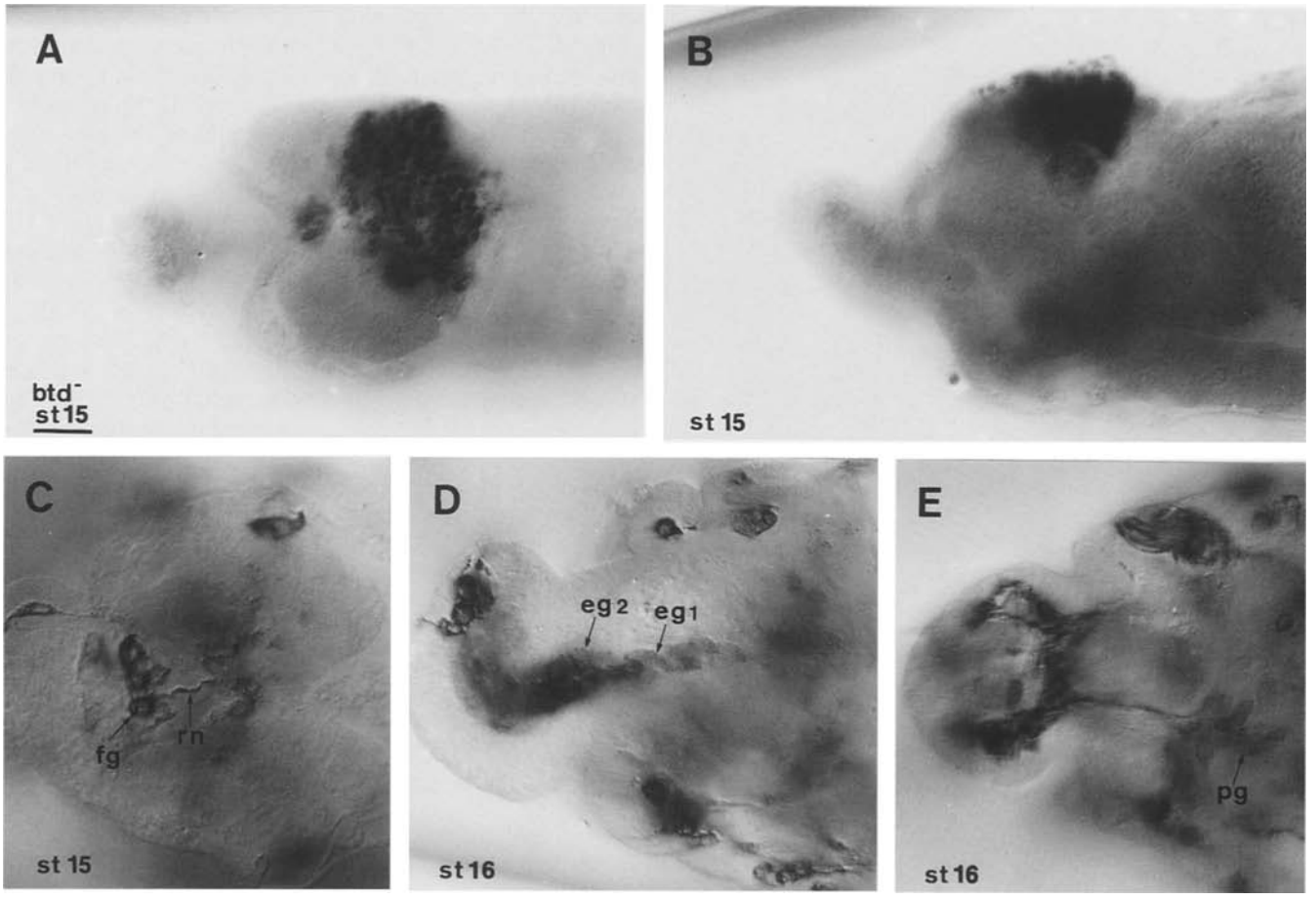


Fig. 9A–E Expression of *lac*Z in the head of *A6-2-45* in *btd* mutant embryos (**A, B**). The optic lobes are initially separated but often fuse by late stage 15. Their dorsal position results from an uplifting of the free (posterior) end of the brain hemispheres. The embryos in **C–E** were stained with 22C10 antibody Anterior to the left, dorsal (**A, C, E**) and lateral views (**B, D**). (For abbreviations see Fig. 4; *bar* 19 μm **A, B,** 15 μm – **C–E**)

Discussion

Our results provide evidence that the optic lobes of the *Drosophila* embryo derive from the ocular segment anteriorly adjacent to the antennal segment and that no labral segment is interspersed between them. On the basis of the segmentation phenotype of *tor, tll, otd, ems* and *btd* mutant embryos, neural elements of the PNS, SNS and CNS can be assigned to different head segments (Table 1; Cohen and Jürgens 1990; Schmidt-Ott et al. 1994b). This approach is based on the correlation of missing head structures in the lack-of-function mutants and the blastodermal expression domains of the respective genes in wild-type embryos (Fig. 1b, Table 1). Although not strictly proven, this correlation seems likely since it is supported by the following observations. The segmental assignment of sensory organs based on the mutants is consistent with the direct mapping of some of them to different head segments, such as the hypophysis and labial organ to the labial segment, the ventral and terminal organ to the maxillary segment, the dorsal organ to the antennal segment and the epiphysis to the clypeolabrum (Jürgens et al. 1986). Furthermore, the deletion of neural elements in distinct regions of *tor, otd, ems* or *btd* mutant embryonic heads suggests that the activities of the respective genes are required for their formation at an early stage of development. Otherwise, remnants or altered structures would be expected. Finally, the expression of *tor, otd, ems* and *btd* in single stripes of the head blastoderm correlates with a single gap in the respective mutants when assuming the anteroposterior order along the longitudinal body axis of neural progenitors shown in Table 1.

Markers which are missing in *otd* mutant embryos belong to the ocular and antennal segments, in *ems* mutant embryos to the posterior ocular, antennal and intercalary segments and in *btd* mutant embryos to the antennal, intercalary and mandibular segments. The phenotype of *tor* mutant embryos is complementary to the phenotype of *otd, ems* and *btd,* mutant embryos, i.e. elements which are deleted in the absence of *tor* activity are present in the other mutants. Only the supraoesophageal commissure is missing in *tor* as well as in *otd* mutant embryos. However, the missing supraoesophageal commissure in *tor* might be a secondary effect since the brain hemispheres form as a medially fused mass of cells and leave no space for the formation of the commissure. Consequently, primary deletions in *tor* might be restricted to the clypeola-

brum and stomodeum with respect to our set of markers. They do not include the optic lobe anlagen, the Bolwig organs or the *en* expression of the ocular segment. In *tll*, the neural markers are altered but not deleted and all En spots in the head are present suggesting that this mutant does not cause a "gap-phenotype" in the head.

The optic lobes are affected in all five mutants (Figs. 5D, E, 6I, 7F, 8B, 9A, B). However, the epithelial pouches of the optic lobe anlagen (and Bolwig organs) are missing only in *otd* and *ems* mutants. Furthermore, in *otd* only small remnants of the optic lobes are present (Fig. 7F). Consistently, only in *otd* mutant embryos are the expression domains of *en* and *wingless (wg)* in the ocular segment deleted (Table 1). These results suggest that the optic lobes are part of the ocular segment which occupies a position anteriorly adjacent to the antennal segment.

The En pattern in the head of various crustaceans, which like the insects belong to the Mandibulata, is compatible with our conclusion of an ocular segment anteriorly adjacent to the antennal segment. In crustaceans, the first En stripe lies in the optic ganglia and the second can be assigned to the first antennal segment (Scholtz 1994). Thus, neither in insects nor in crustaceans does a labral segment seem to be interspersed between head regions.

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