Dendritic refinement of an identified neuron in the Drosophila CNS is regulated by neuronal activity and Wnt signaling

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SUMMARY
The dendrites of neurons undergo dramatic reorganization in response to developmental and other cues, such as stress and hormones. Although their morphogenesis is an active area of research, there are few neuron preparations that allow the mechanistic study of how dendritic fields are established in central neurons. Dendritic refinement is a key final step of neuronal circuit formation and is closely linked to emergence of function. Here, we study a central serotonergic neuron in the Drosophila brain, the dendrites of which undergo a dramatic morphological change during metamorphosis. Using tools to manipulate gene expression in this neuron, we examine the refinement of dendrites during pupal life. We show that the final pattern emerges after an initial growth phase, in which the dendrites function as ‘detectors’, sensing inputs received by the cell. Consistent with this, reducing excitability of the cell through hyperpolarization by expression of Kᵥ2.1 results in increased dendritic length. We show that sensory input, possibly acting through NMDA receptors, is necessary for dendritic refinement. Our results indicate that activity triggers Wnt signaling, which plays a ‘pro-retraction’ role in sculpting the dendritic field: in the absence of sensory input, dendritic arbors do not retract, a phenotype that can be rescued by activating Wnt signaling. Our findings integrate sensory activity, NMDA receptors and Wingless/Wnt5 signaling pathways to advance our understanding of how dendritic refinement is established. We show how the maturation of sensory function interacts with broadly distributed signaling molecules, resulting in their localized action in the refinement of dendritic arbors.

KEY WORDS: Dendritic refinement, Drosophila, Neuronal activity, NMDA receptors, Wnt signals

INTRODUCTION
In the central nervous system (CNS), dendrites are major sites of synaptic input from partner neurons, and their development is intimately linked to the emergence of a functional network. The molecular and cellular mechanisms that regulate dendritic growth and refinement are an area of intense research (Corty et al., 2009; Wong and Ghosh, 2002). The development of precise dendritic patterns is believed to be regulated by interplay between an intrinsic genetic program, extrinsic factors and neuronal activity.

The mechanisms that underlie the activity-dependent regulation of dendritic growth and refinement could be the same as those that regulate neuronal plasticity in the mature nervous system. Activity levels are maintained at circuit-specific set points by homeostatic mechanisms: the system tries to counterbalance any deviations by adjusting overall synaptic strength in multiple ways. Recently, Tripodi et al. suggested that during development, dendrites in embryonic Drosophila motoneurons act as homeostatic devices that regulate their size and shape in response to inputs (Tripodi et al., 2008). Refinement, by contrast, involves the strengthening of optimum connections between pre- and postsynaptic neurons and the elimination of suboptimal connections. The mechanisms of synaptic maturation and refinement are suggested to be similar to Hebbian plasticity, which underlies learning and memory (Espinosa et al., 2009; Constantine-Paton and Cline, 1998). Thus, overall dendritic patterns are established through a combination of homeostatic plasticity, which maintains the functional range of excitability, and Hebbian plasticity, which could act to regulate activity-dependent refinement within this range (Turrigiano and Nelson, 2004). The value of these distinctions and their extent of overlap will only become clear as the molecular and cellular mechanisms of dendritic patterning are revealed.

The molecular mechanisms by which some neurites are stabilized while others are removed are not yet fully understood. One view is that this could depend on the ability of certain connections to ‘capture’ trophic molecules released in the vicinity of their targets (Poo, 2001). The relative contribution of ‘activity-dependent’ versus ‘activity-independent’ processes (Hebb’s rule versus Sperry’s chemoaffinity hypothesis) during circuit formation has been a matter of some debate (Cline, 2003), although there is a growing body of evidence to suggest the recruitment of developmental signaling pathways in response to activity. Recent observations that Wnts are secreted at the synapse in response to neural activity (Ataman et al., 2008; Chen et al., 2006) suggest a novel role for the Wnt signaling pathway downstream of activity during nervous system development and plasticity (Tang, 2007). Activity-dependent Wnt release and concomitant β-catenin stabilization have been shown to influence dendritic branching (Yu and Malenka, 2003) and, recently, Hayashi et al. have implicated the release of Wnt as a trophic agent regulating synaptic stability (Hayashi et al., 2009). The fact that Wnts have been shown to act as pro- as well as anti-synaptogenic factors in different contexts (Klassen and Shen, 2007; Packard et al., 2002) makes them interesting candidates for examination in the context of dendritic refinement.
In the current study, we identify a wide-field serotonergic neuron (CSDn) in the CNS of Drosophila as a model with which to study dendritic growth and refinement. Dendrites of the CSDns in Drosophila undergo remodeling during metamorphosis and, as the adult neuron has very few dendrites projecting to the antennal lobe, deviations from the wild-type pattern can be quantified easily. We demonstrate a key role for neuronal activity in the refinement of dendritic branches of the CSDn during metamorphosis. Cell-autonomous reduction in excitability results in a lack of refinement of dendritic arbors. Dendritic refinement and maturation of the aristal-derived presynaptic inputs are temporally coincident, suggesting that these neurons could trigger activation of the CSDns. Consistent with this observation, phenotypes observed in mutants lacking aristal neurons can be rescued by manipulations that have been shown to lead to increased levels of activity. Cell-autonomous knockdown of N-methyl-D-aspartate-type glutamate receptors (NMDARs) in the CSDn also affects the refinement process. We show that target interactions are likely to lay the field for the action of Wnt signaling. We find that Wingless (Wg; Drosophila Wnt1) levels are elevated in response to increased neural activity in the developing antennal lobe, and compromising Wg and Wnt5 signaling phenocopies reduced excitability, suggesting that the Wnt signaling cascade is recruited by neural activity to drive dendritic refinement. In summary, we present a new and valuable neuron preparation for the genetic dissection of dendritic patterning mechanisms. Using this identified central neuron, we demonstrate that neuronal activity, mediated through identified receptors and sensory inputs, along with Wnt signaling, acts to refine dendritic pattern to its ultimate functional state in the adult animal.

MATERIALS AND METHODS

Drosophila strains
RN2fly, tub>CD2-Ga4, UASSmCD8::GFP and RN2fly, tub>CD2-Ga4, UAS-nRFP (Roy et al., 2007) flies were used to uniquely label the CSDn. UAS-Apc2-GFP, UAS-nod-GFP, UAS-Syr-GFP, UAS-pan.dTCPßN, UAS-axn.GFP, UAS-arg-sN4 1 MB14, UAS-Trap-At dshß, dsßß, wgßß, paraßß, T(2;3)C202, alßß/S1M1 and alßß dpß bß prß cß prß were obtained from the Bloomington Drosophila Stock Center, Indiana University, IN, USA. UAS-Kcßß was kindly provided by Richard Baines (Baines et al., 2001), UAS-fasßß/ßßGFP by Roel Nusse (Cadijan et al., 1998), UAS-dsßß and UAS-dsßß 2 by Tim Tully (Wu et al., 2007; Xia et al., 2005), UAS-Calpain A RNAi (v35262) from the Vienna Drosophila RNAi Center (Dietzl et al., 2007), UAS-Calpain B RNAi from the National Institute of Genetics, Japan [courtesy of Lucas Waltzer (Osman et al., 2009)] and UAS-lgsßßß 1 E by Konrad Basler (Kramps et al., 2002; Mosimann et al., 2006). The UAS-eag-ßß, UAS-Skt-ßß recombinant was provided by Subhabrata Sanyal (Hartwig et al., 2008) and Sgßßß 1-Ga4 by B. V. Shyamala (Shyamala and Chopra, 1999). The mutant stocks Wntßßßß, dpßßßß and fßßßßß/TMßßßß were obtained from Bassem Hassan (Srahana et al., 2006). To generate alßß homoyogous animals, either alßß dpß bß prß cß prß or homoyogotes or the progeny of T(2;3)C202, alßß/S1M1 and alßß dpß bß prß cß prß were used. Both combinations gave similar results and are referred to throughout as alßß homoyogotes.

All flies were maintained under standard conditions at 25°C unless otherwise indicated. For pupal timing, white pupa [0 hours after puparium egression (APF)] were reared at 25°C until 48 hours APF and then placed at 34°C for 2 hours. Dissected brains were subjected to spaced depolarization in hemolymph-like saline (HL3) solution containing 90 mM KCl (high K+-HL3) at room temperature. Controls were treated as the test animals but in HL3 without K+. Brains were fixed immediately after treatment and stained with anti-Wg. Images were background subtracted and the Wg staining intensity was measured in the antennal lobe neuropil and cortex adjacent to the antennal lobe using ImageJ. In each case, intensity was measured from ten regions of interest (ROIs; 15×15 pixels). The ratio of Wg intensity between neuropil and cortex was calculated for each sample. The average ratio was calculated from 16 control and test samples and the mean ± s.e.m. plotted.

RESULTS

Dendrites of the CSDn undergo a defined pattern of activity-dependent refinement during metamorphosis

A pair of serotonergic neurons associated with the antennal lobes are labeled in the RN2fly, tub>CD2-Ga4, UASSmCD8 GFP strain (Roy et al., 2007) (see Fig. S1 in the supplementary material). FLP recombinase activity labeled either both or a single neuron in each animal. Analysis of preparations bearing a unilateral ‘flip-out’ allowed us to analyze dendrites, which show a characteristic change in pattern from larval to adult stages. Processes close to the soma were identified as dendrites based on selective localization of the well-established markers Apc2-GFP and nod-GFP (see Fig. S1B-D in the supplementary material) (Rolls et al., 2007; Sanchez-Soriano et al., 2005). Synaptotagmin-GFP is excluded from the dendrites and is enriched specifically in the presynaptic terminals (see Fig. S1C in the supplementary material).

The sparse dendrites innervating the adult antennal lobe, as compared with that seen in the larva, makes the CSDn a promising system for analysis of the regulation of dendritic patterning during metamorphosis (Fig. 1). We quantified the cumulative length of dendritic branches by measuring the number of bins occupied by the neurites (see Fig. S2A, A’ in the supplementary material). There was some variability in the dendritic pattern of the adult CSDn across animals (see Fig. S2B-D in the supplementary material), although this was not so large that it precluded quantitative analysis.

At the larval stage, the dendritic field is composed of several branches (Fig. 1A,M), which are pruned such that at 10 hours APF only a few fine arbors remain (Fig. 1B,M). At 30 hours APF, there is an increase in fine, long arbors (Fig. 1C,M; see Fig. S3A1-6 in the supplementary material). At this time, the cumulative dendritic length is at its maximum and begins to show a reduction by 50 hours APF (Fig. 1D,M; see Fig. S3B1-6 in the supplementary material).
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that, as in vertebrates, refinement in (Rodrigues and Hummel, 2008). It is therefore tempting to speculate APF; glomerular formation continues until ~50 hours APF neurons, which are known to invade the antennal lobe by ~30 hours the main trunk of the axon. which occurs earlier during pupal life to remove larval arbors from refer to this process as ‘refinement’ to distinguish it from pruning, and resembles that of the adult by ~70 hours APF (Fig. 1E,F,M). We to further probe the involvement of neuronal activity during and resembles that of the adult by ~70 hours APF (Fig. 1E,F,M). We refer to this process as ‘refinement’ to distinguish it from pruning, which occurs earlier during pupal life to remove larval arbors from the main trunk of the axon. The refinement period coincides with the ingrowth of sensory neuronal innervation (Wong and Ghosh, 2002; Zhang and Poo, 2001). In order to test the role of activity, we silenced the CSDn by targeted expression of the inward rectifying human K+ channel K_{i2.1} (also known as KCNJ2 and IRK1 – Human Genome Nomenclature Database), which hyperpolarizes membrane potential (Baines et al., 2001). In these animals, development of the larval dendritic arbors occurred normally (compare Fig. 1G with 1A,M; P=0.12) and these were pruned as in the wild-type controls (compare Fig. 1H with 1B,M; P=0.1). Morphology appeared normal even at 30 hours APF (compare Fig. 1I with 1C,M; P=0.2), but was dramatically different in later pupal phases (Fig. 1D,F,J,M; P<0.01) and in adults (Fig. 1L,M; P=0.0001). Thus, in the absence of activity, ‘excess’ dendritic arbors are not removed and as a result the adult CSDn has an enlarged dendritic field. Autonomously activity within the CSDn is therefore necessary to regulate refinement of the dendritic arbors during metamorphosis. To further probe the involvement of neuronal activity during dendritic refinement, we analyzed a conditional allele of paralytic (para). para encodes a voltage-sensitive Na+ channel and at restrictive temperatures (≥29°C) para^{26B} animals undergo paralysis and exhibit reduced nerve excitability (Sidigqi and Benzer, 1976). para^{26B}; RN2>flp, tub>CD2>G4l4, UAsmCD8::GFP/+ animals were reared at permissive temperature until the third larval instar and then shifted to 29°C. In adults, these animals had an increased dendritic field (Fig. 2C,E; P=0.03) as compared with controls grown under similar conditions (Fig. 2A,E; P=0.82). This observation supports our conclusion of a requirement for neuronal activity during dendritic remodeling. Since these experiments suggest that activation of the CSDn regulates dendritic form, we expected that its hypereexcitability could alter, perhaps hasten, the course of dendritic remodeling. To test this, we targeted expression of the Sh$_{DN}$ and eago$_{DN}$ channels, which is known to result in increased membrane excitability (Broughton et al., 2004; Mosca et al., 2005), into the CSDn and examined its metamorphosis. We found that the morphology in the adult was unchanged (Fig. 2D,E; P=0.3) and that the timecourse of refinement in the CSDn expressing Sh$_{DN}$ and eago$_{DN}$ channels was indistinguishable from that of the wild type (Fig. 2F-H). This suggests that autonomous activity, although
nec, is not sufficient for dendritic refinement, raising the possibility that additional inputs might be required from presynaptic partners, which arrive at the lobe ~30 hours APF.

Taken together, these results suggest that the dendritic pruning that occurs during the early phase of metamorphosis is independent of neuronal activity. Neuronal activity is essential for dendritic refinement at the time when synapse formation and maturation occur in the developing antennal lobe.

**Sculpting of the dendrites of the CSDn requires NMDA receptors and presynaptic input**

Dendritic refinement is thought to follow Hebb’s postulate, whereby coincident pre- and postsynaptic firing results in strengthening of synaptic connections, whereas non-coincident pre- and postsynaptic activity results in weakening of synapses (Constantine-Paton and Cline, 1998). N-methyl-D-aspartate-type glutamate receptors (NMDARs) have been suggested to act as ideal coincident detectors, as opening of these channels requires simultaneous release of glutamate from the presynaptic neuron and depolarization of the postsynaptic neuron (Espinosa et al., 2009; Zhang and Poo, 2001).

The *Drosophila* NMDAR is composed of two subunits, dNR1 and dNR2 (Nmdar1 and Nmdar2 – FlyBase), which are widely expressed in the brain, and RNAi-mediated knockdown of these subunits results in memory defects (Wu et al., 2007). Simultaneous knockdown of both subunits in the CSDn using RNAi resulted in an increase in the dendritic field in adult flies (Fig. 3B,C, compare with 3A; see Fig. S4B in the supplementary material; \(P<0.0001\)). This suggests a requirement for a presynaptic glutamatergic input that is sensed by NMDARs on the CSDn. What could be the source of the input to the NMDARs?

Dendrites of the CSDn invade the coarse neuropile in the posterior region of the antennal lobe, where glomerular identity is not very clear with normal synaptic markers. Axonal projections from aristal sensory neurons are known to project to this region to glomeruli VP2 and VP3 (Stocker et al., 1983). We backfilled the arista with rhodamine-dextran (Fig. 3E, magenta) and noted that the terminals in VP3 lie in close proximity to the CSDn dendrites (Fig. 3E,F). However, the sensory neurons from the arista are likely to be cholinergic in nature and therefore their activity is unlikely to directly activate the NMDARs. The resolution of our methods does not allow us to assess whether connections with the aristal neurons are direct or are through local interneurons, most of which are known to be GABAergic (Wilson and Laurent, 2005) and several of which express glutamate (R. Priya and A. Chiang, personal communication) (Daniels et al., 2008). We examined the morphology of the CSDn dendrites in *aristaless* (*al*\(^2\)) mutants, which have poorly developed aristae and reduced sensory input (Fig. 3G, H). Cell bodies of aristal neurons could be clearly seen inside the aristae in wild-type animals (Fig. 3D, arrow; see Fig. S5 in the supplementary material). In *al*\(^2\) animals, however, cell bodies of aristal neurons were not seen in the rudimentary aristae (Fig. 2G; see Fig. S5B,C,E-H in the supplementary material). The dendritic field of the CSDn in *al*\(^2\) adults was significantly increased as compared...
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**Inhibiting Wnt signaling results in increased dendritic arbor**

Work in *Drosophila* and in mouse has suggested a role for the activity-dependent recruitment of Wnt at the synapse (Chiang et al., 2009; Ataman et al., 2008; Chen et al., 2006; Wayman et al., 2006). Wingless (Wg) and the receptor Frizzled 2 (Fz2) can be detected by immunostaining in the developing glomeruli (see Fig. S6A-B in the supplementary material) and levels of Wg were found to increase in the neuropile upon KCl-induced global depolarization (see Fig. S6C-E in the supplementary material). The observation that Wg levels at the terminals of neurons are influenced by activity even during development, led us to test the role of Wnt signaling during dendritic morphogenesis.

We expressed Fz2-GPI, a GPI-anchored form of the Wg receptor that acts in a dominant-negative manner by titrating away the ligand (Cadigan et al., 1998). Compared with wild type (Fig. 4A), the Fz2-GPI-expressing CSDn showed an increase in the dendritic field (Fig. 4B,I; \(P<0.0001\)). Signaling downstream of Wnt receptors occurs through the cytoplasmic protein Dishevelled (Dsh), which is an essential component of canonical as well as non-canonical Wnt signaling pathways (Wallengford and Habas, 2005). Heterozygotes of \(wg^{1114}\); (Fig. 4C) as well as of \(dsh^{h\beta/}\); Fig. 4D) showed mild, but significant, dendritic phenotypes (Fig. 4I; \(P<0.05\)). Fz2 heterozygotes \(fz2^{[E4]/+}\) were normal (Fig. 4E; \(P=0.38\)). Transheterozygotes of \(dsh^{h\beta/}\) and \(fz2^{[E4]/+}\); Fig. 4F) showed an enhanced defect in dendritic morphology that was more severe than a cumulative effect of each heterozygote alone (Fig. 4I; \(P<0.0001\)). This result further establishes the role for Wnt signaling, but importantly identifies that the levels of signaling activity within the neuron are critical. The requirement of Wg signaling in the regulation of dendritic refinement was further confirmed by targeted overexpression of the known negative regulators Axin (Fig. 4G,I; \(P=0.006\)) and activated Shaggy (Sgg; Fig. 4H,I; \(P<0.0001\); see Fig. S4D in the supplementary material).

Downregulation of Wnt signaling by ectopic expression of activated Sgg in the CSDn did not affect the branching pattern of larval dendrites (see Fig. S7A in the supplementary material) or the pruning of larval arbors during early metamorphosis (see Fig. S7B in the supplementary material). However, dendritic refinement during later pupal stages was compromised and ectopic arbors were seen to persist at 50 hours APF (see Fig. S7C in the supplementary material).

These results suggest that Wnt signaling impinges upon the mechanisms that culminate in the refinement of dendritic processes. Recent studies (Abe and Takeichi, 2007) have demonstrated that neuronal activity can also trigger Wnt signaling by activation of Calpain proteases, which result in \(\beta\)-catenin/TCF-dependent nuclear activity. In order to test whether this mechanism operates in our system, we examined the dendritic field of the CSDn after RNAi-mediated knockdown of Calpain A (Dietzl et al., 2007) or Calpain B (Osman et al., 2009). The dendritic arbors were normal in adults, demonstrating that downregulation of the Calpain pathway does not affect the refinement process (see Fig. S8A-C,G in the supplementary material; \(P=0.5\)). Furthermore, abrogation of the transcriptional route of the Wnt pathway by ectopic expression of dTCFAN (van de Wetering et al., 1997) or a mutant form of legless \((lgs^{17E})\) that has weak Armadillo-binding capacity (Mosimann et al., 2006; Kramps et al., 2002), did not compromise dendritic refinement (see Fig. S8D,E,G in the supplementary material). The dendritic field was also normal in \(dsh^{h}\) mutants (see Fig. S8F,G in the supplementary material), in which the planar cell polarity pathway is compromised but Dsh function in the canonical pathway is unaffected (Boutros et al., 1998). This observation implicates a role for the canonical Wnt pathway in normal refinement. However, as TCF-dependent nuclear activity is also dispensable in this process, we suggest that refinement of dendritic arbors requires the action of the divergent canonical pathway downstream of Wnt action (Salinas and Zou, 2008).
Wg and Wnt5 signaling genetically interact in refining dendrites of CSDn

Wnt5, an additional ligand, is expressed together with Wg in the developing antennal lobe (Yao et al., 2007). We find that Wnt5 and its receptor Derailed (Drl) (Bonkowsky et al., 1999) participate in dendritic refinement of the CSDn. drlRed2 (Fig. 5B) and Wnts400 (Fig. 5C; see Fig. S4E in the supplementary material) mutants showed mild, but significant, increases in dendritic fields (Fig. 5H; P<0.0001). Wnts400/+ heterozygotes were comparable to wild-type animals (Fig. 5D,H; P=0.09). Wnts400/+; wg1114/+ transheterozygotes (Fig. 5E) demonstrated haploinsufficient interactions (Fig. 5H; P<0.0001), suggesting that signaling by Wnt5 and Wg ligands collaborate during dendritic refinement. Further genetic evidence for this conclusion arose from experiments in which either the dosage of wg (Wnts400; wg1114/+; Fig. 5F,H; see Fig. S4F in the supplementary material; P<0.0001) or that of fz2 (Wnts400; fz2[E4]/+; Fig. 5G,H; P=0.0001) was reduced in a Wntsnull background. Together, these genetic data lead us to conclude that signaling via Wnt5/Drl and Wg/Fz2 act synergistically to regulate dendritic refinement.

The aristal sensory neurons trigger activation of the CSDn and Wnt signaling is recruited to regulate dendritic refinement

What is the link between presynaptic input, neuronal activity and the Wnt signaling pathway in regulating dendritic refinement? The experiments described above do not allow us to decipher whether these are contributing factors that act independently on the refinement process resulting in incremental changes in phenotype.

In order to test for a link between presynaptic input and Wnt signaling, we targeted overexpression of Dsh, which is known to trigger Wnt signaling (Wehrli et al., 2000), in the CSDn of animals mutant for al1. As expected, al1 control progeny from a cross between al1/CyO; UAS-dsh and al1/CyO; RN2flp, tub>CD2=Gal4, UASmCD8::GFP showed a greatly enlarged dendritic field (Fig. 6B; P=0.005). Experimental siblings (al1/al1; UAS-dsh/RN2flp, tub>CD2=Gal4, UASmCD8::GFP) had entirely normal morphology, indicating complete rescue of the al phenotype (Fig. 6C; P=0.15). Ectopic expression of Dsh in a wild-type neuron did not affect the dendritic morphology (Fig. 6D; P=0.5). This means that the arrival of the aristal sensory neurons within the antennal lobe recruits Wnt signaling in the CSDn, either by inducing postsynaptic activity or by the physical presence of these terminals within the lobe.

In order to distinguish between these possibilities, we exploited the temperature-sensitive dTrp-A1 (TrpA1 – FlyBase) channel, which, upon ectopic expression, has been shown to trigger activity in animals exposed to temperatures above 25°C (Pulver et al., 2009; Hamada et al., 2008). al1 homozygous animals expressing dTrp-A1 in the CSDn were reared at 22°C (at which temperature dTrp-A1 is not active) until adulthood (Fig. 6G) was not rescued by Wnts400 (Fig. 6G; P=0.18 compared with Wnts5). The dendritic field in this genotype (al1/al1; UAS-Trp-A1/RN2flp, tub>CD2=Gal4, UASmCD8::GFP) showed strong rescue of the al1 phenotype (Fig. 6E compared with 6B) and was similar to that of the wild type (P=0.03). Ectopic expression of dTrp-A1 in a wild-type background had no effect (Fig. 6F,J). The ability of autonomous activation of CSDn to compensate for a lack of sensory innervation provides evidence for the triggering of neuronal activity by the aristal neurons, either directly or through the action of local interneurons.

How does activation of the CSDn relate to Wnt signaling? We activated the CSDn by targeted expression of the eag-DN and Sh-DN transgenes in Wnts400 homozygous mutants. The dendritic phenotype seen in Wnts5 mutants (Fig. 6G) was not rescued by activation of the CSDn (Fig. 6H; P=0.18 compared with Wnts5). Expression of eag-DN and Sh-DN has been used in several recent studies (Chiang et al., 2009; Duch et al., 2008; Hartwig et al., 2008) and has been found to cause hyperactivation. Two possible explanations for this observation are that Wnt5 signaling is
downstream of CSDn activation or that both Wnt5 and activity act in parallel to trigger dendritic refinement. This needs to be investigated further.

The results from experiments involving epistatic interactions allow us to propose the model shown in Fig. 7. The aristal sensory neurons, acting either directly or through local circuits, trigger neuronal activity in the CSDn. The activity of the aristal neurons, acting either directly or through local circuits, trigger dendritic refinement. This needs to be investigated further.

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Discusison

In this study, we focus on a specific phase during the metamorphosis of the dendrites of a central serotonergic neuron, in which excess growth is removed by a process that we term refinement. Genetic analyses using loss-of-function mutants and RNAi-mediated knockdown of specific genes allow us to postulate a link between neuronal activity, synaptic input and Wnt signaling in this process (Fig. 7). The dendrites present on the CSDn during the larval stage are removed early in pupation by pruning, followed by a period of exuberant growth. The activation of sensory neurons at the antennal lobe correlates well with when growth of the CSDn dendrites ceases and removal of the excess branches occurs. The CSDn must be active for the refinement process to occur, as refinement fails when neuronal activity is inhibited or when the sensory neurons are absent. Phenotypes observed in the latter case can be rescued by ectopic activation of the neuron using the temperature-sensitive dTrp-A1 channel. We suggest that activity within the CSDn, possibly together with activity in presynaptic neurons, acts to provide the correlated activity required to trigger activation of NMDARs. We know that knockdown of NMDARs affects the refinement process, although identifying its specific action requires further study. A possible consequence of the activity-dependent process is activation of the Wg pathway, as the phenotype observed in mutants can also be rescued by ectopic expression of Dsh in the CSDn. It seems unlikely that activity within the CSDn leads to the release of Wnt ligands, but rather that dendrites respond locally to Wnt ligands in the region of a dendrite that is receiving input. Although other interpretations of the data are possible, we favor a hypothesis whereby specific synapses are stabilized as a result of correlated neuronal activity, and that excess dendritic branches are removed by Wnt signaling (Fig. 7C).

Neuronal activity shapes dendritic form

Perturbations in neuronal activity can be compensated by changes at multiple levels, including alterations in the expression of ion channels and in synaptic strength (for reviews see Davis, 2006; Turrigiano and Nelson, 2004). Tripodi et al. provide evidence for structural homeostasis whereby alterations in afferent input during development can be compensated by changes in dendritic geometry (Tripodi et al., 2008). This suggests that dendritic arbors serve as sensors for input levels, thus allowing the self-organization of circuits that is necessary for robust behavioral outputs (Tripodi et al., 2008). Our studies in the CSDn support these observations: reduced activation of the cell by targeted expression of Kir2.1 results in a greatly enlarged dendritic field in the adult. This phenotype can be explained by a mechanism in which the absence of electrical activity results in a failure of the signaling mechanisms that stop growth of the arbors and that remove additional branches. Reduced excitability could also drive the homeostatic mechanisms towards making more arbors and to suppress the refinement program.

Dendritic remodeling requires NMDAR function

Dendritic growth and refinement are closely associated with input activity and synapse formation during development. Activity-dependent development of circuits is thought to utilize mechanisms similar to those involved in Hebbian learning and plasticity. NMDARs are ideal candidates for detecting correlated pre- and postsynaptic activity, which is crucial in the Hebbian model of learning and plasticity. Here, strengthening of synapses leads to the stabilization and extension of dendrites, whereas weakening of synapses leads to the destabilization and elimination of dendritic branches (Espinosa et al., 2009; Cline and Haas, 2008; Constantine-Paton and Cline, 1998). During vertebrate hippocampal development, NMDAR activation has been shown to limit synapse
number and reduce dendritic complexity (Luthi et al., 2001). The stabilization of a particular synapse or arbor possibly attenuates the formation of new branches or synapses, thus limiting further dendritic growth. In such a scenario, knocking down NMDAR levels would be expected to result in increased dendritic complexity, as indeed we have observed. The mechanism by which ‘appropriately connected’ synapses are strengthened, whereas suboptimal contacts are eliminated, needs to be studied in our system. In other systems, Ca^{2+}, which is released upon NMDAR activation, impinges on various intracellular effectors that regulate dendritic morphogenesis (Konur and Ghosh, 2005). In addition, selective stabilization/destabilization of dendritic arbors could be affected by the local release of growth factors in response to activity.

**Wnt signaling in dendritic refinement**

We demonstrate that activity-dependent activation of the Wnt pathway facilitates retraction of dendritic arbors. Arbors that receive appropriate input are somehow protected and stabilized. Our experiments suggest that Wnt-dependent refinement mechanisms through a non-nuclear pathway and could act by impinging directly on cytoskeletal dynamics (Schlessinger et al., 2009; Salinas and Zou, 2008). Disruption of the microtubule cytoskeleton is a key feature of dendritic pruning in *Drosophila* during metamorphosis (Williams and Truman, 2005). GSK3β (Shaggy in *Drosophila*) is an intracellular inhibitor of the Wnt pathway, has been shown to act as a sensor of inputs for neuronal activity (Chiang et al., 2009) and a potent regulator of microtubule dynamics in axons (Ciani et al., 2004). In the *Drosophila* embryonic CNS, the Src family of tyrosine kinases (SFKs) is required for Wnt5/Drl-mediated signaling (Wouda et al., 2008). Interestingly, SFKs seem to act as a crucial point of convergence for multiple signaling pathways that enhance NMDAR activity and hence are thought to act as molecular hubs for the control of NMDARs (Salter and Kalia, 2004). It is tempting to envisage a scenario in which there is cross-talk between Wnt5/Drl signaling-mediated activation of SFKs and NMDAR signaling during refinement.

In summary, we show that the dendritic refinement of a central modulatory serotonergic neuron is regulated by electrical activity, NMDAR and Wnt signaling. Similar mechanisms have been implicated in dendritic growth and refinement of excitatory neurons in vertebrates (Wong and Ghosh, 2002; Luthi et al., 2001; Constantine-Paton and Cline, 1998). We provide a model neuron preparation in which the dendritic growth and refinement of a modulatory neuron can be analyzed genetically. We demonstrate that the dendrites of CSDn receive input from sensory neurons from the arista, supporting previous suggestions (Hill et al., 2002) that mechanosensory input could alter sensitivity to odorant stimuli. In both *Drosophila* (Dacks et al., 2009) and the mammalian olfactory bulb (Petzold et al., 2009), serotonin gates the odor-evoked sensory response. CSDn sends projections to higher brain centers and multiglomerular projections to the contralateral antennal lobe and hence it is likely to influence the overall properties of the olfactory circuit. Our study suggests that the structural and resulting functional properties of this neuron emerge from an interaction between partner neurons, together with input from intrinsic and extrinsic cues.

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**Competing interests statement**

The authors declare no competing financial interests.

**Supplementary material**

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**References**


