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Spatial and Temporal Control of Gene Expression in Drosophila Using the Inducible GeneSwitch GAL4 System. I. Screen for Larval Nervous System Drivers

Louise Nicholson,* Gunisha K. Singh,* Thomas Osterwalder,*,[1] Gregg W. Roman,[†] Ronald L. Davis[‡] and Haig Keshishian*,[2]

**MCDB Department, Yale University, New Haven, Connecticut 06520-8103, [†]Biology and Biochemistry Department, University of Houston, Houston, Texas 77204 and [‡]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030*

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ABSTRACT

There is a critical need for genetic methods for the inducible expression of transgenes in specific cells during development. A promising approach for this is the GeneSwitch GAL4 system of Drosophila. With GeneSwitch GAL4 the expression of upstream activating sequence (UAS) effector lines is controlled by a chimeric GAL4 protein that becomes active in the presence of the steroid RU486 (mifepristone). To improve the utility of this expression system, we performed a large-scale enhancer-trap screen for insertions that yielded nervous system expression. A total of 204 GeneSwitch GAL4 lines with various larval expression patterns in neurons, glia, and/or muscle fibers were identified for chromosomes I–III. All of the retained lines show increased activity when induced with RU486. Many of the lines reveal novel patterns of sensory neurons, interneurons, and glia. There were some tissue-specific differences in background expression, with muscles and glia being more likely to show activity in the absence of the inducing agent. However, >90% of the neuron-specific driver lines showed little or no background activity, making them particularly useful for inducible expression studies.

TARGETED gene expression is a powerful technique for analyzing neural development and function in Drosophila. For example, the selective expression of constructs such as modified ion channels, which alter the electrical properties of neurons, can be used to control neural activity (Paradis *et al.* 2001; White *et al.* 2001; Nitabach *et al.* 2002; Mosca *et al.* 2005; Luan *et al.* 2006). However, these constructs often have potent and systemic effects when expressed constitutively, and their effective use requires fine spatial and temporal control of expression. The aim of this study is to develop molecular tools for confining transgene expression to well-defined subsets of neurons, muscle fibers, or glia at specific times during development. We have focused on the larval nervous system of Drosophila as it provides a favorable model system for studying neuronal circuitry and synaptic plasticity.

In Drosophila, several techniques exist that give both temporal and spatial control over gene expression (McGuire *et al.* 2004). Many of these approaches are derived from the widely used bipartite GAL4-upstream activating sequence (UAS) system (Brand and Perrimon 1993). In this system, the transcriptional activator GAL4 is expressed in a spatially restricted pattern using a tissue-specific promoter. GAL4 then activates the expression of transgenes under UAS control in tissues that express GAL4. While this system provides no independent control over the timing of transgene expression, a number of methods that add temporal control to the GAL4-UAS expression system have now been developed (Han *et al.* 2000; Osterwalder *et al.* 2001; Roman *et al.* 2001; Mcguire *et al.* 2003). These either use modified steroid-activated versions of GAL4 to achieve inducible expression or introduce a temperature-sensitive GAL4 repressor ($GAL80^{ts}$) to regulate GAL4 expression. Both of these approaches show significant promise for controlling gene expression in both time and space. Alternative tetracycline-inducible systems have also been developed (Bello *et al.* 1998; Bieschke *et al.* 1998; Stebbins and Yin 2001; Stebbins *et al.* 2001), but are limited by the small number of tetO-dependent transgenes currently available.

Steroid-activated chimeric GAL4 proteins have been independently developed by three different groups (Han *et al.* 2000; Osterwalder *et al.* 2001; Roman *et al.* 2001). Each protein combines the GAL4 DNA-binding domain with a steroid hormone receptor transcriptional activation domain, which requires ligand binding to become transcriptionally active. The GAL4-estrogen-receptor fusion protein (GAL4-ER) becomes

[1]Deceased.

[2]*Corresponding author:* MCDB Department, Yale University, POB 208103, New Haven, CT 06520-8103. E-mail: haig.keshishian@yale.edu

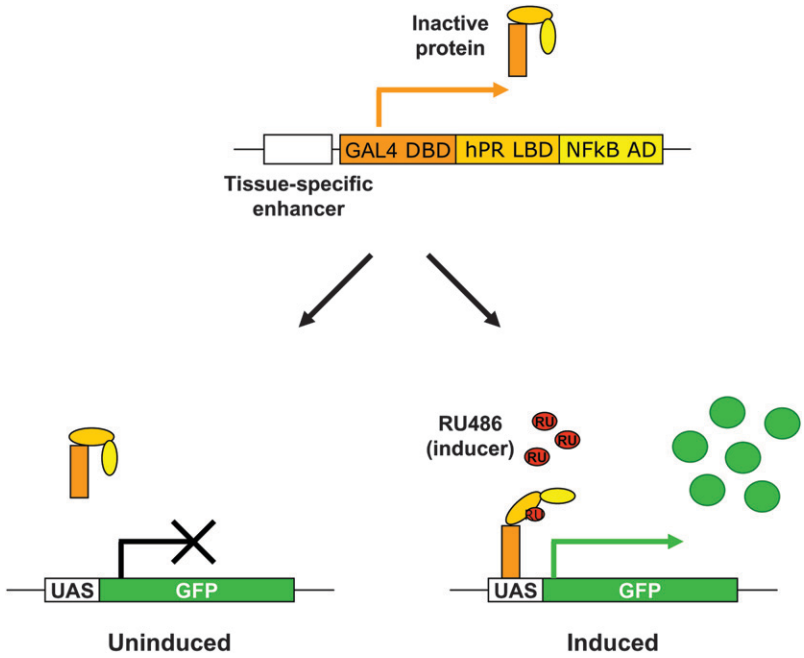

Figure 1.—The GeneSwitch GAL4 system. In the absence of an activator (uninduced), the GeneSwitch GAL4 protein is expressed in target tissues but remains transcriptionally silent; no expression of downstream UAS-linked genes therefore occurs. However, after systemic application of RU486 (induced), the binding of the RU486 ligand causes the GeneSwitch GAL4 protein to become transcriptionally active, resulting in expression of UAS-linked genes (shown here as UAS-GFP).

active in the presence of estrogen (Han *et al.* 2000), while GAL4-progesterone-receptor fusion proteins (GeneSwitch, here referred to as GeneSwitch GAL4; Figure 1) are activated by RU486 (mifepristone) (Osterwalder *et al.* 2001; Roman *et al.* 2001). Tissue-specific promoters are used to control the spatial expression of GAL4-ER or GeneSwitch GAL4, and the timing of steroid exposure provides ligand-inducible activation of UAS transgenes. The steroid ligand can be administered by either feeding or immersing the animals in a steroid solution. Transgene expression is detectable after 3–5 hr using GeneSwitch GAL4 and maximal expression is reached in 21–48 hr, with a somewhat slower time course being reported for GAL4-ER (Han *et al.* 2000; Osterwalder *et al.* 2001; Roman *et al.* 2001). Ligand-inducible systems also provide the benefit of being able to control the level of transgene expression by varying the dosage of the steroid ligand (Osterwalder *et al.* 2001).

An alternative approach uses temperature to regulate GAL4-UAS-mediated gene expression through GAL80^{ts}, a temperature-sensitive variant of the endogenous GAL4 repressor from *Saccharomyces cerevisiae* (McGuire *et al.* 2003). Ubiquitous expression of GAL80^{ts} represses GAL4 at 19°, while shifting to 30° derepresses GAL4 and activates expression of downstream transgenes. Tissue specificity is provided by the choice of GAL4 driver. The advantage of the GAL80^{ts} system is that it can be combined with existing GAL4 drivers and UAS effectors, whereas the GeneSwitch GAL4 and GAL4-ER systems require the creation of new tissue-specific GeneSwitch GAL4 or GAL4-ER driver lines. However, rearing animals at elevated temperatures (28°–29°) has direct effects on larval locomotion and neural activity. The high temperature results in both elevated synaptic transmission and enlarged neuromuscular junctions (Sigrist *et al.* 2003; Zhong and Wu 2004), similar to the effects observed in larvae with hyperactivity mutations, such as *eag* and *Sh* (Budnik *et al.* 1990; Zhong *et al.* 1992; Renger *et al.* 2000; Sigrist *et al.* 2003; Mosca *et al.* 2005). Elevated temperature has also been shown to alter voltage-gated potassium currents in larval muscles (Chopra and Singh 1994). For the temporal and spatial regulation of gene expression in larvae, we have therefore focused on the ligand-inducible gene expression systems.

While there are only a limited number of tissue-specific GeneSwitch GAL4 and GAL4-ER lines available, both approaches capitalize on the vast array of existing UAS constructs that can be used for ectopic expression, RNAi knockdown of gene function, or manipulation or reporting of cellular function. To date, GAL4-ER has been successfully tested in oocytes (Han *et al.* 2000), while GeneSwitch GAL4 has been expressed using pan-neuronal, pan-muscle, mushroom body-specific, eye-specific, and ubiquitous promoters (Osterwalder *et al.* 2001; Roman *et al.* 2001; Roman and Davis 2002; Mao *et al.* 2004; Ford *et al.* 2007).

Using an enhancer-detector GeneSwitch GAL4 construct (Roman *et al.* 2001), we have conducted a large-scale enhancer-trap screen in third instar larvae and the adult head. Here we summarize our results from the larval screen and describe a subset of these lines in detail. From this screen we isolated 204 new GeneSwitch GAL4 driver lines with expression in neurons, glia, and/or muscle. We report that while all lines show increased activity when induced, muscles and glia are more likely to show uninduced activity than neurons. These inducible driver lines are valuable tools for the developmental and functional analysis of the larval nervous system.

MATERIALS AND METHODS

Fly stocks: All stocks were raised on cornmeal medium at room temperature (22°). GeneSwitch GAL4 enhancer-detector lines were balanced over either *w−; Sp/CyO; Dr/Tm6b,Hu* or *w−; Sp/CyO; Dr/Tm3,Sb* chromosomes. The dicistronic enhanced green fluorescent protein (UAS-2xEGFP) fly stock contained an insert on both the second and the third chromosome (Halfon *et al.* 2002).

***P*-element mobilization:** Several pilot mobilizations of the X-linked *P*{Switch2}19-2 element were performed using the TMS, *Sb*, Δ2-3 balancer chromosome as a transposase source. Two new insertions were identified on the CyO balancer chromosome from these screens and these were used for large-scale screening. For the enhancer-detector screen, single males of the genotype w^{1118}*; CyO, P{Switch2}/+; TMS, Sb,* Δ*2-3/+* were crossed to multiple virgin w^{1118} females in single vials. A total of 4343 independent *P*{Switch2} insertions were selected as w^+, non-CyO progeny from 11,453 dysgenic crosses. These new *P*{Switch2} insertions were used for expression screening in third instar larvae as described here and in the adult head (our unpublished data).

Ru486 induction: Crosses were raised at room temperature on fly food containing RU486 (Sigma, St. Louis). A stock solution of 10 mg/ml RU486 in ethanol was added during fly food preparation to a final concentration of 5 μg/ml.

F_1 screen of GeneSwitch GAL4 enhancer-detector lines: Expression was monitored by crossing males from each GeneSwitch GAL4 insertion to virgin females from the UAS-2xEGFP fly stock. Each cross was raised on food containing 5 μg/ml RU486 and the F_1 progeny were examined as third instar larvae. For the primary F_1 whole-mount screen seven third instar larvae were examined from each enhancer trap line. Larvae were placed in a drop of glycerol on a coverslip and killed by briefly heating to 70° on a hot plate. The coverslips were then mounted on a microscope slide and examined under fluorescent epiillumination using a 10× 0.45 NA planapochromatic objective. For the secondary F_1 screen, four to six filleted larval body wall preparations (Halpern *et al.* 1991) from each enhancer-trap line retained after the primary screen were examined by whole-field epifluorescence.

Immunohistohemistry: Filleted larval body wall preparations (Halpern *et al.* 1991) were fixed in 4% paraformaldehye for 1 hr, followed by 4 × 15-min washes in phosphate-buffered saline (0.9% NaCl, 10 mM NaH_2PO_4, pH 7.2) containing 0.3% Triton X-100 (TBS), blocked for 1 hr in 1% BSA in TBS, and incubated in primary antibody overnight at 4°. After 4 × 15-min washes in TBS, larvae were incubated in secondary antibody for 4 hr at room temperature, washed 4 × 15 min in TBS, and mounted in antifade (Invitrogen, San Diego). Primary antibodies were Mab1D4 anti-FasII (1:10; Developmental Studies Hybridoma Bank, DSHB), Mab8D12 anti-Repo (1:5; DSHB), rabbit anti-FMRFamide (1:1000; P. H. Taghert), and rabbit anti-eve (1:1000; M. Frasch). Secondary antibodies were purified anti-mouse IgGs labeled either with $Alexa^{488}$, $Alexa^{568}$, or $Alexa^{647}$ or with an anti-rabbit IgG labeled with $Alexa^{568}$ (Molecular Probes, Eugene, OR), all at 1:500 dilutions. Fillet preps were imaged either by whole-field epifluorescence or by laser-scanning confocal microscopy [Bio-Rad (Hercules, CA) 1024]. Images were compiled using Adobe Photoshop CS.

***P*-element insertion localization:** Genomic DNA was recovered, digested with either *Hin*PI or *Sau*3A, ligated to Vectorette linkers, and used for nested PCR with primers PF2, PF3, and Vrev1 or Vrev2 as described (Eggert *et al.* 1998). The PF2 and PF3 primers recognize sequences within the 31-bp *P*-element-terminal repeat, while Vrev1 and Vrev2 both recognize the Vectorette linker and are used for *Sau*3A- and *Hin*PI-digested DNA, respectively. PCR products were recovered from a 1% agarose gel and sent for sequencing using the PF3 primer. The flanking sequences were BLASTed against the *Drosophila melanogaster* genome on FlyBase (Gelbart *et al.* 2003) to identify insertion locations.

RESULTS

We screened 3034 GeneSwitch GAL4 enhancer-detector lines for expression in the third instar larvae, using a dicistronic UAS-2xEGFP reporter construct. In the primary screen of F_1 whole-mount larvae, 433 lines (14.3% of the screen) showed detectable expression in neurons of the CNS and/or the PNS, in muscle fibers, or in glia. Each of these positive lines was then rescreened as a dissected fillet preparation. We retained 204 lines (6.7% of the screen) on the basis of consistent, strong, and/or restricted inducible expression patterns. Of these lines, 132 showed expression in neurons, 58 were expressed in glia, and 27 drove expression in muscle. A summary of the tissues in which expression was observed is given in Table 1, while the expression pattern for each line is provided in supplemental Table S3 at http://www.genetics.org/supplemental/. Images for 96 of these lines are given in supplemental Table S4 at http://www.genetics.org/supplemental/. Note that numerous lines showed expression in multiple tissues. Furthermore, 25 lines were retained with expression outside the nervous system, in tissues such as trachea, heart, epidermis, or fat bodies (Table 1, supplemental Figure S1 at http://www.genetics.org/supplemental/)

We divided the neuronal drivers into five classes (Table 1). Lines were classified as motoneuronal if peripheral EGFP expression was detected at neuromuscular junctions (NMJs) and as sensory if only sensory neurons showed expression. Those that labeled CNS cell bodies but showed no detectable peripheral expression were provisionally classified as interneuronal, although some of these lines may also include efferents with expression too weak to reveal their peripheral arbors. The remaining two classes consist of sensory neuron lines that combined expression with motoneurons and/or interneurons.

Widespread larval nervous system expression was observed in 33 lines. These included 7 lines that showed additional glial expression and a further 2 lines that expressed in neurons, glia, and muscle. While 7 of these lines showed strong expression in large subsets of motoneurons, sensory neurons, and interneurons, we did not isolate any truly pan-neuronal drivers. However, a pan-neuronal GeneSwitch GAL4 driver is available, based on the use of the elav promoter (ELAV-GeneSwitch GAL4; Osterwalder *et al.* 2001).

For simplicity we classified glial drivers into one of three categories, on the basis of whether glia showed expression in the periphery, in the CNS, or in both. Each of these categories includes multiple types of glia. However, none of the lines identified in this screen

TABLE 1

Summary of GeneSwitch GAL4 enhancer-trap expression patterns

Expression pattern category		Expression class	
Primary tissue showing expression	No. of lines	Cell type showing expression	No. of lines
Neurons	102	Motoneurons	10[a]
		Sensory neurons	10
		Interneurons	43
		Motor and sensory neurons	26
		Interneurons and sensory neurons	13
Neurons and glia	23	Motoneurons and glia	3
		Sensory neurons and glia	6
		CNS and glia	4
		Motor/sensory and glia	9
		CNS/sensory and glia	1
Neurons and muscle	4		
Neurons and muscle and glia	6		
Glia	26	PNS glia	3
		CNS glia	15
		Mixed	8
Glia and muscle	6		
Muscle	12		
Other cell types	25		
Total	204		

[a] Includes three lines that also express in the lateral bipolar dendritic (lbd) cells.

labeled all glial cell types. Insertions that drive expression in muscle were the smallest class recovered. We also found that these lines frequently exhibited far more variability in expression pattern than either glial or neuronal GeneSwitch GAL4 drivers, in the number and/or identity of the muscle fibers labeled and in their level of activity (see below).

GeneSwitch GAL4 insertions with strong levels of expression were found for all tissues examined. This indicates that RU486 can diffuse freely throughout the larva after ingestion and that its availability is not a limiting factor for GeneSwitch GAL4 expression, in accord with previous observations for neuron- and muscle-specific GeneSwitch GAL4 drivers (Osterwalder *et al.* 2001). As a rule, the lines with the weakest expression also showed the most variability in their expression pattern.

Patterns of expression in the CNS: The ventral nerve cord and brain of the larval CNS respectively contain ~3500 and ~1000–2000 embryonically produced neurons (Bossing *et al.* 1996; Younossi-Hartenstein *et al.* 1996; Schmidt *et al.* 1997; Schmid *et al.* 1999; Urbach and Technau 2003). These form the functional circuitry of the larval nervous system. In the ventral nerve cord (VNC), each abdominal hemisegment contains ~250 local/axonless interneurons, ~60 intersegmental interneurons, and ~35 motoneurons (Bossing *et al.* 1996; Schmidt *et al.* 1997; Schmid *et al.* 1999). We identified 116 GeneSwitch GAL4 insertions with expression in VNC cells. Of these, 27 lines labeled intersegmental interneurons, on the basis of the presence of longitudinal intersegmental processes, and 51 lines expressed in motoneurons, as judged by consistent peripheral NMJ expression (see below). In 18 lines expression was observed both in motoneurons and in intersegmental interneurons. The identity of the cells in the remaining GeneSwitch GAL4 lines that show VNC expression has not been determined.

Of the 116 lines that expressed in VNC cells, 53 insertions also expressed either in specific structures or in specific cells within the brain, or in cells throughout the brain. We identified 12 lines that expressed in the mushroom bodies, one of the most prominent structures in the larval brain. An additional 20 lines showed expression patterns limited to specific neurons or clusters of neurons in the brain. We did not recover any insertions that were exclusively expressed in the brain.

To further characterize the expression patterns of selected GeneSwitch GAL4 insertions, lines expressing UAS-2xEGFP were counterstained with anti-Fasciclin II (FasII). The axon tract scaffold labeled by FasII provides three-dimensional positional markers for the larval CNS (Grenningloh *et al.* 1991; Lin *et al.* 1994; Landgraf *et al.* 2003; Nassif *et al.* 2003). We also used markers such as anti-FMRF (Schneider *et al.* 1993) and anti-Even-skipped (Patel *et al.* 1989) to identify cells in which GeneSwitch GAL4 lines are expressed and to provide additional spatial landmarks for describing GeneSwitch GAL4 expression patterns.

Lines GSG1142 and GSG3315-1 both show expression in interneurons but not in motoneurons. GSG1142 is expressed in a small cluster of segmentally repeated

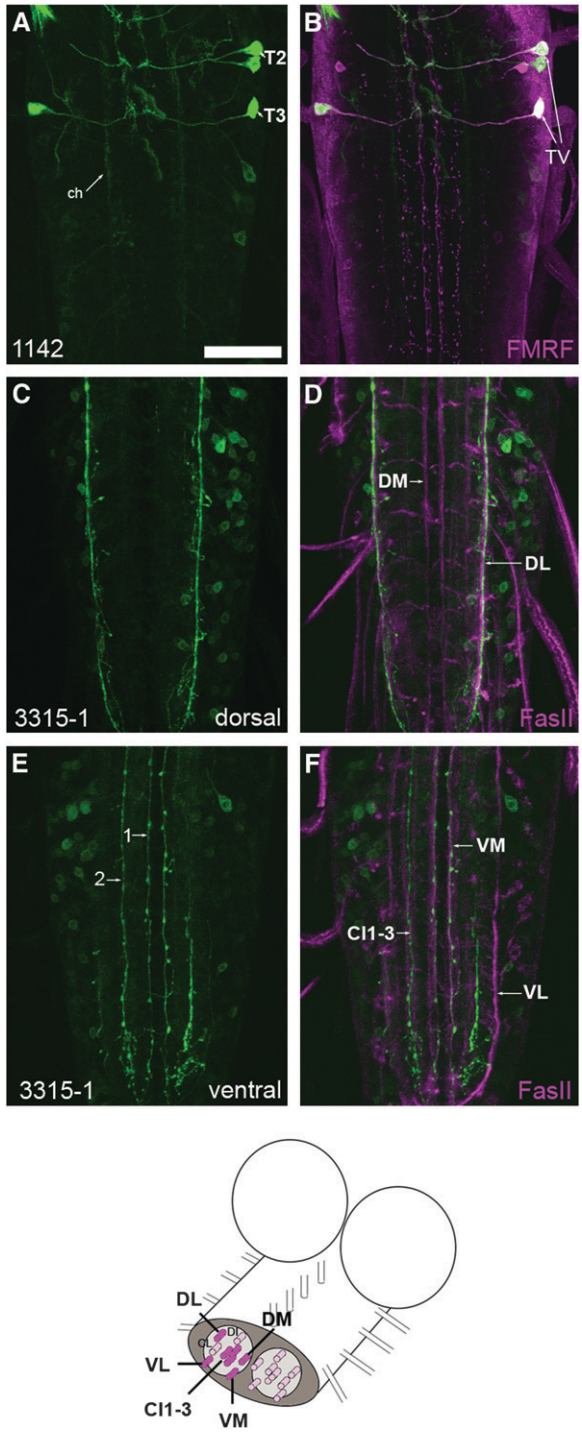

FIGURE 2.—GeneSwitch GAL4 lines with expression in VNC interneurons. Z-projection of confocal sections of the L3 VNC. (A and B) UAS-2xEGFP expression driven by GSG1142 (green) and double labeled with anti-FMRF (magenta). (C–F) UAS-2xEGFP expression driven by GSG3315-1 and double labeled with anti-FasII (magenta). A schematic view of the axon tracts labeled by FasII is shown at the bottom. Tracts referred to in this figure are highlighted in magenta. (A and B) GSG1142 is strongly expressed in single cells in thoracic (T) segments that extend processes toward the midline. Weak expression is also observed in chordotonal sensory neurons (ch). Double labeling with anti-FMRF shows that GSG1142 is expressed in the neuroendocrine Tv neurons. (C and D) GSG3315-1 expression in the dorsal VNC. Intersegmental processes extend along the DL FasII axon tract. (E and F) GSG3315-1 expression in the ventral VNC. Intersegmental interneuron processes extend (1) along the VM FasII axon tract and (2) lateral to the CI1-3 FasII axon tract. Anterior is up in this and all other figures, unless otherwise stated. Bar, 50 μm.

lateral VNC cells, including one ventral neuron in each thoracic hemisegment that shows particularly strong expression (Figure 2A). Each of these neurons projects to the midline. Counterstaining with anti-FMRF (Figure 2B) identified these strongly labeled cells as the neuroendocrine FMRF-positive Tv neurons (SCHNEIDER *et al.* 1993). Expression also occurs in chordotonal sensory neurons. No motoneuron expression was detected. GSG3315-1 is expressed in a lateral cluster of segmentally repeated VNC cells. Its expression pattern includes intersegmental interneurons, which produce lateral longitudinal processes within the dorsal neuropil (Figure 2, C and D), and multiple ventrally located intersegmental processes (Figure 2, E and F). While the ventral–medial intersegmental process is closely associated with FMRF-positive processes, there is no colocalization with FMRF (not shown). Strong expression is also observed in a single pair of large terminal abdominal cells (not shown). There is no labeling of motoneurons or sensory neurons.

Within the brain, GSG1802-2 (Figure 3C) and GSG5961 (Figure 3D) show very restricted expression patterns, while GSG3516 (Figure 3A) and GSG6124 (Figure 3B) show more widespread expression. Both GSG3516 and GSG6124 label multiple clusters of neurons within the central brain region, while brain expression in GSG1802-2 and GSG5961 is limited to a few cells. GSG5961 is also expressed in the optic lobe primordium (OLP) (Figure 3D). Of the lines that show mushroom body expression, GSGB15-1 is exclusively expressed in the mushroom bodies within the brain (Figure 3F), while other lines, such as GSG11,194-1 (not shown), label the mushroom bodies and additional cells scattered throughout the brain. GSG6098 labels a single cluster of neurons in addition to the mushroom bodies (Figure 3E). These neurons have processes that appear to be associated with the mushroom bodies (Figure 3E, arrow).

Patterns of expression in motoneurons: There are four types of larval abdominal motoneuron, each of which contacts a specific target muscle or muscle group and can be recognized by its NMJ morphology and physiology (JOHANSEN *et al.* 1989; ATWOOD *et al.* 1993; JIA *et al.* 1993; KESHISHIAN *et al.* 1996). Every abdominal hemisegment contains ~30 type Ib motoneurons, 2–3 type Is motoneurons, and 2 type II motoneurons (HOANG and CHIBA 2001). A single type III motoneuron innervates muscle fiber 12 in segments A2–A5 (GORCZYCA *et al.* 1993). The majority of motor axons exit the CNS via the peripheral nerve, which splits into five subbranches (SNa, SNc, ISN, ISNb, and ISNd). A few axons exit via a second nerve, the transverse nerve (TN). Each

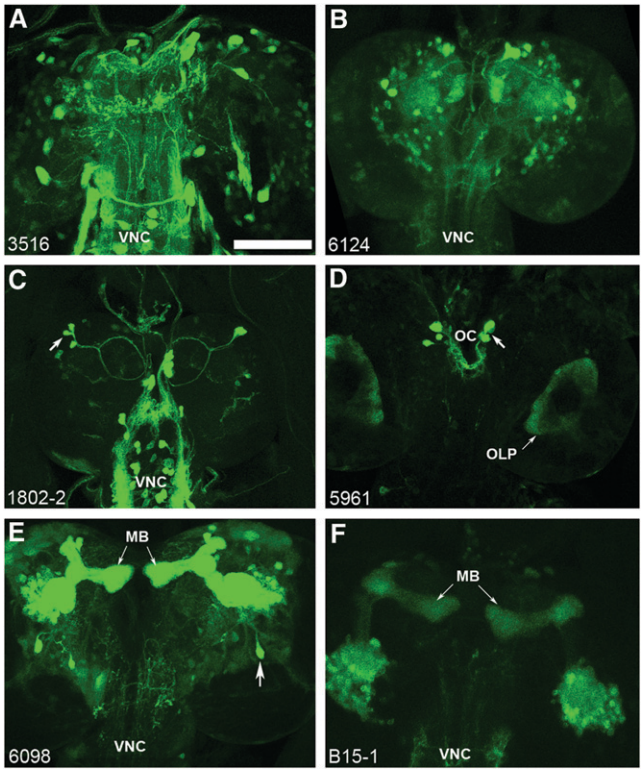

FIGURE 3.—GeneSwitch GAL4 lines with expression in the larval brain. Z-projections of confocal sections of the L3 brain. GeneSwitch GAL4 expression patterns were visualized using UAS-2xEGFP (green). (A) GSG3516 and (B) GSG6124 are broadly expressed in the central brain region and inner brain lobes. (C) GSG1802-2 is expressed in a few clusters of neurons, including a prominent lateral cluster (arrow). (D) GSG5961 is expressed in a small cluster of neurons surrounding the esophageal canal (OC) and in the optic lobe primordia (OLP). (E) GSG6098 is expressed in the mushroom bodies (MB) and in a few additional neurons (arrow) with processes that are associated with the mushroom bodies. (F) GSGB15-1 is expressed in the mushroom bodies (MB). VNC, ventral nerve cord. Bar, 100 μm.

type Ib motoneuron innervates a single muscle fiber or muscle cleft. Most muscles are also innervated by type Is and type II motoneurons, which contact multiple muscle fibers.

NMJ expression was observed at most/all muscle fibers in 67% of GeneSwitch GAL4 lines with peripheral motoneuron expression (Table 2). We examined the number of VNC cell bodies labeled to determine if expression was occurring in a few type Is or type II motoneurons, in a large number of type Ib motoneurons, or in a combination of motoneuron types. We focused on the small group of cells at the dorsal midline where larval cell body location has been correlated with muscle fiber innervation (CHOI *et al.* 2004). This group comprises one type Is motoneuron (MNISN-Is or RP2), which innervates all dorsal muscles via the intersegmental nerve, and four Ib motoneurons (MN1-Ib, MN14-Ib, MN6/7-Ib, and MN30-Ib), which innervate muscle fibers 1, 14, 6/7, and 30, respectively. GeneSwitch

TABLE 2

Lines with expression at neuromuscular junctions (NMJs)

Muscle fiber NMJ location	Total	Motoneuron type	No. of lines
Most/all muscle fibers	34	Type Ib + Is + II	2
		Type Ib + Is	12
		Type Ib	3
		Type Is	2
		Not determined	15
Muscle fiber subsets	3	SNa/SNc	1
		mf 25	1
		ISN	1
Variable expression	14		
Total	51		

GAL4 lines that showed either panmotoneuron or subtype-specific expression were counterlabeled with anti-Even-skipped (Eve), which is expressed by RP2/MNISN-Is in the dorsal cluster (PATEL *et al.* 1989), to confirm motoneuron identity. Anti-FasII, which labels all motoneuron endings, was used as a marker to distinguish NMJ morphology in the periphery (VAN VACTOR *et al.* 1993).

When dorsal midline expression was examined, three lines (GSG1802-2, GSG3516, and GSG5255) labeled type Ib motoneurons but not MNISN-Is, while two lines (GSG3621-1 and GSGB15-1) showed preferential or exclusive expression in MNISN-Is. Both GSG1802-2 (Figure 4, G and H) and GSG5255 (not shown) showed peripheral expression exclusively in type Ib NMJs. Additional VNC expression was observed in dorsal midline cells, in lateral cell clusters, and in ventral cells for both lines. GSGB15-1 expressed in a single dorsal midline cell body corresponding to MNISN-Is/RP2 (Figure 4, I and J) and showed exclusive peripheral expression at type Is NMJs on most/all muscle fibers (Figure 4, K and L). Expression was also observed in a small number of lateral and ventral cells in the VNC.

The majority of lines expressed in both type Is and type Ib motoneurons (Table 2). Line GSG3705 showed VNC expression in all dorsal midline neurons (Figure 4, A and B), in a lateral cluster of cells, and in cells at the ventral midline. Strong peripheral expression is detected at all type Is and type Ib NMJs (Figure 4, C and D), with weak expression also being observed at type II NMJs (Figure 4, C and D, arrow). No sensory neuron expression was observed. Expression at type II endings was also observed in GSG3473 (not shown), which showed widespread motoneuron expression but did not label all type Ib motoneurons.

Two of the GeneSwitch GAL4 lines expressed at NMJs on specific muscles only. GSG2451-1 labeled axons in the TN, SNa, and SNc nerve branches and showed peripheral NMJ expression at muscle fibers 26, 27, and 29 (Figure 4, O and P) and muscle fibers 5, 8, 23, and 25

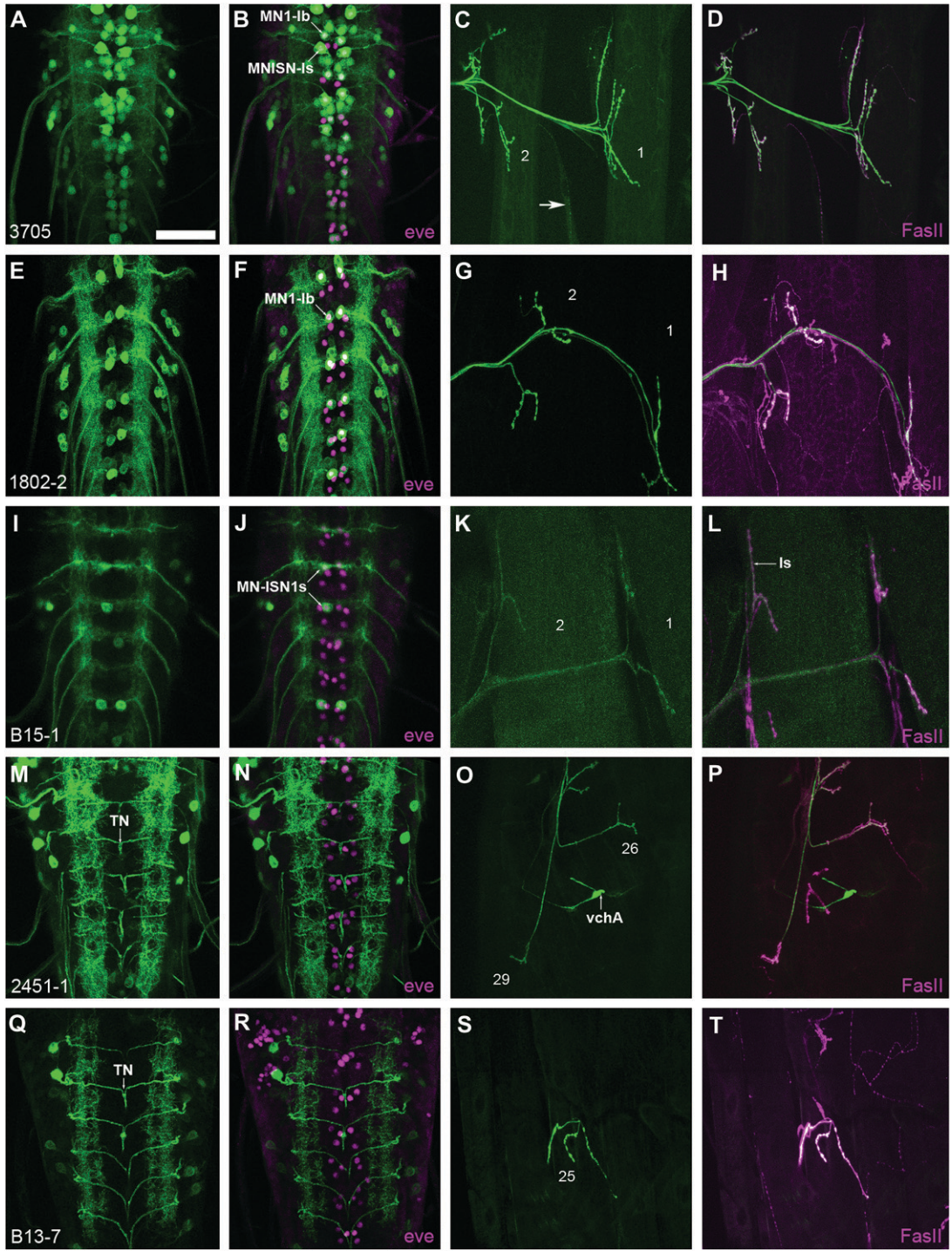

FIGURE 4.—GeneSwitch GAL4 lines with expression in motoneurons. Each GeneSwitch GAL4 expression pattern was visualized using UAS-2xEGFP (green). Columns 1 and 2 show Z-projections of confocal sections of L3 ventral nerve cord (VNC) expression. Column 2 shows anti-Eve expression (magenta), which recognizes three dorsal midline neurons: the motoneurons aCC/MN1-Ib and RP2/MNISN-Is and the pCC interneuron. Columns 3 and 4 show Z-projections of confocal sections of peripheral NMJ expression. Muscle fibers are numbered. Column 4 shows anti-FasII expression (magenta), which recognizes all motoneuron endings. (A–D) GSG3705 is expressed by a cluster of dorsal midline neurons in the VNC, including both Eve-positive motoneurons but not the Eve-positive interneuron (A and B), and at all NMJs (C and D), including type II endings (arrow). (E–H) GSG1802-2 is expressed by type Ib motoneurons in the dorsal VNC (E and F) and shows peripheral NMJ expression at type Ib endings (G and H). (I–L) GSGB15-1 is expressed in one Eve-positive dorsal midline neuron in the VNC, RP2/MNISN-Is (I and J), and exclusively at type Is NMJs in the periphery (K and L). (M–P) GSG2451-1 is expressed in lateral VNC cells (M and N) and shows peripheral NMJ expression at a subset of muscle fibers innervated by SNa, SNc (O and P), and the TN. Peripheral expression also occurs in the chordotonal sensory neuron vchA. (Q–T) GSGB13-7 is expressed in a single lateral VNC motoneuron cell body (Q and R) that innervates muscle fiber 25 via the TN (S and T). Anterior is up and dorsal is to the left in columns 3 and 4. Bar, 50 μm.

(not shown). VNC expression is observed in a lateral cluster of cell bodies (Figure 4, M and N) and in chordotonal sensory axons. The cell bodies of the embryonic motoneurons innervating muscles 5, 8, 25, 26, 27, and 29 also occupy a lateral position in the VNC. GSGB13-7 shows expression in just one motoneuron per hemisegment (Figure 4, Q–T), which innervates muscle fiber 25 via the TN. There is a single type Ib ending on muscle 25 (Figure 4, S and T), so this motoneuron supplies the sole innervation to this muscle. The motoneuron in GSGB13-7 occupies a lateral VNC cell body position (Figure 4, Q and R), similar to the embryonic VT1 motoneuron that innervates mf25 (Gorczyca *et al.* 1994; Landgraf *et al.* 1997), and probably corresponds to VT1. A few additional lateral VNC cells also show expression.

Patterns of expression in sensory neurons: There are three distinct classes of sensory neuron present in the larval abdominal sensory nervous system: external sensory, chordotonal, and multidendritic neurons (Dambly-Chaudiere and Ghysen 1986; Ghysen *et al.* 1986; Bodmer *et al.* 1989). Each abdominal hemisegment contains 8 chordotonal (ch) neurons, 15 external sensory (es) neurons, and 21 multidendritic (md) neurons. The md neurons include 15 md-da neurons, which are divided into class I/II, class III, and class IV md-da's on the basis of dendritic complexity and epidermal tiling (Grueber *et al.* 2002); 3 md-bd neurons with bipolar dendrites; and 3 md-td neurons, which have dendrites associated with tracheal branches. Sensory neuron expression was observed in 74 GeneSwitch GAL4 lines, with 54% of these lines being expressed in a single class (ch, md, or es) of sensory neuron.

In lines where multiple classes of sensory neuron were detected, expression rarely occurred among groups of neurons that shared a common developmental origin. For example, 25 lines showed expression in ch neurons and a subset of md neurons. Just two ch neurons (vchA and vchB) and two md neurons (the md-td neurons v′td1 and -2) are derived from common precursors (Brewster and Bodmer 1995), yet 23 lines expressed in md-da or md-bd neurons in conjunction with most or all ch neurons. The remaining 2 lines expressed in v′td neurons and a subset of lch5 neurons. Only six GeneSwitch GAL4 insertions showed expression in es neurons. Of these, lines GSGB62b2-1, GSG125, and GSG5793 expressed in most or all sensory neurons. Both GSGB62b2-1 and GSG125 showed very little expression in other cells. Only one line, GSG2553, showed exclusive expression in es neurons and labeled just two chemosensory neurons in each thoracic hemisegment (Figure 5, A and B).

Approximately 85% of lines with sensory neuron expression labeled ch neurons. Of these lines, three were solely expressed in ch neurons. GSG3938 showed strong expression exclusively in all ch neurons (Figure 5, C and D). While there are no recognized subclasses of ch neuron, functional subdivision within compound ch organs is observed in other invertebrates and is suspected to occur within the larval compound ch organ, lch5 (Merritt and Whitington 1995; Schrader and Merritt 2000). We identified 12 GeneSwitch GAL4 insertions that expressed in two to five of the eight ch neurons in each hemisegment. However, expression was weak in each line, and the subset of ch neurons that showed expression was variable. For these lines, expression in subsets of ch neurons probably reflects low levels of GeneSwitch GAL4 activity, rather than gene expression in distinct subtypes of ch neurons.

Approximately 85% of GeneSwitch GAL4 insertions with md neuron expression labeled md-da neurons. We identified two lines (GSG734 and GSG5722) that expressed in all md-da neurons, with GSG734 being exclusively expressed in all md-da and md-bd neurons (Figure 5, E and F). Five lines showed exclusive or predominant expression in specific subsets of md-da neurons. Lines GSG12-1, GSG3147-1, GSG2935, and GSG2295 expressed in class III and IV md-da neurons. Both GSG3147-1 (Figure 5, G and H) and GSG2295 (not shown) exclusively labeled md neurons, while GSG2935 and GSG12-1 also expressed in peripheral glia and in a small number of cells in the brain, respectively (not shown). GSG5961 expressed in just two class I md-da neurons, vpda and ddaE (Figure 5, I and J). The md-bd neurons, some ch neurons, and a few motoneurons were also labeled. Expression in md-bd or md-td neurons always occurred in conjunction with other sensory neuron expression.

Patterns of expression in muscle: There are 30 muscles in each abdominal segment A2–A7, which form a stereotyped array of longitudinal, oblique, and transverse fibers. The muscles are grouped into internal, external, and superficial muscle layers. We identified 28 GeneSwitch GAL4 lines that expressed in muscles, with 75% showing expression in all muscle fibers. GSG329-1 (Figure 6, A and B), GSGB94b1-1, and GSGB3b1-3 (not shown) all showed strong pan-muscular expression. The remaining 25% (7 lines) showed preferential or exclusive expression in subsets of muscle fibers, primarily either ventral oblique or transverse muscle fibers. GSG907 labeled ventral oblique muscles 17 and 29 (Figure 6, C and D), while GSG8942 showed strong expression in ventral oblique muscle 28 and weak expression in other muscle fibers (not shown). Selective expression in thoracic transverse muscle fibers 18 and 24 was observed in GSG3678 (Figure 6, E and F).

Muscle expression frequently occurred in conjunction with expression in other tissues. GeneSwitch GAL4 insertions that labeled muscles showed additional glial expression in 43% of lines and/or neuronal expression in 36% of lines. We found that GeneSwitch GAL4 expression patterns in muscles were frequently more variable than expression patterns in other tissues. While lines that labeled all muscles generally always expressed

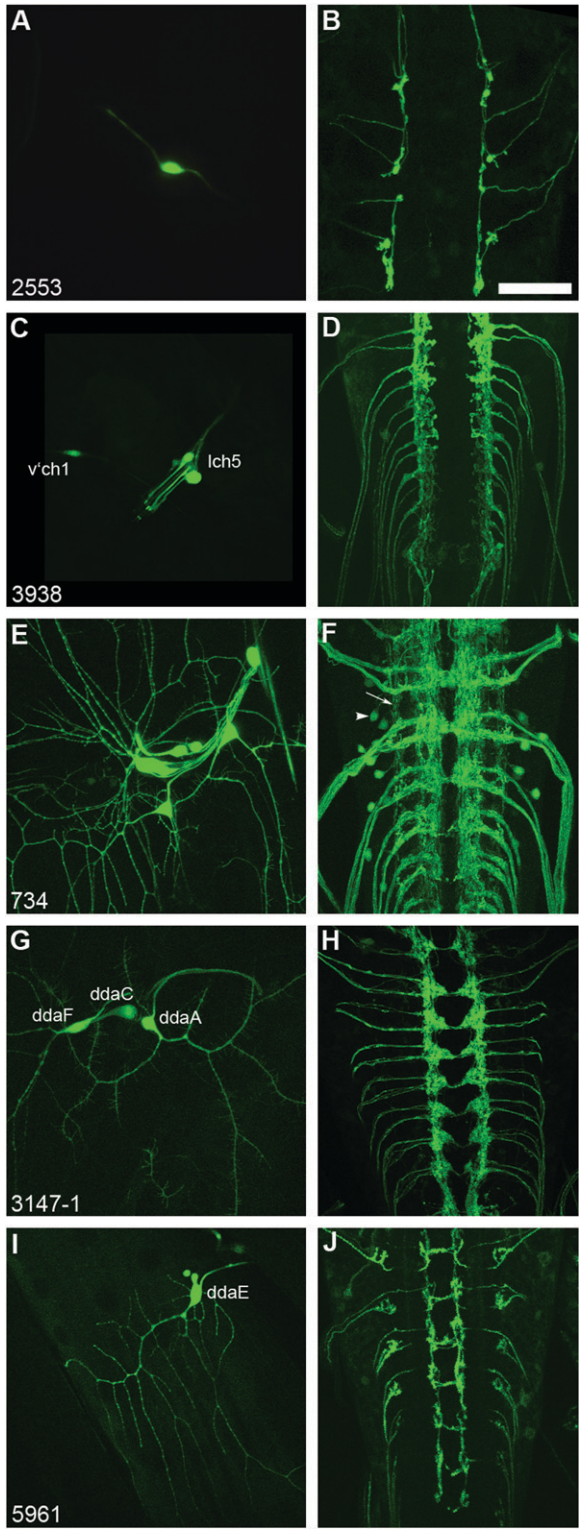

Figure 5.—GeneSwitch GAL4 lines with expression in sensory neurons. Each GeneSwitch GAL4 line expression pattern was visualized using UAS-2xEGFP. Z-projections of confocal sections of peripheral sensory expression (left column) and sensory axons projections within the VNC (right column) in L3 larva are shown. (A and B) GSG2553 expression in thoracic es neurons. (A) Peripheral cell body expression in one of the two es neurons labeled in each hemisegment is shown. (C and D) GSG3938 expresses in all ch neurons. C shows the lateral compound chordotonal organ (lch5) and v′ch1. (E and F) GSG734 expression in all md-da neurons. E shows expression in the dorsal cluster of md-da neurons. (F) In the VNC there is also expression in a small number of lateral cells (arrowhead) that form longitudinal axon projections at a lateral position (arrow). (G and H) GSG3147-1 expression in class III and IV md-da neurons. G shows expression in class III and IV md-da neurons in the dorsal cluster. (I and J) GSG5961 expression in class I md-da neurons. I shows expression in ddaE, a class I md-da neuron in the dorsal cluster. Anterior is up and dorsal is left in all PNS images. Bar, 50 μm.

in all fibers, there were often differences between larvae in the penetrance of expression and/or the level of expression in individual muscle fibers. In lines with preferential or specific GeneSwitch GAL4 expression in groups of muscles, the exact subset of muscle fibers that showed expression frequently varied between larvae and also from segment to segment within an individual larva.

Patterns of expression in glia: In the CNS there are ~30 glial cells per abdominal hemisegment (Ito *et al.* 1995). These have been grouped into three classes—surface, cortex, and neuropil—on the basis of their position within the CNS. The CNS is also surrounded by a thin sheath of perineurial cells (Ito *et al.* 1995; Carlson and Hilgers 1998). Within each peripheral nerve, there are ~8–10 large ensheathing glia (also called peripheral glia), which wrap motor and sensory axons. The smaller and more numerous perineurial cells form an outer sheath around each nerve. Glial sheath cells are also associated with peripheral sensory organs.

We identified 61 GeneSwitch GAL4 insertions that expressed in glial cells. Of these, 85% showed expression in CNS glia. Approximately 75% showed expression in glial cells surrounding the CNS, and 13% labeled glial cells at the midline. Expression in nerve-associated glia was observed in 39% of glia cell drivers, and 7% showed expression in sense organ sheath cells. We examined five lines that showed expression in surface glia in greater detail, using anti-Repo as a glial cell marker to confirm glial cell identity (Campbell *et al.* 1994; Xiong *et al.* 1994; Alfonso and Jones 2002). Among these lines, we observed GeneSwitch GAL4 expression in at least two different populations of cells ensheathing the CNS. GSG8302-2 (Figure 7, A and B), GSG317 (not shown), and GSG1201-2 (Figure 7, F and G) expressed in a small number of large repo-negative cells that formed a regular array covering the surface of the VNC and in channel glia (not shown). GSG2797 and GSG3285-1 (not shown) both expressed in irregularly shaped repo-positive cells that also covered the surface of the VNC and in cortex glia. We believe the larger outer cells correspond to perineurial cells and the smaller inner glial cells correspond to subperineurial glia (Ito *et al.* 1995).

We used a membrane-targeted CD8:GFP reporter construct (Lee and Luo 2001) to examine GeneSwitch

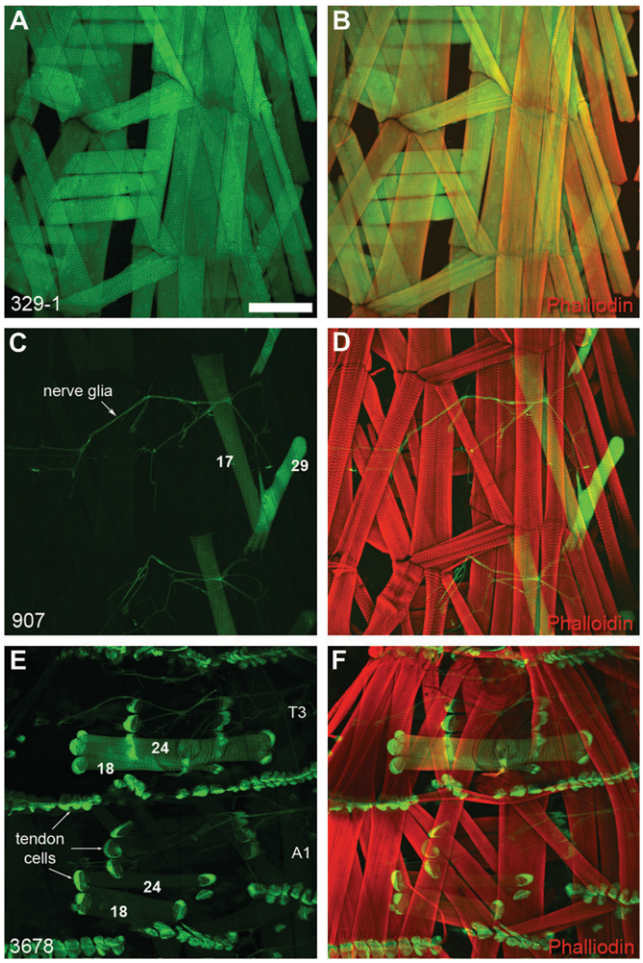

Figure 6.—GeneSwitch GAL4 lines with expression in muscles. Each GeneSwitch GAL4 expression pattern was visualized using UAS-2xEGFP (green) and stained with rhodamine phalloidin (red), which labels all muscle fibers. (A and B) GSG329-1 is expressed in all muscle fibers. (C and D) GSG907 is expressed specifically in muscle fibers 17 and 29. Expression also occurs in peripheral nerve glia. (E and F) GSG3678 is strongly expressed in transverse muscles 18 and 24 in thoracic (T) segments, but not in abdominal (A) segments. Expression is also observed in tendon cells, the epidermal attachment cells at the end of all muscle fibers. Bar, 200 μm.

GAL4 expression in cross-sections through peripheral nerves in five lines with expression in differing numbers of peripheral nerve glia. Of these, GSG8302-2 (Figure 7, C–E) and GSG1687 (not shown) expressed only in the outer perineurial cells, while GSG1202-2 (Figure 7, H–J), GSG2797, and GSG4356 (not shown) showed expression only in ensheathing glia.

Dose-dependent control of transgene expression levels: Using the pan-neuronal ELAV-GeneSwitch GAL4 driver, EGFP reporter protein expression has been demonstrated by Western blot to show dose dependence when larvae are reared on food containing 1.2–12 μg/ml of RU486, with no substantial increase in expression levels being reported for concentrations of 12–120 μg/ml of RU486 (Osterwalder *et al.* 2001). We used a GeneSwitch GAL4 line with strong expression in multidendritic sensory neurons, GSG734, and observed EGFP reporter protein expression after a 24-hr exposure to food containing RU486 concentrations ranging from 0.2 to 10 μg/ml. All multidendritic neurons showed strong expression in cell bodies and in axon terminals at 10 μg/ml, while at 0.2 μg/ml cell body expression was barely detectable and no axon terminal expression was apparent (Figure 8). EGFP expression showed dose dependence in cell body expression levels at all concentrations tested from 0.2 to 10 μg/ml, although expression levels in axon terminals were more variable between 1 and 5 μg/ml. We found that the age of the larva affected the level of expression observed after a 24-hr exposure to RU486. Larvae transferred to food containing 0.2–10 μg/ml RU486 as second instars showed brighter expression at each dosage compared to larvae exposed to RU486 as early third instars. These differences likely reflect differences in the amount of food consumed by each larva. More accurate dosing could be achieved by immersing the larva in a solution of the appropriate RU486 concentration. When larvae were reared throughout development on food containing 0.5–10 μg/ml of RU486, no substantial differences in EGFP expression levels were observed by confocal microscopy (not shown). Reduced reporter protein expression resulted from rearing larvae on 0.2 μg/ml of RU486.

RU486-independent activation of GeneSwitch GAL4 in different larval tissues: To determine whether GeneSwitch GAL4 insertions showed activity without induction, each line was crossed to the UAS-2xEGFP reporter on food without RU486. Third instar larvae from each cross were scored for EGFP expression in the absence of RU486 (supplemental Figure S2 at http://www.genetics.org/supplemental/). In 91% of lines with neuronal GeneSwitch GAL4 expression, little or no detectable EGFP expression occurred in the absence of RU486 (43.3% showed barely detectable levels of expression in a small number of cell bodies and were classified as showing little detectable expression, while no expression was detected at all in 47.3%). Background activity in neuronal GeneSwitch GAL4 lines most frequently occurred in a small number of segmentally repeated VNC cell bodies close to the dorsal midline, even if no expression was apparent at the dorsal midline when RU486 was present. As a control, we examined expression of the UAS-2xEGFP reporter construct alone crossed to wild-type CS flies. No EGFP expression was detected at the VNC dorsal midline or anywhere else in the larva (not shown).

While 61.7% of lines that expressed in glia showed little or no detectable EGFP expression in the absence of RU486, 23.6% showed moderate levels of expression in part of the induced expression pattern with 14.7% showing widespread background expression. A total of 55.6% of lines that expressed in muscle showed

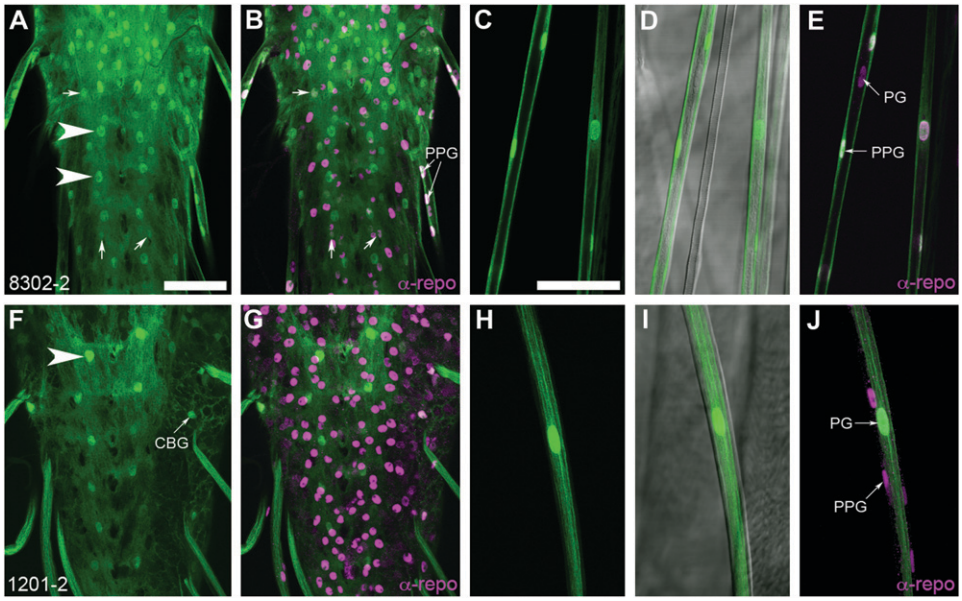

Figure 7.—GeneSwitch GAL4 lines with expression in glia. Z-projections of confocal sections of GeneSwitch GAL4 lines visualized using UAS-2xEGFP (green) and labeled with anti-Repo (magenta). (A–E) GSG8302-2. (A and B) Expression is observed in repo-negative cells on the surface of the VNC (arrowheads) and in a number of repo-positive surface glia (arrows). (C–E) Peripheral perineurial cells (PPG) surrounding the outer edge of the peripheral nerves are labeled. There is no expression in ensheathing glia (PG). (F–J) GSG1201-2. (F and G) Expression occurs in repo-negative cells on the surface of the VNC (arrowhead), as with GSG8302-2, and in repo-positive cortex glia (CBG-cell body glia). (H–J) In peripheral nerves, expression is observed in the inner ensheathing glia, but not in the outer peripheral perineurial cells. Bars: A, B, F, and G, 50 μm; C–E and H–J, 25 μm.

detectable expression without RU486 and showed either moderate expression in a partial expression pattern (16.7%) or strong expression throughout muscles (38.9%). Most lines that showed strong uninduced expression were discarded. There was a correlation between the observed level of uninduced expression and the proportion of the induced expression pattern that showed expression without RU486. Where weak levels of uninduced expression were detected, expression was almost always confined to a small subset of the induced expression pattern. Moderate levels of uninduced expression showed broader expression patterns, while lines with strong expression levels labeled most or all of the cells visible with RU486 induction.

Molecular characterization of GeneSwitch GAL4 insertion sites: We analyzed the genomic sequences surrounding 47 of the GeneSwitch GAL4 insertions (Table 3). This included virtually all lines shown in detail in this article. Thirty-four of the GeneSwitch GAL4 enhancer-detector elements landed within or adjacent to known or predicted genes. These include genes with known or potential roles in transcriptional or translational regulation, such as transcription factors, microRNAs, and RNA-binding proteins; a number of unknown genes with no ascribed functions; known or predicted enzymes including a GTPase, a protein kinase, and a metalloprotease; a neuropeptide; and a tubulin gene. We found that 12 of these genes have already been described in the literature as having loss- or gain-of-function phenotypes that affect some aspect of the nervous system, although only 3 have a described phenotype in the tissue in which GeneSwitch GAL4 expression occurs. GSGB13-7 and GSG4064-1 interrupted *pumilio* and *kuzbanian*, respectively. Both genes are known to alter the phenotypic properties of motoneurons (Fambrough *et al.* 1996; Schweers *et al.* 2002), and both insertions are expressed in a subset of motoneurons. The third gene, *couch potato* (*cpo*), is expressed in the embryonic PNS (Bellen *et al.* 1992a,b). Both GeneSwitch GAL4 insertions that landed in *cpo* are expressed in sensory neurons. One line, GSG5793, a lethal insert into β-*tubulin-56D*, is expressed in the mushroom bodies and in all sensory neurons. The endogenous β-*tubulin-56D* gene is preferentially expressed in the embryonic nervous system (Buttgereit *et al.* 1991). However, there are no published nervous system phenotypes associated with β-*tubulin-56D* due to its embryonic lethality.

In 12 lines the transposon landed between genes, with the nearest annotated gene between 1.1 and 10 kb away. Several of these lines showed very specific expression patterns. For example, GSG2553 is inserted 5.7 kb 5′ of the *spineless* start site and is expressed in just two es neurons per thoracic hemisegment. Restricted GeneSwitch GAL4 expression patterns were also seen when transposons landed in or near a gene. GSGB13-7 landed in *pumilio* and shows expression in a single motoneuron that innervates mf25.

Of the lines analyzed, two insertions landed in the 5′ regulatory region of *RluA-1*, a predicted diaminohydroxyphosphoribosylaminopyrimidine deaminase. Both GSG734 and GSG2935 insertions are within 125 bp of the *RluA-1* start site, with insertion sites that differ by just 4 bp, and both are expressed in md neurons. Interestingly, they

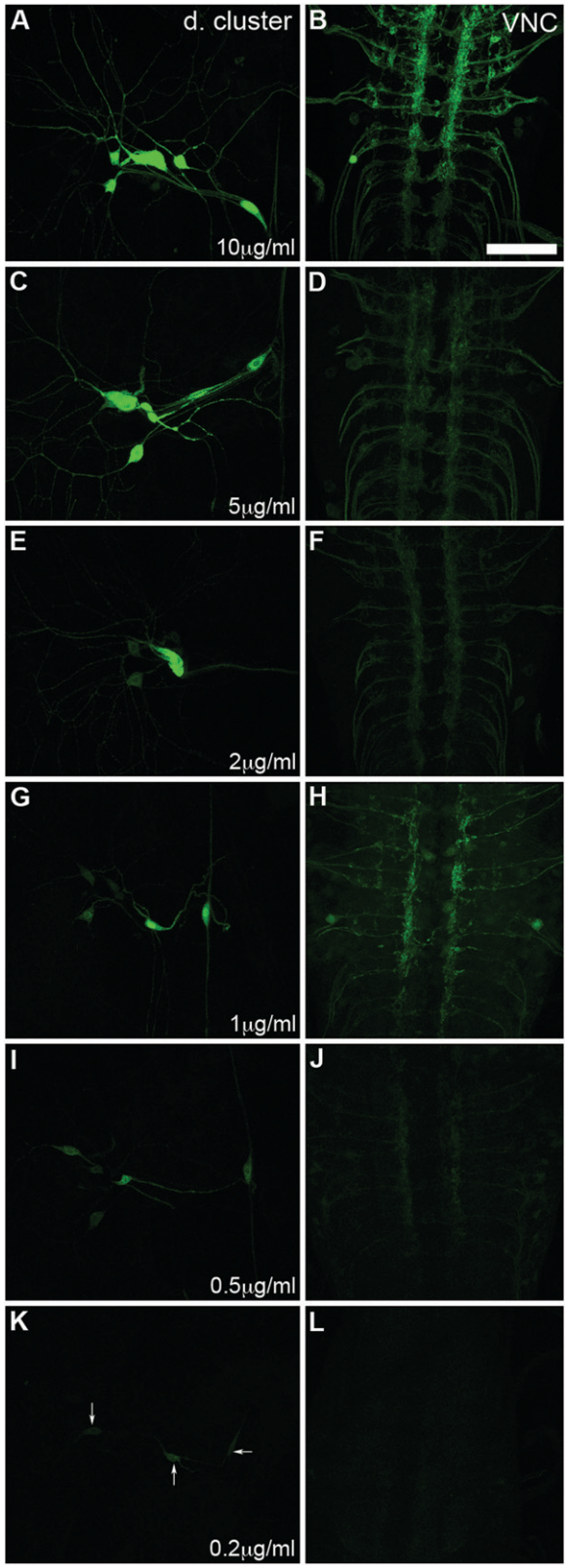

FIGURE 8.—Transgene expression level can be controlled by varying RU486 concentration. GSG734 drives expression in multidendritic sensory neurons. Z-projections of confocal sections of peripheral cell body expression (left) and sensory axon expression in the VNC (right) in L3 larvae are shown. Expression was visualized after a 24-hr exposure to varying concentrations of RU486 using a UAS-2xEGFP reporter gene. (A and B) After a 24-hr exposure to 10 μg/ml RU486 strong expression is visible in peripheral cell bodies (A) and in axon terminals in the VNC (B). (C and D) At 5 μg/ml, strong expression is observed in cell bodies (C), with weaker axonal expression (D). (E–H) Peripheral cell body expression is variable at 2 μg/ml (E) and 1 μg/ml (G), although some cells show strong expression. Axon expression is also variable (F and H). (I and J) At 0.5 μg/ml weak cell body expression is visible (I), and axon expression is barely detectable (J). (K and L) At 0.2 μg/ml, cell body expression is barely detectable (K, arrows) and no axon expression is observed (L). Bar, 50 μm.

show overlapping but not identical patterns of expression. While GSG734 is expressed in all md neurons, GSG2935 shows expression in a subset of md neurons and in peripheral nerve glia. Similarly, GSGB62b2-1 and GSGB38b3-2 both have insertions in the same hotspot in the second intron of *cpo*, and both are expressed in sensory neurons. GSGB62b2-1 is expressed in all sensory neurons (md, ch, and es), while GSGB38b3-2 is expressed by a subset of sensory neurons (ch and md). This is in accord with previous analysis of multiple pLacZ insertions within a single promoter region, which showed that, while there are slight variations in pattern, the core expression pattern remains relatively consistent between different insertion lines (HAN *et al.* 1996).

DISCUSSION

In this article we describe an enhancer-detector screen for GeneSwitch GAL4 lines that show inducible expression in specific subsets of larval nervous system cells. We found that 71% of GeneSwitch GAL4 lines showed expression in at least one tissue, excluding salivary glands, with ~15% showing expression in the larval nervous system and/or musculature and ~7% showing more specific expression in neurons, glia, and/or muscle. This is comparable to embryonic enhancer-detector screens using pLacZ, which report expression in ~65% of lines, with 5–10% of those lines having more specific expression patterns that provide useful cell or tissue markers (BELLEN *et al.* 1989; BIER *et al.* 1989; HARTENSTEIN and JAN 1992). Likewise, GAL4 enhancer-detector screens report larval nervous system expression in ~20–30% of lines (GUSTAFSON and BOULIANNE 1996; MANSEAU *et al.* 1997).

In contrast, a screen of >6000 pLacZ enhancer-detector lines in the adult brain reported CNS expression in >80% of lines examined, with 15% being selectively expressed in subsets of neurons (HAN *et al.* 1996). If both GeneSwitch GAL4 and pLacZ enhancer detectors reflect the activity of nearby genes, then the difference in CNS expression between the two stages suggests that many more genes are expressed in the adult CNS than in the larval CNS. The brain increases in

TABLE 3

Genomic, expression, and phenotypic information for selected GeneSwitch GAL4 insertions

Line	Expression pattern	Cytological location	Transposon location		Molecular information	Nervous system expression and/or phenotype	Viability
			In/near gene	Between genes			
3687-1	Mushroom bodies, motoneuron, peripheral glia	45F1	151 bp 5′ of *mir-14*		MicroRNA	—	Viable
8308	Interneuron	63C1	334 bp 5′ of *mir-282*		MicroRNA	—	Lethal
B13-7	Single motoneuron	85D1	*pumilio* (intron)		RNA-binding protein, translational inhibitor	Expressed in CNS and PNS. Mutation causes neuronal hyperexcitability (Schweers *et al.* 2002), affects long-term memory (Dubnau *et al.* 2003), and affects dendrite morphogenesis and NMJ morphology (Menon *et al.* 2004; Ye *et al.* 2004).	Viable
125	Sensory	90D1	*couch potato* (intron)		RNA-binding protein	Expressed in CNS and PNS. Mutation affects PNS development and adult behavior (Bellen *et al.* 1992a,b).	Viable
B38b3-2	Sensory	90D1	*couch potato* (intron)		RNA-binding protein (see line 125)	See line 125.	Viable
5961	Sensory	33E7	*bunched* (intron)		Transcription factor	Delayed photoreceptor differentiation (Treisman *et al.* 1995).	Viable
B3b1-3	Muscle	67B5	*MTF-1* (first exon)		"Metal-responsive transcription factor"	—	ND
B13-5	Glia	88B1	*Trithorax* (intron)		Transcription factor	Mutations cause homeotic transformations and affect homeotic gene expression in the nervous system (Ingham and Whittle 1980; Mazo *et al.* 1990).	ND
2451-1	SNa/SNc motoneuron	92F2	*bonus* (first exon)		Transcription cofactor, nuclear receptor cofactor	Mutations affect PNS development (Prokopenko *et al.* 2000).	Lethal
2797	Glia	48A3	*toutatis* (intron)		DNA/chromatin-binding protein	Mutations affect proneural gene activity/bristle formation (Vanolst *et al.* 2005).	ND
3938	Sensory	11E8	*HDAC4* (intron)		Histone deacetylase activity	—	Viable

(*continued*)

TABLE 3

(Continued)

Line	Expression pattern	Cytological location	Transposon location		Molecular information	Nervous system expression and/or phenotype	Viability
			In/near gene	Between genes			
12-1	Sensory	85D18	13 bp 5′ of *Rho-like* in antisense direction		Rho family small GTPase	Overexpression phenotype in motor axons (Kraut *et al.* 2001), DN RhoL does not affect dendritic morphology (Emoto *et al.* 2004).	Viable
734	Sensory	31F1	121 bp 5′ of *RluA-1* in antisense direction		Inferred diaminohydroxy-phosphoribosylaminopyri-midine deaminase activity	—	Viable
2935	Sensory, peripheral glia	31F1	117 bp 5′ of *RluA-1* in antisense direction		See line 734	—	Viable
1802-2	Type Ib motoneuron	39F1	*CG31619* (intron)		Inferred procollagen N-endopeptidase activity	—	Lethal
1142	Neuroendocrine, sensory	86F6	*LK6* (intron)		Protein kinase, functions in translational regulation	—	Viable
1687	Peripheral glia	46E1	54 bp 5′ of *eiger*		TNF superfamily ligand	Predominantly expressed in the nervous system (Igaki *et al.* 2002).	Viable
3173-3	Motoneuron	18F4	*amnesiac*		Neuropeptide	Memory phenotype (Quinn *et al.* 1979), altered electrophysiological properties of larval neurons (Yu *et al.* 1999).	Viable
3285-1	Glia, sensory	93A4	*ATPα* (intron)		Na^+ pump aα-subunit	Neural physiology and behavioral phenotype (Schubiger *et al.* 1994; Palladino *et al.* 2003).	ND
3705	Motoneuron, interneuron	41F9	18 bp 5′ of *gp210*		Nucleoporin	Memory phenotype (Dubnau *et al.* 2003).	Viable
4064-1	Motoneuron, sensory	34C4	Start site of *kuzbanian*		Metalloprotease	Expressed in CNS and PNS. Neurogenic gene, also required for axon extension in the embryonic CNS (Fambrough *et al.* 1996; Rooke *et al.* 1996).	Viable
4102	Motoneuron, sensory	61B3	98 bp 5′ of *E(bx)* in antisense direction		Nucleosome remodeling factor (NURF) complex component	RNAi affects dendrite morphogenesis in md-da neurons (Parrish *et al.* 2006).	Viable

(*continued*)

TABLE 3

(Continued)

Line	Expression pattern	Cytological location	Transposon location		Molecular information	Nervous system expression and/or phenotype	Viability
			In/near gene	Between genes			
5793	Mushroom bodies, sensory	56D7	β-*tubulin56D* (intron)		Tubulin	Expressed in the SOG and ventral nerve cord (Gasch *et al.* 1988).	Lethal
8942	Glia	57F6	*CG10082*(intron)		Putative inositol hexakisphosphate kinase	—	Viable
10703	Glia	48B6	*Roc2* (intron) and 226 bp 5′ of *CG30035*		Ubiquitin ligase (roc2) and glucose transporter (CG30035)	—	ND
B12-6	Motoneuron, interneuron	93D4	71 bp 5′ *Heat-shock RNA* ω		Unknown (apparently untranslated)	—	Viable
3183	Muscle	88D1	70 bp 5′ of *CG33967* in antisense direction		Unknown	—	ND
3330-2	Motoneuron, sensory, interneuron	9B1	*CG15312* (intron)		Unknown	—	Viable
3630	Mushroom bodies	67E6	35–80 bp 5′ of *CG32066* in antisense direction; 386–431bp 5′ of *CG8003*		Unknown	—	Viable
4356	Peripheral glia	47C3	*CG30015* (intron)		Unknown	—	Viable
5255	Motoneuron, interneuron	42E5	*Tetraspanin 42eF* (intron)		Unknown	—	Viable
8708	Muscle	60E10	*CG2765* (intron)		Unknown	—	ND
B94b1-1	Muscle	38F5	*CG9339* (intron)		Unknown	—	Viable
317	Glia	58F4		499 bp 3′ of *CG13512* and 1.8 kb 5′ of *CG4250*			Lethal
329-1	Muscle	30C5		172 bp 5′ of *Pka-C1* in antisense direction; 369 bp 5′ of *hoip*			ND
907	Glia, muscle	35B2		1.2 kb 5′ of *tRNA:G3:35Ba*			ND
176	Motoneuron	28B1		5.3 kb 5′ of *Spn7*; 1.6 kb 3′ of *CG13791* and 7.8 kb 5′ of *CG13790* in antisense direction			Viable
1201-2	Glia	48B1		2.7 kb 5′ of *CG9005* in antisense direction			Viable
2553	Sensory	89B15		5.7 kb 5′ of *spineless* in antisense direction			Viable
3147-1	Sensory	36F2		17.1 kb 5′ of *Fas3* and 5.7 kb 3′ of *CG5790*			Viable

(*continued*)

TABLE 3

(Continued)

Line	Expression pattern	Cytological location	Transposon location		Molecular information	Nervous system expression and/or phenotype	Viability
			In/near gene	Between genes			
3315-1	Interneuron	8B2		3.5 kb 5′ of *CG32710*; 10 kb 5′ of *lim1* in antisense direction			Viable
3473	Motoneuron, sensory	86C7		2 kb 3′ of *CG4509* and 2.2 kb 5′ of *CG4565*; 584 bp 3′ of *CG6567* in antisense direction			Viable
3516	Motoneuron, interneuron	90E2		8 kb 3′ of *CG7794*; 11.8 kb 3′ of *htl* in antisense direction			Viable
6124	Neuron	95C1		174 bp 3′ of snRNA:U1:95Cc and 1199 bp 5′ of snRNA:U1:95CB			ND
8302-2	Glia	46F1		1.1 kb 5′ of *gem*; 1.6 kb 5′ of *CCS* in antisense direction			Viable
8892	Neuron	16B9		17 bp 3′ of *CG8408* in antisense direction; 17 kb 3′ of *X11L*			Viable
6098	Neuron	—		In heterochromatin on 3, did not map			Viable

size from ~2000 neurons to 100,000 neurons in the transition from larva to adult and is required to generate increasingly complex behaviors such as courtship and flight. One might therefore expect the adult brain to require more genes to be expressed. It is also possible that the lower incidence of CNS expression in GeneSwitch GAL4 lines arises from differences between the *P*-element constructs.

The aim of this screen is to provide useful tools for inducible expression in specific cells or tissues within the larval nervous system. In addition to identifying strains with useful expression patterns, we also determined how much basal transcriptional activity each line possessed without RU486. All GeneSwitch GAL4 lines showed an increase in expression upon application of RU486, with little or no detectable reporter gene expression in the absence of RU486 in 82% of lines. We found tissue-specific differences in the likelihood of GeneSwitch GAL4 activity in the absence of RU468, with little or no uninduced expression observed in 91% of neuronal lines and in 61.7% of glial lines. However, significant uninduced expression was observed in more than half of lines that expressed in muscles. This has also been reported when the myosin heavy chain (MHC) promoter is used to express GeneSwitch GAL4 specifically in muscles (Osterwalder *et al.* 2001). With this construct, expression occurred during late third instar in the absence of the inducer. By contrast, a pan-neuronal ELAV-GeneSwitch GAL4 construct gave little uninduced expression. This suggests that RU486-independent activation of GeneSwitch GAL4 frequently occurs in third instar muscle fibers and, to a lesser degree, in glial cells, but only rarely occurs in neurons. In the adult, a screen of several hundred GeneSwitch GAL4 insertions reported little or no expression in the absence of the ligand (Roman *et al.* 2001). The uninduced activity may be therefore both stage and tissue specific.

One possibility is that an alternative ligand present in some third instar larval tissues can activate GeneSwitch GAL4. GeneSwitch GAL4 contains the ligand-binding domain of the human progesterone receptor, a nuclear steroid hormone receptor. There are no orthologs of the nuclear steroid hormone receptors in Drosophila (Maglich *et al.* 2001). The most closely related nuclear receptors present in Drosophila are the estrogen-related receptors (ERR), and ERR ligands are widely available during mid-third instar (Palanker *et al.* 2006). Although GeneSwitch GAL4 is reported to have very low transcriptional activity in the absence of RU486 (Burcin *et al.* 1998), it is also possible that uninduced GeneSwitch GAL4 activity occurs if GeneSwitch GAL4 is present at very high levels. Larval muscles degenerate during metamorphosis, while neurons largely become incorporated into the adult nervous system, and changes in DNA structure and regulation in tissues that undergo histolysis may provide a permissive environment for residual GeneSwitch GAL4 transcriptional activity in the absence of RU486. It should also be noted that, while RU486 causes no obvious phenotypic effects at doses used for activation (Osterwalder *et al.* 2001; Roman *et al.* 2001; Etter *et al.* 2005; Mosca *et al.* 2005), microarray analysis has identified five mRNAs in Drosophila whose expression changes in response to RU486, three of unknown function and two with predicted roles in transmembrane transport (Etter *et al.* 2005). These mRNAs may be regulated as part of a general drug-response mechanism or as steroid hormone-responsive genes that can be activated by RU486.

Ligand-inducible systems like GeneSwitch GAL4 provide an opportunity to control not only the timing of gene expression but also the level of expression, simply by varying the concentration of the ligand. For example, one could select a level of transgene expression that produces a partial genetic rescue, creating a sensitized background for testing genetic interactions. We analyzed EGFP expression levels using confocal microscopy and found that EGFP expression levels showed dose dependence after a 24-hr exposure to different concentrations of RU486 (0.2–10 μg/ml). When larvae were reared throughout development on food containing RU486, concentrations of 0.2 μg/ml of RU486 resulted in limited EGFP expression, while no substantial difference in EGFP expression was observed for concentrations of 0.5–10 μg/ml of RU486. Western blot analysis has previously shown reporter gene induction by ELAV-GeneSwitch to be dose dependent from 1.2 to 12 μg/ml of RU486, when larvae are fed RU486 throughout development (Osterwalder *et al.* 2001). The different methods of detection used, confocal fluorescence microscopy or Western blot, partly account for the difference in the ability to detect changes in EGFP levels resulting from continual feeding of RU486 over similar concentration ranges. However, such differences also reflect the different expression levels of the respective GeneSwitch GAL4 drivers used (ELAV-GS and GSG734) and their different expression patterns (pan-neuronal *vs.* md-neuron specific). To generate low levels of expression and subtle phenotypes with a strong, specific GeneSwitch GAL4 driver, a shorter induction time or a lower dosage (0.2 μg/ml) may be required.

We determined the genomic insertion sites for 47 GeneSwitch GAL4 enhancer-detector lines, including most lines referred to in this article. Information on genomic insertion sites is useful in determining whether there may be unwanted phenotypes associated with a given GeneSwitch GAL4 insertion. In the majority of lines (72%), the *P* element is inserted into the 5′-regulatory region or within an intron of a known or a predicted gene. Approximately 85% landed in hotspots or close to previously documented *P*-element insertions, while the remaining 15% represent unique insertions. Of these genes, 6% have associated phenotypes affecting the cells showing GeneSwitch GAL4 expression,

while a further 21% have associated phenotypes affecting other stages or other cells within the nervous system. However, insertion within or close to a gene with a known phenotype does not mean that those insertions themselves will cause a phenotype. Only 11–13% of all *P*-element insertions result in an evident phenotype or lethality (Bellen 1999). We found that ~10% of GeneSwitch GAL4 insertion lines caused lethality, as judged by the lack of viable homozygous adults, consistent with previous enhancer-detector screens.

During the course of the screen we identified several lines with very restricted expression patterns. These included GSGB13-7, which is expressed in a single motoneuron innervating muscle fiber 25, and GSG2553, which is expressed in two external sensory neurons in each thoracic segment. In one, GSGB13-7, the *P* element was inserted into the third intron of *pumilio*, while in the other, GSG2553, the closest known or predicted gene to the *P*-element insertion site is *spineless*, >5.6 kb away. Although the GeneSwitch GAL4 expression patterns associated with these insertions are limited, both *pumilio* and *spineless* are widely expressed throughout the larval nervous system and in the PNS, respectively (Menon *et al.* 2004; Ye *et al.* 2004; Kim *et al.* 2006). The GSGB13-7 insertion, although within an intron of *pumilio*, is >10.8 kb away from the *pumilio* start site. As GSGB13-7 and GSG2553 insertion sites are a considerable distance from a transcriptional start site, both may be less likely to detect all enhancers that regulate the endogenous gene.

While the major goal of this screen was to provide tools for inducible transgene expression in the larval nervous system, enhancer-detector elements were developed to identify novel genes on the basis of their expression pattern, without screening for a mutant phenotype (Casadaban and Cohen 1979; O'Kane *et al.* 1986; O'Kane and Gehring 1987). Enhancer-detector elements that land in or near a gene are generally expressed in tissues or cells that express the endogenous gene. We can therefore use the expression pattern of lines that have inserted into or adjacent to unknown genes to provisionally assign neuronal, glial, or muscle expression to these genes. Endogenous genes are frequently more widely expressed than the enhancer-detector element, as shown in the GSGB13-7 insertion into *pumilio*, and so the function of these unknown genes may not be restricted to the tissues that show GeneSwitch GAL4 expression.

In summary, the lines described in this screen provide new GeneSwitch GAL4 strains for inducible gene expression in specific cells or tissues of the larval nervous system.

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LITERATURE CITED

Alfonso, T. B., and B. W. Jones, 2002 gcm2 promotes glial cell differentiation and is required with glial cells missing for macrophage development in Drosophila. Dev. Biol. **248:** 369–383.

Atwood, H. L., C. K. Govind and C. F. Wu, 1993 Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in Drosophila larvae. J. Neurobiol. **24:** 1008–1024.

Bellen, H. J., 1999 Ten years of enhancer detection: lessons from the fly. Plant Cell **11:** 2271–2281.

Bellen, H. J., C. J. O'Kane, C. Wilson, U. Grossniklaus, R. K. Pearson *et al.*, 1989 P-element-mediated enhancer detection: a versatile method to study development in Drosophila. Genes Dev. **3:** 1288–1300.

Bellen, H. J., S. Kooyer, D. Develyn and J. Pearlman, 1992a The Drosophila couch potato protein is expressed in nuclei of peripheral neuronal precursors and shows homology to RNA-binding proteins. Genes Dev. **6:** 2125–2136.

Bellen, H. J., H. Vaessin, E. Bier, A. Kolodkin, D. Develyn *et al.*, 1992b The Drosophila couch-potato gene: an essential gene required for normal adult behavior. Genetics **131:** 365–375.

Bello, B., D. Resendez-Perez and W. J. Gehring, 1998 Spatial and temporal targeting of gene expression in Drosophila by means of a tetracycline-dependent transactivator system. Development **125:** 2193–2202.

Bier, E., H. Vaessin, S. Shepherd, K. Lee, K. McCall *et al.*, 1989 Searching for pattern and mutation in the Drosophila genome with a P-Lacz vector. Genes Dev. **3:** 1273–1287.

Bieschke, E. T., J. C. Wheeler and J. Tower, 1998 Doxycycline-induced transgene expression during Drosophila development and aging. Mol. Gen. Genet. **258:** 571–579.

Bodmer, R., R. Carretto and Y. N. Jan, 1989 Neurogenesis of the peripheral nervous-system in Drosophila embryos: DNA-replication patterns and cell lineages. Neuron **3:** 21–32.

Bossing, T., G. Udolph, C. Q. Doe and G. M. Technau, 1996 The embryonic central nervous system lineages of Drosophila melanogaster. 1. Neuroblast lineages derived from the ventral half of the neuroectoderm. Dev. Biol. **179:** 41–64.

Brand, A. H., and N. Perrimon, 1993 Targeted gene-expression as a means of altering cell fates and generating dominant phenotypes. Development **118:** 401–415.

Brewster, R., and R. Bodmer, 1995 Origin and specification of type-II sensory neurons in Drosophila. Development **121:** 2923–2936.

Budnik, V., Y. Zhong and C. F. Wu, 1990 Morphological plasticity of motor axons in Drosophila mutants with altered excitability. J. Neurosci. **10:** 3754–3768.

Burcin, M. M., B. W. O'Malley and S. Y. Tsai, 1998 A regulatory system for target gene expression. Frontiers Biosci. **3:** c1–c7.

Buttgereit, D., D. Leiss, F. Michiels and R. Renkawitzpohl, 1991 During Drosophila embryogenesis the beta-1 tubulin gene is specifically expressed in the nervous-system and the apodemes. Mech. Dev. **33:** 107–118.

Campbell, G., H. Goring, T. Lin, E. Spana, S. Andersson *et al.*, 1994 Rk2, a glial-specific homeodomain protein required for embryonic nerve card condensation and viability in Drosophila. Development **120:** 2957–2966.

Carlson, S. D., and S. L. Hilgers, 1998 Perineurium in the Drosophila (Diptera: Drosophilidae) embryo and its role in the blood-brain/nerve barrier. Int. J. Insect Morphol. Embryol. **27:** 61–66.

Casadaban, M. J., and S. N. Cohen, 1979 Lactose genes fused to exogenous promoters in one-step using a Mu-Lac bacteriophage: in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA **76:** 4530–4533.

Choi, J. C., D. Park and L. C. Griffith, 2004 Electrophysiological and morphological characterization of identified motor neurons in the Drosophila third instar larva central nervous system. J. Neurophysiol. **91:** 2353–2365.

Chopra, M., and S. Singh, 1994 Developmental temperature selectively regulates a voltage-activated potassium current in Drosophila. J. Neurobiol. **25:** 119–126.

Dambly-Chaudiere, C., and A. Ghysen, 1986 The sense-organs in the Drosophila larva and their relation to the embryonic pattern of sensory neurons. Rouxs Arch. Dev. Biol. **195:** 222–228.

Dubnau, J., A. S. Chiang, L. Grady, J. Barditch, S. Gossweiler *et al.*, 2003 The staufen/pumilio pathway is involved in Drosophila long-term memory. Curr. Biol. **13:** 286–296.

Eggert, H., K. Bergemann and H. Saumweber, 1998 Molecular screening for *P*-element insertions in a large genomic region of *Drosophila melanogaster* using polymerase chain reaction mediated by the vectorette. Genetics **149:** 1427–1434.

Emoto, K., Y. He, B. Ye, W. B. Grueber, P. N. Adler *et al.*, 2004 Control of dendritic branching and tiling by the tricornered-kinase/furry signaling pathway in Drosophila sensory neurons. Cell **119:** 245–256.

Etter, P. D., R. Narayanan, Z. Navratilova, C. Patel, D. Bohmann *et al.*, 2005 Synaptic and genomic responses to JNK and AP-I signaling in Drosophila neurons. BMC Neurosci. **6:** 39.

Fambrough, D., D. J. Pan, G. M. Rubin and C. S. Goodman, 1996 The cell surface metalloprotease disintegrin Kuzbanian is required for axonal extension in Drosophila. Proc. Natl. Acad. Sci. USA **93:** 13233–13238.

Ford, D., N. Hoe, G. N. Landis, K. Tozer, A. Luu *et al.*, 2007 Alteration of Drosophila life span using conditional, tissue-specific expression of transgenes triggered by doxycyline or RU486/Mifepristone. Exp. Gerontol. **42:** 483–497.

Gasch, A., U. Hinz, D. Leiss and R. Renkawitzpohl, 1988 The expression of beta-1 and beta-3 tubulin genes of Drosophila-melanogaster is spatially regulated during embryogenesis. Mol. Gen. Genet. **211:** 8–16.

Gelbart, W., L. Bayraktaroglu, B. Bettencourt, K. Campbell, M. Crosby *et al.*, 2003 The FlyBase database of the Drosophila genome projects and community literature. Nucleic Acids Res. **31:** 172–175.

Ghysen, A., C. Dambly-Chaudiere, E. Aceves, L. Y. Jan and Y. N. Jan, 1986 Sensory neurons and peripheral pathways in Drosophila embryos. Rouxs Arch. Dev. Biol. **195:** 281–289.

Gorczyca, M., C. Augart and V. Budnik, 1993 Insulin-like receptor and insulin-like peptide are localized at neuromuscular-junctions in Drosophila. J. Neurosci. **13:** 3692–3704.

Gorczyca, M. G., R. W. Phillis and V. Budnik, 1994 The role of tinman, a mesodermal cell fate gene, in axon pathfinding during the development of the transverse nerve in Drosophila. Development **120:** 2143–2152.

Grenningloh, G., E. J. Rehm and C. S. Goodman, 1991 Genetic-analysis of growth cone guidance in Drosophila: Fasciclin-II functions as a neuronal recognition molecule. Cell **67:** 45–57.

Grueber, W. B., L. Y. Jan and Y. N. Jan, 2002 Tiling of the Drosophila epidermis by multidendritic sensory neurons. Development **129:** 2867–2878.

Gustafson, K., and G. L. Boulianne, 1996 Distinct expression patterns detected within individual tissues by the GAL4 enhancer trap technique. Genome **39:** 174–182.

Halfon, M. S., S. Gisselbrecht, J. Lu, B. Estrada, H. Keshishian *et al.*, 2002 New fluorescent protein reporters for use with the Drosophila Gal4 expression system and for vital detection of balancer chromosomes. Genesis **34:** 135–138.

Halpern, M. E., A. Chiba, J. Johansen and H. Keshishian, 1991 Growth cone behavior underlying the development of stereotypic synaptic connections in Drosophila embryos. J. Neurosci. **11:** 3227–3238.

Han, D. D., D. Stein and L. M. Stevens, 2000 Investigating the function of follicular subpopulations during Drosophila oogenesis through hormone-dependent enhancer-targeted cell ablation. Development **127:** 573–583.

Han, P. L., V. Meller and R. L. Davis, 1996 The Drosophila brain revisited by enhancer detection. J. Neurobiol. **31:** 88–102.

Hartenstein, V., and Y. N. Jan, 1992 Studying Drosophila embryogenesis with P-Lacz enhancer trap lines. Rouxs Arch. Dev. Biol. **201:** 194–220.

Hoang, B., and A. Chiba, 2001 Single-cell analysis of Drosophila larval neuromuscular synapses. Dev. Biol. **229:** 55–70.

Igaki, T., H. Kanda, Y. Yamamoto-Goto, H. Kanuka, E. Kuranaga *et al.*, 2002 Eiger, a TNF superfamily ligand that triggers the Drosophila JNK pathway. EMBO J. **21:** 3009–3018.

Ingham, P., and R. Whittle, 1980 Trithorax: a new homoeotic mutation of Drosophila-melanogaster causing transformations of abdominal and thoracic imaginal segments. 1. Putative role during embryogenesis. Mol. Gen. Genet. **179:** 607–614.

Ito, K., J. Urban and G. M. Technau, 1995 Distribution, classification, and development of Drosophila glial-cells in the late embryonic and early larval ventral nerve cord. Rouxs Arch. Dev. Biol. **204:** 284–307.

Jia, X. X., M. Gorczyca and V. Budnik, 1993 Ultrastructure of neuromuscular-junctions in Drosophila: comparison of wild-type and mutants with increased excitability. J. Neurobiol. **24:** 1025–1044.

Johansen, J., M. E. Halpern, K. M. Johansen and H. Keshishian, 1989 Stereotypic morphology of glutamatergic synapses on identified muscle-cells of Drosophila larvae. J. Neurosci. **9:** 710–725.

Keshishian, H., K. Broadie, A. Chiba and M. Bate, 1996 The Drosophila neuromuscular junction: a model system for studying synaptic development and function. Annu. Rev. Neurosci. **19:** 545–575.

Kim, M. D., L. Y. Jan and Y. N. Jan, 2006 The bHLH-PAS protein Spineless is necessary for the diversification of dendrite morphology of Drosophila dendritic arborization neurons. Genes Dev. **20:** 2806–2819.

Kraut, R., K. Menon and K. Zinn, 2001 A gain-of-function screen for genes controlling motor axon guidance and synaptogenesis in Drosophila. Curr. Biol. **11:** 417–430.

Landgraf, M., T. Bossing, G. M. Technau and M. Bate, 1997 The origin, location, and projections of the embryonic abdominal motorneurons of Drosophila. J. Neurosci. **17:** 9642–9655.

Landgraf, M., N. Sanchez-Soriano, G. M. Technau, J. Urban and A. Prokop, 2003 Charting the Drosophila neuropile: a strategy for the standardised characterisation of genetically amenable neurites. Dev. Biol. **260:** 207–225.

Lee, T. M., and L. Q. Luo, 2001 Mosaic analysis with a repressible cell marker (MARCM) for Drosophila neural development. Trends Neurosci. **24:** 251–254.

Lin, D. M., R. D. Fetter, C. Kopczynski, G. Grenningloh and C. S. Goodman, 1994 Genetic-analysis of Fasciclin-II in Drosophila: defasciculation, refasciculation, and altered fasciculation. Neuron **13:** 1055–1069.

Luan, H. J., W. C. Lemon, N. C. Peabody, J. B. Pohl, P. K. Zelensky *et al.*, 2006 Functional dissection of a neuronal network required for cuticle tanning and wing expansion in Drosophila. J. Neurosci. **26:** 573–584.

Maglich, J. M., A. Sluder, X. Guan, Y. Shi, D. D. McKee *et al.*, 2001 Comparison of complete nuclear receptor sets from the human, Caenorhabditis elegans and Drosophila genomes. Genome Biol. **2:** research0029.0021–0029.0027.

Manseau, L., A. Baradaran, D. Brower, A. Budhu, F. Elefant *et al.*, 1997 GAL4 enhancer traps expressed in the embryo, larval brain, imaginal discs, and ovary of Drosophila. Dev. Dyn. **209:** 310–322.

Mao, Z. M., G. Roman, L. Zong and R. L. Davis, 2004 Pharmacogenetic rescue in time and space of the rutabaga memory impairment by using Gene-Switch. Proc. Natl. Acad. Sci. USA **101:** 198–203.

Mazo, A. M., D. H. Huang, B. A. Mozer and I. B. Dawid, 1990 The trithorax gene, a trans-acting regulator of the bithorax complex in Drosophila, encodes a protein with zinc-binding domains. Proc. Natl. Acad. Sci. USA **87:** 2112–2116.

McGuire, S. E., P. T. Le, A. J. Osborn, K. Matsumoto and R. L. Davis, 2003 Spatiotemporal rescue of memory dysfunction in Drosophila. Science **302:** 1765–1768.

McGuire, S. E., G. Roman and R. L. Davis, 2004 Gene expression systems in Drosophila: a synthesis of time and space. Trends Genet. **20:** 384–391.

Menon, K. P., S. Sanyal, Y. Habara, R. Sanchez, R. P. Wharton *et al.*, 2004 The translational repressor Pumilio regulates presynaptic morphology and controls postsynaptic accumulation of translation factor elF-4E. Neuron **44:** 663–676.

Merritt, D. J., and P. M. Whitington, 1995 Central projections of sensory neurons in the Drosophila embryo correlate with sensory modality, soma position, and proneural gene-function. J. Neurosci. **15:** 1755–1767.

Mosca, T. J., R. A. Carrillo, B. H. White and H. Keshishian, 2005 Dissection of synaptic excitability phenotypes by using a

dominant-negative Shaker K+ channel subunit. Proc. Natl. Acad. Sci. USA **102:** 3477–3482.

Nassif, C., A. Noveen and V. Hartenstein, 2003 Early development of the Drosophila brain: III. The pattern of neuropile founder tracts during the larval period. J. Comp. Neurol. **455:** 417–434.

Nitabach, M. N., J. Blau and T. C. Holmes, 2002 Electrical silencing of Drosophila pacemaker neurons stops the free-running circadian clock. Cell **109:** 485–495.

O'Kane, C., M. A. Stephens and D. Mcconnell, 1986 Integrable alpha-amylase plasmid for generating random transcriptional fusions in Bacillus-subtilis. J. Bacteriol. **168:** 973–981.

O'Kane, C. J., and W. J. Gehring, 1987 Detection in situ of genomic regulatory elements in Drosophila. Proc. Natl. Acad. Sci. USA **84:** 9123–9127.

Osterwalder, T., K. S. Yoon, B. H. White and H. Keshishian, 2001 A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. USA **98:** 12596–12601.

Palanker, L., A. S. Necakov, H. M. Sampson, R. Ni, C. Hu *et al.*, 2006 Dynamic regulation of Drosophila nuclear receptor activity in vivo. Development **133:** 3549–3562.

Palladino, M. J., J. E. Bower, R. Kreber and B. Ganetzky, 2003 Neural dysfunction and neurodegeneration in Drosophila Na+/K+ ATPase alpha subunit mutants. J. Neurosci. **23:** 1276–1286.

Paradis, S., S. T. Sweeney and G. W. Davis, 2001 Homeostatic control of presynaptic release is triggered by postsynaptic membrane depolarization. Neuron **30:** 737–749 (erratum: Neuron **31:** 167).

Parrish, J. Z., M. D. Kim, L. Y. Jan and Y. N. Jan, 2006 Genome-wide analyses identify transcription factors required for proper morphogenesis of Drosophila sensory neuron dendrites. Genes Dev. **20:** 820–835.

Patel, N. H., B. Schafer, C. S. Goodman and R. Holmgren, 1989 The role of segment polarity genes during Drosophila neurogenesis. Genes Dev. **3:** 890–904.

Prokopenko, S. N., Y. C. He, Y. Lu and H. J. Bellen, 2000 Mutations affecting the development of the peripheral nervous system in Drosophila: a molecular screen for novel proteins. Genetics **156:** 1691–1715.

Quinn, W. G., P. P. Sziber and R. Booker, 1979 Drosophila memory mutant amnesiac. Nature **277:** 212–214.

Renger, J. J., A. Ueda, H. L. Atwood, C. K. Govind and C. F. Wu, 2000 Role of cAMP cascade in synaptic stability and plasticity: ultrastructural and physiological analyses of individual synaptic boutons in Drosophila memory mutants. J. Neurosci. **20:** 3980–3992.

Roman, G., and R. L. Davis, 2002 Conditional expression of UAS-transgenes in the adult eye with a new gene-switch vector system. Genesis **34:** 127–131.

Roman, G., K. Endo, L. Zong and R. L. Davis, 2001 P{Switch}, a system for spatial and temporal control of gene expression in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA **98:** 12602–12607.

Rooke, J., D. Pan, T. Xu and G. M. Rubin, 1996 KUZ, a conserved metalloprotease-disintegrin protein with two roles in Drosophila neurogenesis. Science **273:** 1227–1231.

Schmid, A., A. Chiba and C. Q. Doe, 1999 Clonal analysis of Drosophila embryonic neuroblasts: neural cell types, axon projections and muscle targets. Development **126:** 4653–4689.

Schmidt, H., C. Rickert, T. Bossing, O. Vef, J. Urban *et al.*, 1997 The embryonic central nervous system lineages of Drosophila melanogaster. 2. Neuroblast lineages derived from the dorsal part of the neuroectoderm. Dev. Biol. **189:** 186–204.

Schneider, L. E., E. T. Sun, D. J. Garland and P. H. Taghert, 1993 An immunocytochemical study of the fmrfamide neuropeptide gene-products in Drosophila. J. Comp. Neurol. **337:** 446–460.

Schrader, S., and D. J. Merritt, 2000 Central projections of Drosophila sensory neurons in the transition from embryo to larva. J. Comp. Neurol. **425:** 34–44.

Schubiger, M., Y. Y. Feng, D. M. Fambrough and J. Palka, 1994 Mutation of the Drosophila sodium-pump alpha-subunit gene results in bang-sensitive paralysis. Neuron **12:** 373–381.

Schweers, B. A., K. J. Walters and M. Stern, 2002 The Drosophila melanogaster translational repressor pumilio regulates neuronal excitability. Genetics **161:** 1177–1185.

Sigrist, S. J., D. F. Reiff, P. R. Thiel, J. R. Steinert and C. M. Schuster, 2003 Experience-dependent strengthening of Drosophila neuromuscular junctions. J. Neurosci. **23:** 6546–6556.

Stebbins, M. J., and J. C. P. Yin, 2001 Adaptable doxycycline-regulated gene expression systems for Drosophila. Gene **270:** 103–111.

Stebbins, M. J., S. Urlinger, G. Byrne, B. Bello, W. Hillen *et al.*, 2001 Tetracycline-inducible systems for Drosophila. Proc. Natl. Acad. Sci. USA **98:** 10775–10780.

Treisman, J. E., Z. C. Lai and G. M. Rubin, 1995 Shortsighted acts in the Decapentaplegic pathway in Drosophila eye development and has homology to a mouse Tgf-beta-responsive gene. Development **121:** 2835–2845.

Urbach, R., and G. M. Technau, 2003 Molecular markers for identified neuroblasts in the developing brain of Drosophila. Development **130:** 3621–3637.

Vanolst, L., C. Fromental-Ramain and P. Ramain, 2005 Toutatis, a TIP5-related protein, positively regulates Pannier function during Drosophila neural development. Development **132:** 4327–4338.

Van Vactor, D., H. Sink, D. Fambrough, R. Tsoo and C. S. Goodman, 1993 Genes that control neuromuscular specificity in Drosophila. Cell **73:** 1137–1153.

White, B. H., T. P. Osterwalder, K. S. Yoon, W. J. Joiner, M. D. Whim *et al.*, 2001 Targeted attenuation of electrical activity in Drosophila using a genetically modified K+ channel. Neuron **31:** 699–711.

Xiong, W. C., H. Okano, N. H. Patel, J. A. Blendy and C. Montell, 1994 Repo encodes a glial-specific homeo domain protein required in the Drosophila nervous-system. Genes Dev. **8:** 981–994.

Ye, B., C. Petritsch, I. E. Clark, E. R. Gavis, L. Y. Jan *et al.*, 2004 Nanos and pumilio are essential for dendrite morphogenesis in Drosophila peripheral neurons. Curr. Biol. **14:** 314–321.

Younossi-Hartenstein, A., C. Nassif, P. Green and V. Hartenstein, 1996 Early neurogenesis of the Drosophila brain. J. Comp. Neurol. **370:** 313–329.

Yu, D. H., C. H. Feng and A. Guo, 1999 Altered outward K+ currents in Drosophila larval neurons of memory mutants rutabaga and amnesiac. J. Neurobiol. **40:** 158–170.

Zhong, Y., and C. F. Wu, 2004 Neuronal activity and adenylyl cyclase in environment-dependent plasticity of axonal outgrowth in Drosophila. J. Neurosci. **24:** 1439–1445.

Zhong, Y., V. Budnik and C. F. Wu, 1992 Synaptic plasticity in Drosophila memory and hyperexcitable mutants: role of camp cascade. J. Neurosci. **12:** 644–651.

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