Does bouton morphology optimize axon length?

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The total length of cortical axons could be reduced if the parent axons maintained straight trajectories and simply connected to dendritic shafts via spine-like *terminaux* boutons and to dendritic spines via bead-like *en passant* boutons. Cortical axons from cat area 17 were reconstructed from serial electron micrographs and their bouton morphology was correlated with their synaptic targets. *En passant* or *terminaux* boutons did not differ in the proportion of synapses they formed with dendritic spines and shafts, and thus, the two morphological variants of synaptic bouton do not contribute directly to optimizing axon length.

The bulk of the brain consists of axonal 'wiring.' In the gray matter of the neocortex, each cubic millimeter contains about 4 km of axon[1], so removing axonal zigzags is not trivial: if only 0.1 mm were pruned off each axon in cat area 17, it would save 3 km of 'wire.' It has been proposed that one role of dendritic spines is to help optimize the length of axonal wire[2,3]. The idea is that instead of zigzagging through the neuropil to contact their specific target dendrites, axons simply grow in economically straight trajectories and form *en passant* synaptic boutons, leaving to dendrites the task of emitting spines to 'catch' the passing axons. This gives spiny dendrites the active role in selecting particular axons during development and learning[4]. New evidence pointing to the involvement of dendritic spines in forming connections has come from reports of motile spines[5] and of new spines appearing during long-term potentiation[6–8]. However, one-fifth of cortical neurons lack spines and thus cannot exploit such a mechanism, so some degree of axonal zigzagging would seem inevitable.

In all these discussions of spines, it has been completely overlooked that cortical axons too can produce spine-like structures, called *terminaux* boutons, whose dimensions match those of dendritic spines. Do *terminaux* boutons exist to prevent zigzags in the parent axons by connecting to dendritic shafts by means of axonal 'spines'? Evidence in support of this comes from the pyramidal cells of layer 6, which, unusually for pyramidal cells, form synapses mainly with dendritic shafts[9] and whose axons bear mainly *terminaux* boutons[9,10]. This is the mirror-image of the pattern for spiny cells in other cortical layers whose axons bear mainly *en passant* boutons and form synapses mainly with spines. These observations suggest an 'only connect' hypothesis[3] in which both dendritic and axonal spines are specializations that allow axons to maintain economically straight trajectories. We tested this hypothesis by correlating the target (dendritic spine/shaft) with the bouton type in the same local region of neuropil.

We examined the axons of 3 spiny neurons, which were recorded in area 17 of anesthetized cats (protocols approved by the Veterinary Department of the Canton of Zurich; for details, see ref. 10). The neurons were filled intracellularly with horseradish peroxidase. Two were layer 3 pyramidal cells with extensive axon collaterals in layers 2, 3 and 5. The third cell was a spiny stellate cell from layer 4A whose axon arborized in layers 2, 3 and 4. We selected collateral segments located in layer 3 that had a mix of both bouton types.

Eighty-five boutons were serially sectioned, photographed in the electron microscope (EM), and reconstructed together with their targets. The morphological type of each bouton was determined from the serial EM reconstructions. The synaptic targets of the labeled boutons were positively identified using established criteria. Spines and dendritic shafts were the only synaptic targets and were readily distinguished. Forty-five of the boutons were *en passant* and formed 49 synapses, and 40 boutons were *terminaux* and formed 43 synapses. As is typical for spiny cortical neurons, spines were the major target. Of the 34 synapses formed by the spiny stellate axon, 24 (70%) were with spines and similarly, of 58 synapses formed by the two pyramidal cell axons, 37 (64%) were spines.

In one reconstruction of a 20-μm length of the spiny stellate axon (**Fig. 1a**), all but one of the boutons were located in a tight cluster, where they formed synapses with dendritic shafts and spines. This cluster illustrates the morphological variety of *terminaux* boutons and the size of their necks relative to the parent axon. The target spines were of the same dimension as the presynaptic boutons. At the center of the cluster was a single *en passant* bouton. *Terminaux* boutons were formed even when the parent axon actually touched the target. In the example in Fig. 1b, the synaptic target (a spine) of the spiny stellate axon was directly on the path of the parent axon. Yet, instead of forming an *en passant* bouton, the axon formed a *terminaux* bouton, whose slender neck had to wrap around a myelinated axon (approximately 0.8 μm diameter) to reach its target spine. Even when the targets were aligned along the same path as the axon, multiple synapses were rarely formed. In the only two cases found, two closely spaced *en passant* boutons formed two synapses with the dendritic shafts of smooth neurons, before the trajectories of the axon and dendrites deviated (see supplementary reconstructions, available on the *Nature Neuroscience* web site).

In the analysis of the distribution of all the targets of the 3 axons (**Fig. 1c**), spines formed 63% of the targets of *terminaux* boutons and 69% of *en passant* boutons. Dendritic shafts formed

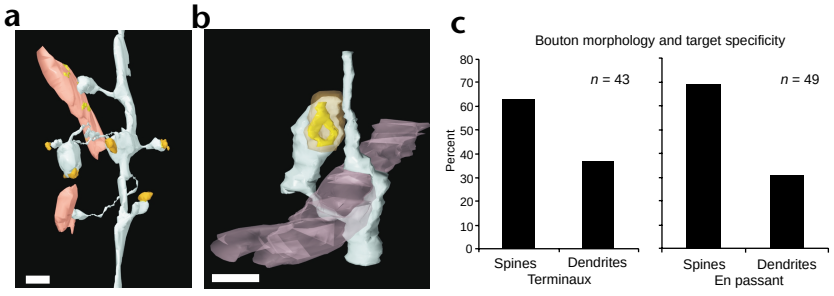

Fig. 1. Reconstructions from serial electron microscope sections of segments of axon and summary histograms. (**a**) Axon, light blue; target dendritic shafts, red; target spines, orange; dendritic postsynaptic densities, yellow. (**b**) Axon, light blue; spine, transparent brown; postsynaptic density, yellow; unlabeled myelinated axon, transparent mauve. Scale bars (**a**, **b**), 1 μm. (**c**) Histograms of all identified targets of the two bouton types for all 92 synapses. Electron microscope reconstructions created using Nuages and Blue Moon Rendering Tools.

the remainder of the targets. The distributions for the two bouton types were not significantly different (chi-square test). The morphology of the bouton is not correlated with the type of synaptic target. It seems that axons and dendrites have no trouble finding each other, regardless of bouton type or whether the target is dendritic spine or shaft. Thus, the 'only connect' hypothesis fails to account for the data.

It is tempting to write off the differences between the two bouton types as being of any particular significance. Are they simply an expression of some developmental quirk that produces one or the other kind of bouton? However, the morphological similarity of the *terminaux* boutons to dendritic spines prompts another interpretation. Perhaps, as with dendritic spines, which compartmentalize calcium[11–13], the fine necks of these axonal spines also prevent calcium from diffusing rapidly into the parent axon during an action potential? Because each bouton would retain more residual calcium after each impulse, synapses formed by *terminaux* boutons might show more presynaptic facilitation than the *en passant* boutons. The layer 6 pyramidal synapses, which are formed mainly by *terminaux* boutons, show strong facilitation due to an increased probability of transmitter release[14]. If bouton morphology does indeed influence synaptic dynamics, it will be a revision of the present view that cortical axons exist only to connect.

Note: Supplementary reconstructions are available on the Nature Neuroscience web site (http://neuroscience.nature.com/web_specials).

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Passive eye displacement alters auditory spatial receptive fields of cat superior colliculus neurons

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The superior colliculus (SC) is thought to use a set of superimposed, topographically organized neural maps of visual, auditory, somatosensory and motor space to direct the eyes toward novel stimuli[1,2]. Auditory spatial response fields (SRFs) of SC neurons may change when an animal moves its eyes, presumably to compensate for the resulting misalignment of visual and auditory sensory spatial reference frames[3–6], but the mechanisms responsible for these SRF changes remain unknown. Here we report that passive deviation of the eye in anesthetized, paralyzed animals can profoundly affect the auditory responsiveness of SC neurons, but seems insufficient by itself to provide adaptive shifts of auditory SRFs.

In awake animals, changes in eye position either shift the rostral edge or the center of auditory SRFs in the SC[3–6], or modulate the overall strength of auditory responses while not systematically shifting the SRF[5,6]. It is unknown whether these SRF changes are mediated through efference copy of eye movement commands, through sensory feedback from the oculomotor plant, or through some combination of the two. Extraocular proprioceptive signals reach the SC[8,9], but modeling studies have so far emphasized the importance of efference copy and visual feedback in SC motor function (for example, see ref. 10). Furthermore, the motor aspects of SC function can operate accurately when proprioceptive feedback is abolished[11]. We assessed the involvement of proprioceptive feedback in sensory processing within the SC by testing whether passive eye displacement alters auditory SRFs in anesthetized (~1% halothane, 66% N_2O), paralyzed (pancuronium bromide, 1 mg/kg every 3 h) cats in complete darkness. In this preparation, neither efference copy nor visual input could contribute to any observed changes. Experimental protocols were approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Twenty neurons from 11 cats were tested for the effects of passive eye movements. The position of the contralateral (left) eye was manipulated by tension on four sutures (6-0 silk) passed through the nasal, temporal, ventral and dorsal margin of the sclera. The sutures were attached to a mechanical device which held the eye securely either in its straight-ahead (control) position, or displaced by ~23° in a contralateral (temporal) and downward direction. This displacement was chosen for maximal proprioceptive activation (stretching both the superior and medial recti, and possibly the superior oblique, while relaxing the inferior and lateral recti). Tension on the sutures was maintained for the control and the deviated conditions. We used short (10–100 ms) Gaussian noise bursts delivered in virtual acoustic space (VAS) and standard extracellular recordings[12] to map auditory SRFs of single SC neurons. SRFs were constructed from 3 to 5 randomly interleaved stimulus presentations at each of 324 different VAS directions. In some cases, we repeated SRF measurements in the eye-deviated and the control position several times, and at different sound intensities. Recording sites were confirmed histologically to be distributed evenly throughout the central two-thirds of the intermediate and deep layers of the SC. With the eyes in their control position, SC neurons exhibited circumscribed