Supplementary Materials

Title: **Discrete neuronal population coordinates brain-wide developmental activity**

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Additional References

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Supplementary Discussion

Instructive role for PSINA

What is PSINA's role in the development of the connectome? While the significance of PSINA to synaptic specificity and circuit function remain unknown, *trpγ* activity phenotypes (Fig. 2) are consistent with Hebbian mechanisms: given the global participation in PSINA, cell- and circuit-specific patterns of activity may be critical for temporally isolating and matching synaptic partners to fire together. In *trpγ* mutants, the general trends are for cells of a type to decrease their participation in PSINA and to become active more synchronously (Fig. 2). These shifts would decrease the co-incidence of synaptic partner activity and—with many cell types losing their distinctive activity waves—reduce the temporal corralling of elements of emerging neural circuits from surrounding activity. If so, we would expect to find more off-circuit connections, altered synaptic weights, and a more variable connectome to result from perturbing PSINA. As the activity patterns are derived from the Trpγ+ relay, tracing the origins of these patterns for elements of a defined circuit to a small number of Trpγ+ neurons would provide the tools to test this temporal corralling model.

The Source of PSINA

The data indicate that Trpγ+ neurons are not the source of PSINA: Residual periodic activity is still present when the Trpγ+ domain is silenced (Fig. 5b, Extended Data Fig. 7b-d). Notably, 2PM shows that 57C10-GAL4 driven Kir2.1 expression also fails to completely silence PSINA (Extended Data Fig. 7b-d). This 'breakthrough' signal may be due to persistent activity in the presumptive PSINA CPG. In the pan-neuronal case, residual activity likely originates from cells that are either outside the 57C10-GAL4 domain, are not expressing Kir2.1 at high enough levels, or are insensitive to or can compensate for this hyperpolarizing channel. Given these considerations, the large neuropeptide cells of the fly brain with their expansive projections are attractive candidates to be elements or modulators of the PSINA CPG. Compared to the pan-neuronal case, residual activity amplitude is greater with Trpγ+ silencing, which suggests that there may be additional PSINA relay elements that do not express *Trpγ*. These putative Trpγ- relay elements and the CPG can still maintain coordination of the residual activity across the brain (Extended Data Fig. 7g-j)

Role for developmental activity in invertebrates

Previous data indicate that circuit formation in the fly visual and olfactory systems can broadly develop normally in the absence of sensory input. In the absence of phototransduction, photoreceptors form their expected complement of synapses[11], and visual projection neurons elaborate processes normally[54]. Further, blocking odor-evoked activity does not significantly affect the formation of the glomerular map in the antennal lobe[55]. Based on these data, a commonly held notion was that activity in general was dispensable for the development of the adult nervous system[10].

Since the presence of stimulus-independent activity in fly development has been observed only recently[3], previous studies have not focused on the role of stimulus-independent activity in synapse formation. However, photoreceptor morphology has been studied in the absence of both stimulus-independent and evoked activity, via tetrodotoxin injection or *paralytic* mutants[11]. In these conditions, the photoreceptor cell R7 elaborates processes similar to wildtype animals, although synapse formation was not assessed in the context of these interventions. Here we show that photoreceptor synapse counts, like those of other visual processing cells, are indeed altered by activity.

The fly visual system is suitable for understanding cell-type-specific roles of developmental activity in circuit formation. Many of the ~100 cell types in the medulla have been characterized with respect to connectivity at the EM level in the adult[14] and to transcriptional programs through pupal development[25]. Further, thanks to the presence of GAL4 drivers specific for many visual processing cell types, the PSINA dynamics for many of these cells have been characterized[3]. Here we leveraged these resources to show that stimulus-independent activity influences synaptogenesis in the visual system in a cell-type-specific manner. In the vertebrate visual system, activity after eye opening has been shown to affect synaptogenesis in a cell-type-specific manner[56]. However, it is unknown whether these observations extend to stimulus-independent activity, where differential dynamics in retinal waves has been observed between classes of retinal ganglion cells[57]. The

mammalian retina contains diversity in cell types comparable to the fly visual system[58]. Specific genetic handles for these cell types will make it possible to ask whether retinal waves are differentially interpreted by individual cell types and/or represent a signal that modulates generalized and universal developmental programs.

Mammalian orthologs of *Trpγ*

The two mammalian orthologs of *Trpγ*, TRPC4 and TRPC5, offer important insights into the functional contribution of this channel to PSINA. In thalamic interneurons, TRPC4 is reported to act as a Ca^{2+} influx amplifier downstream of activated serotonin receptors to facilitate the release of dendritic GABA[59]. TRPC5 is required cell-autonomously in the dopamine neurons of the hypothalamic arcuate nucleus to maintain their stereotyped infra-slow bursting oscillations; loss of TRPC5 alters their dynamics[60]. TRPC5 is also necessary for prolactin-evoked tonic excitation of these cells. We found that $Trp\gamma^{G4}$-driven expression of TRPC4 or TRPC5 does not rescue the *trpγ* PSINA phenotype. However, the common theme of acting downstream of non-ionotropic receptors to facilitate excitation may inform efforts to identify the PSINA CPG.

SPARC3-Out-GAL80 produces sparse labeling of neurons

To visualize individual neuronal morphologies, we first turned to established methods of sparse labeling[61] which rely on titrating the expression level of FLP recombinase to turn on cell markers in a small number of cells. Due to constitutive FLP-mediated labeling of some Trpγ+ neurons with expansive projections, these methods did not produce the desired results. SPARC3-Out-GAL80, used in series with another SPARC element, enabled consistent visualization of 5-10 members of the 2000+ neuron strong *Trpγ* expression domain. SPARC moves the control of labeling density from recombinase activity level to the engineered recombination efficiency of the recognition sequences (Extended Data Figure 6a-d). In the absence of phiC31 recombinase, the SPARC3-Out-GAL80 transgenes drive ubiquitous expression of the GAL4 inhibitor GAL80. With the recombinase, the GAL80 open reading frame is 'SPARC'ed' out at a high, intermediate, or low probability, depending on the flanking recognition sequences. Loss of the GAL80 in a cell disinhibits GAL4, which allows for all UAS effectors in that cell to be expressed. We expect that SPARC3-Out-GAL80 will be a useful addition to the set of available SPARC tools.

Supplementary Video Captions

Supplementary Video 1. Wide-field imaging of whole-brain activity and traces in control (top) or *trpγ* null (bottom) pupae expressing GCaMP6s pan-neuronally. Images were sampled at 1 Hz and playback is 60 frames per second (fps). Scale bar is 200 μm. Activity scale is 0.1 $\Delta F/F_0$.

Supplementary Video 2. Composite time series of the cell types analyzed in Figure 2. Three cycles at approximately 60 hAPF are shown for each. Images were sampled at 0.4 Hz and playback is 60 fps. Scale bar is 40 μm.

Supplementary Video 3. 2-photon imaging of $Trp\gamma^{G4}$ expressing GCaMP6s in control (top) or *trpγ* null (bottom) pupae. Three cycles at approximately 60 hAPF are shown for each. Images were sampled at 0.4 Hz and playback is 60 fps. Scale bar is 40 μm.

Supplementary Video 4. Wide-field imaging of whole-brain activity and traces in control (empty-GAL4, top), panN-GAL4>Kir2.1 (middle), and $Trp\gamma^{G4}$>Kir2.1 (bottom) pupae expressing GCaMP6s pan-neuronally. Images were sampled at 1 Hz and playback is 60 fps. Scale bar is 200 μm. Activity scale is 0.1 $\Delta F/F_0$.