



Dark-reared dendritic spines shed light on ocular dominance plasticity

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In the early 1960's, David Hubel and Torsten Wiesel, working in the kitten primary visual cortex, made the remarkable finding that altering visual experience by closing one eye profoundly changes the physiological and anatomical organization of the cortex [1]. Since their pioneering work, ocular dominance plasticity, as this paradigm is known, has become a prominent model for studying the role of experience in patterning cortical representations of sensory input. Ocular dominance plasticity is most rapid during a so-called critical period, which in mice begins around postnatal day 19 and ends around postnatal day 35 [2]. Closure of one eye during this period induces a depression of the deprived eye responses in as little as 3 days [3], and possibly faster in extragranular layers [4]. But what drives this process whereby synapses from deprived eye inputs lose their efficacy? Proposed mechanisms include everything from rapid changes in the phosphorylation state of single neurotransmitter receptors [5] or alterations in second messenger molecules, to large scale anatomical changes in dendritic [6] and axonal branching [7], although the latter may take weeks to develop.

What is the earliest morphological substrate of ocular dominance plasticity?

For three decades now, neuroscientists have looked for a morphological signature associated with the earliest physiological changes seen with ocular dominance plasticity. At last, in the December 2004 issue of the journal *Neuron*, two independent groups have simultaneously pointed their fingers to the same entity: dendritic spines [8, 9]. Spines are the curious appendages that decorate the shafts of dendrites in some neurons. Although their function remains unknown, their shape and location suggests important roles in synaptic transmission and neuronal plasticity. Dendritic spines lie across the synaptic cleft, opposite the presynaptic axon terminals, and contain the receptors for the neurotransmitter glutamate that translate a signal to the postsynaptic cell. Because the majority of spines in the adult brain resemble lollipops (a short narrow stem connects their bulbous tip to the dendrite shaft), many investigators have emphasized their role as biochemical compartments for signaling proteins and second messengers, such as calcium. Spines are also electrical compartments due to the higher neck resistance causing voltage-gated ion channels to be activated in one spine but not its neighbors, thereby isolating axonal inputs from one another [10, 11]. Moreover, spines can be motile [12], particularly early in development [13], a characteristic which enables them to change shape in response to varying stimuli, and perhaps even to sample several nearby axon terminals [14]. It is this motility that endows spines with the ability to participate in various forms of neuronal plasticity. Thus, spines are obvious candidates to be mediators of ocular dominance plasticity.

Spines are more motile when they lose their normal inputs:

Starting with this hypothesis in mind, Oray et al. turned to state-of-the art *in vivo* imaging of dendritic spines of cortical pyramidal neurons in the visual cortex of anesthetized mice. A previous study by the same group

[15] had demonstrated that prolonged binocular deprivation (eyelid suture for at least 8 days) within the critical period led to a 60% increase in spine motility. In the present study the authors go a step further. First, they test a deprivation paradigm that is more relevant to issues of synaptic competition, namely they focus their analysis on the binocular region of primary visual cortex and image dendritic spines after monocular deprivation of the contralateral eye. Second, they look for spine changes very soon after deprivation (2-3 days), which coincides more closely with the time frame when other physiologic changes are seen [2, 3]. Oray et al. again find a significant increase (35%) in spine motility in the binocular region of primary visual cortex at P28 (the height of the critical period), but not at P42, beyond the critical period of ocular dominance plasticity. This further reinforces the concept that spines are one physical locus of the plastic changes in the brain that follow this type of experience dependent plasticity.

But a couple of issues remain unanswered. First, if increased spine motility is triggered by an imbalance of inputs caused by sensory deprivation then one would expect no change in spine motility after binocular deprivation, where no such imbalance exists. So how can we explain Majewska and Sur's findings of increased spine motility after binocular deprivation [15]? If, on the other hand, sensory deprivation causes all deprived spines to be more motile (independent of an input disequilibrium), then surely one would expect that roughly half as many spines would become more motile after monocular deprivation than after binocular deprivation. The difference in percent increases in motility in monocular vs. binocular deprivation (35% vs. 60%) suggests this is the case. But this brings up the second issue, which is whether the observed increase in spine motility reflects a small increase in a large number of spines, or a large increase in a handful of spines? Unfortunately neither Majewska and Sur [15] nor Oray et al. [9] presented their data as a frequency distribution of spine motilities. A bimodal distribution in the case of monocular deprivation would suffice to confirm this theory.

All spines are not equal:

From the *in vivo* observations of Oray et al, it was not clear whether the increase in motility affected all deprived spines in pyramidal neurons, or just subsets of spines located in certain dendrites, for example those in layer 4 that receive direct inputs from thalamus. Unfortunately, *in vivo* two-photon microscopy can only be used to image the most superficial dendrites, known as the subpial arch of the apical dendritic branches. In order to test whether there was a laminar-specificity for the observed change in spine motility, Oray et al. turned to acute coronal brain slices through the visual cortex from the monocularly deprived mice. In such a preparation they could examine not only the subpial tuft of apical dendritic branches but also the entire extent of the apical dendrite. They again found a significant, though more modest, increase (by 20%) in spine motility. Further, they observed that the up-regulation in spine motility was only evident in the infra- and supra-granular layers of visual cortex, and not in the portion of the apical dendrite corresponding to layer 4. This is an important finding because physiological changes in cortical processing are initiated in the extragranular layers II/III and V/VI, and are only later seen in the primary input layer IV [4]. Thus, the laminar specificity of the morphological and the physiological changes that accompany ocular dominance plasticity match one another.

Spines are also lost after monocular deprivation:

Oray et al. convincingly show that spine motility is up regulated after visual deprivation. But how can a slight increase in spine twitching lead to the robust synaptic changes behind ocular dominance plasticity in the mouse brain? Enter the report of Mataga et al. in the same issue of *Neuron* [8]. Also eager to detect physical changes in the structure of the synapse that might precede the pruning of axons and their boutons [7, 16, 17], the authors hypothesized that spines, known for their morphological plasticity in paradigms of learning and memory [18] and during development, might be good candidates. They examined fixed dendrites from visual cortex after 4 days (short term) of monocular deprivation and found a 20-40% decrease in spine density.

Is there a link between spine motility and spine turnover?

Both groups conclude that they are seeing different steps in the same sequence of events in ocular dominance plasticity. Namely, immediately after monocular deprivation, changes in sensory experience lead to an imbalance between deprived-eye and non-deprived eye inputs that causes an increase in spine motility. This increase in spine motility, the authors argue, is then followed by a loss of spines. The two groups also go on to propose how sensory deprivation leads to the spine changes via alterations in the extracellular matrix (this is discussed below). Unfortunately, a direct link between the two phenomena (spine motility and spine turnover) is still missing.

It should be emphasized that the idea that experience dependent synaptic plasticity resides in spines is not novel. Elegant studies led by Karel Svoboda, pioneering the use of *in vivo* two-photon imaging of cortical neurons, have shown that changes in spine motility [13] and in spine turnover [19] are associated with deprivation in the rodent barrel cortex. But, as opposed to the findings of Majewska and Oray [15], Lendvai et al. [13] found a 40% decrease in spine motility in somatosensory cortex following whisker trimming. Do these differences reflect age differences, or differences in barrel versus visual cortex? Perhaps so, as the decrease in motility observed by Lendvai et al. happened at the peak of synaptogenesis in barrel cortex (P13), implying that activity may be required for spine motility during synapse formation. This is certainly in agreement with the observations reported recently by Konur and Yuste [20], who also used acute slices of primary visual cortex (from GFP-expressing mice). They show that dark rearing and enucleation (two forms of binocular deprivation similar to bilateral eyelid suture) at P13 led to a decrease in spine motility. Still, the increase in spine motility reported by Majewska and Sur and Oray et al. is at odds with Konur and Yuste's data for older ages, as the latter group found no change in spine motility. The difference could lie on the location of the dendrite segments analyzed in each study (basal vs. apical), on the cell types examined (layer II/III vs. Layer V), or on the duration of the deprivation (longer for the Konur study).

Unfortunately the controversy does not end there. For example, we find it intriguing that in both of the studies from the Svoboda group there was no change in spine density, in sharp contrast to the spine loss that Mataga et al. report. In fact, none of the studies of living neurons (acute slices or *in vivo* imaging) have shown changes in spine density after deprivation paradigms, in contrast to studies using fixed tissue, including this one by Mataga et al. Among those research papers using fixed preparations are the classic studies of Albert Globus and Arnold Scheibel illustrating that removal of visual experience (through binocular deprivation or lesions in the lateral geniculate nucleus) leads to spine loss along the apical stalk but not along the subpial arch (more on this below) [21-23]. Table 1 summarizes some of the classic experiments examining the impact of visual experience on spine densities. Note that in almost all of the fixed-tissue studies, decreases in spine density were limited to the apical dendritic stalk. So, how can we reconcile these results? The entire field would certainly benefit from a series of more careful and comprehensive *in vivo* imaging studies examining the motility and turnover of spines of pyramidal neurons in several layers, under identical paradigms of sensory deprivation and experimental manipulation. Studies conducted in this manner, such as the elegant one just published in the journal *Neuron* [24], are likely to have longstanding repercussions and resolve many of the remaining controversies.

Table 1

Ref.	Region	Spec.	Prep.	Model	Duration	Layer	Spine Motility	Spine Density
Vital Imaging Studies								
[13]	S1	R	in vivo	WT	P8-10	II/III	NS	NS
					P11-13		40% decrease	NS

					P14-16		NS	NS
[19]	S1	M	in vivo	WT‡	Adult X 1-3 d	V	N/A	NS
[15]	V1	M	in vivo	BD	P13-21	V	NS	NS
					P13-28		60% increase	NS
					P13-42		NS	NS
[9]	V1	M	in vivo	MD	P26-P29	V	35% increase	N/A
					P40-P43		NS	N/A
			acute slice	MD	P26-29		20% increase	N/A
[20]	V1	M	acute slice	DR	P0-P11, -P20	II/II	NS	NS
				DR	P0-28/34		20% decrease	NS
				E	P6-P11		25% decrease	NS
				E	P6-P28/34		NA	NS

Fixed Tissue Studies

[8]	V1	M	Fixed	MD	P25-29	III	N/A	20-40% decrease
					P60-64	III	N/A	NS
[36]	V1	aR	Fixed	MD**	P14-44	V	N/A	16% decrease
					P90-120	V	N/A	NS
[37]	V1	aR	Fixed	MD**	P14-45	V	N/A	19% decrease
[38]	V1	aR	Fixed	MD**	P14-70	V	N/A	25% decrease
[39]	V1	aR	Fixed	MD**	P14-70	V	N/A	20% decrease
[22]	V1	Rb	Fixed	uE	P0-30	V	N/A	30% decrease
[40]	V1	R	Fixed	DR	P3-30	III	N/A	15% decrease

[41]	V1	R	Fixed	E	P1-25	VA	N/A	NS
						VB	N/A	30% decrease
[42]	V1	M	Fixed	DR	P0-25	V	N/A	35% decrease

Table 1: Summary of literature on spine changes after various sensory deprivation paradigms This table lists some of the salient studies on the effects of experience dependent plasticity on spine motility or spine density. It is by no means all-inclusive, but it does underline some of the controversies of these studies. In particular, note how a loss of spines was universally found in apical dendrites after visual deprivation in fixed preparations but not in acute slices or *in vivo*. Also, a decrease in spine motility was reported in one study [20] after visual input deprivation, while another group reported an increase in spine motility in two separate studies [9, 15]. Finally, the model proposed by Mataga et al. and Oray et al., whereby visual deprivation leads to increased spine motility and then to loss of spines is challenged by the report of Makewska and Sur which found no evidence for changes in spine density after prolonged (>8 d) of visual deprivation [15].

Abbreviations: aR: albino rat; BD: binocular deprivation; DR: dark rearing; E: enucleation; M: mouse; MD: monocular deprivation (** These experiments were done in albino rats, which have fully crossed retinogeniculate projections, and thus lack binocular domains). N/A: not available or not measured; NS: not significant; P: postnatal day; R: rat; Rb: rabbit; S1: primary somatosensory (barrel) cortex; uE: unilateral enucleation; V1: primary visual cortex; WT: whisker trimming (‡ indicates checker board pattern of whisker trimming).

Meanwhile, through their use of *in vivo* two-photon microscopy (allowing the imaging of selected dendrites over many days) Oray et al. could have easily resolved this discrepancy by testing whether those spines that exhibit a transient period of increased spine motility after monocular deprivation are indeed lost a few days later. Yet, the absence of spine density changes reported by Majewska and Sur [15] raises doubts about the proposed model whereby visual deprivation leads to increased spine motility and then to spine loss. These are very important issues because there are alternative ways to reconcile the data in these two Neuron articles. For instance, it is possible that Oray and Mataga are looking at two different forms of ocular dominance plasticity, one that involves spine loss and another that involves a rapid spine motility that facilitates sampling of multiple axons by input-deprived dendrites but not necessarily a loss of synapses. In support of this view, binocular enucleation induces spine loss only along the central three quartiles of the apical dendritic stalk with no significant changes in spine density in either the subpial arch, oblique shaft branches or basilar dendrites [22]. Long-term spine imaging experiments might also shed light on the role of spine motility in normal brain function, which is presently unknown. Clearly, a link between spine motility and spine turnover would be most instructive. Another way to address this in intact, non-deprived mice would be to correlate motility in individual spines with their lifetime, since considerable spine turnover still takes place in the adult brain [19, 24, 25]. If Oray, Mataga and their co-workers are correct, one would anticipate that those spines with higher motility would be the ones most likely to be lost subsequently.

How does sensory deprivation translate into the spine changes observed during OD plasticity? The link between the extracellular matrix and spines.

Perhaps one of the most unsettling aspects about the observation that spines twitch or appear and disappear in the adult brain [19, 25] is that the tightly packed ultrastructure revealed by electron microscopy had conditioned neuroscientists to view the mature brain as a rather rigid structure. Now we must ask ourselves, how can adult spines move or disappear, as proposed by these two sets of investigators, when they should be constrained by their stiff environment?

One explanation for the deprivation-induced increases in spine motility and, ultimately, spine retraction is that the “glue” that binds postsynaptic spines to presynaptic boutons could be enzymatically removed by the actions of extracellular proteases. The scaffolding substance that surrounds and holds together neurons and

synapses is none other than the extracellular matrix (ECM), which is rich in proteins that play key roles in everything from cell migration and axon growth to synapse formation and elimination. In the developing cortex, the plasminogen activating system appears to play a central role in regulating extracellular proteolysis. Two serine proteases, urokinase-like plasminogen activator (uPA) and the tissue-type plasminogen activator (tPA) regulate the conversion of plasminogen into its active form, plasmin, a trypsin-like endopeptidase with broad substrate specificity. It is known that neural activity upregulates tPA expression [26]. This induction is dependent upon post-synaptic NMDA receptor activation, which suggests that tPA expression is somehow mediated by retrograde signaling from spine to bouton. In the developing visual cortex, tPA expression is selectively increased in binocular zones after monocular deprivation [27], and ocular dominance plasticity is impaired in mice lacking tPA [27]. Similarly, intracortical infusion of tPA inhibitors blocks plasticity following reverse occlusion in kittens [28]. These observations provide powerful evidence that tPA, and its regulation of plasmin, translate functional changes in neural responsiveness into structural changes in neural connectivity.

But which elements of cortical structure are altered by tPA induction and on what time scale? Oray et al. and Mataga et al. both weigh in on this question, in search for a link between the ECM and their observed spine alterations following visual deprivation. Oray et al. applied tPA directly to the perfusate of their cortical slices. Slices from naïve, P28 cortex showed a pronounced increase in spine motility that mimicked the effect seen after monocular deprivation. Application of tPA to cortical slices from monocularly deprived mice increased motility along dendrites in layer IV, but had no effect on dendrites in the supragranular layers. The absence of an effect on spine motility in the supragranular layers suggests that tPA was already present and active at these dendrites. Conversely, the increased motility observed along layer IV dendrites argues that tPA was absent from these regions in the early days after monocular deprivation. From these observations, Oray et al. conclude that tPA acts to provide a permissive environment for spine motility and, ultimately, retraction.

Mataga et al. approach the question from an altogether different angle. Using wild-type and tPA null mice, they demonstrate that monocular deprivation induces a dramatic loss of spines along the apical dendrites of layer III pyramidal neurons in wild-type mice, but not in the mutant mice. Exogenous application of tPA in the mutant mice rescued the effects of monocular deprivation on reducing spine density. The authors propose a model of synaptic plasticity in which changes in neural firing resulting from monocular deprivation alter the balance of proteases and their inhibitors in the synaptic cleft. In this model, a decrease in presynaptic activity leads to an increase in tPA release or activity and a decrease in the release or activity of its inhibitors, with the result that more active synapses are maintained while less active synapses experience an initial increase in spine motility and, ultimately, retraction.

Holtmaat et al [24] report that spines in the developing and adult cortex belong to one of two kinetic classes: short-lived, which persist for less than 4 days, and stable, which persist for months. The fraction of stable spines increases with age, but at all ages studied, from 2 weeks to 6 months, highly motile, short-lived spines were observed. These observations suggest that if Mataga's model is correct then the effects of tPA likely become restricted to a smaller and smaller subset of axons through aging. Supporting this view, it is well established that cells in different cortical layers have different critical periods [29, 30] and thus, plasticity is lost heterogeneously throughout the cortical circuit. What regulates tPA expression in specific cell types during development and into adulthood remains a mystery.

Summary:

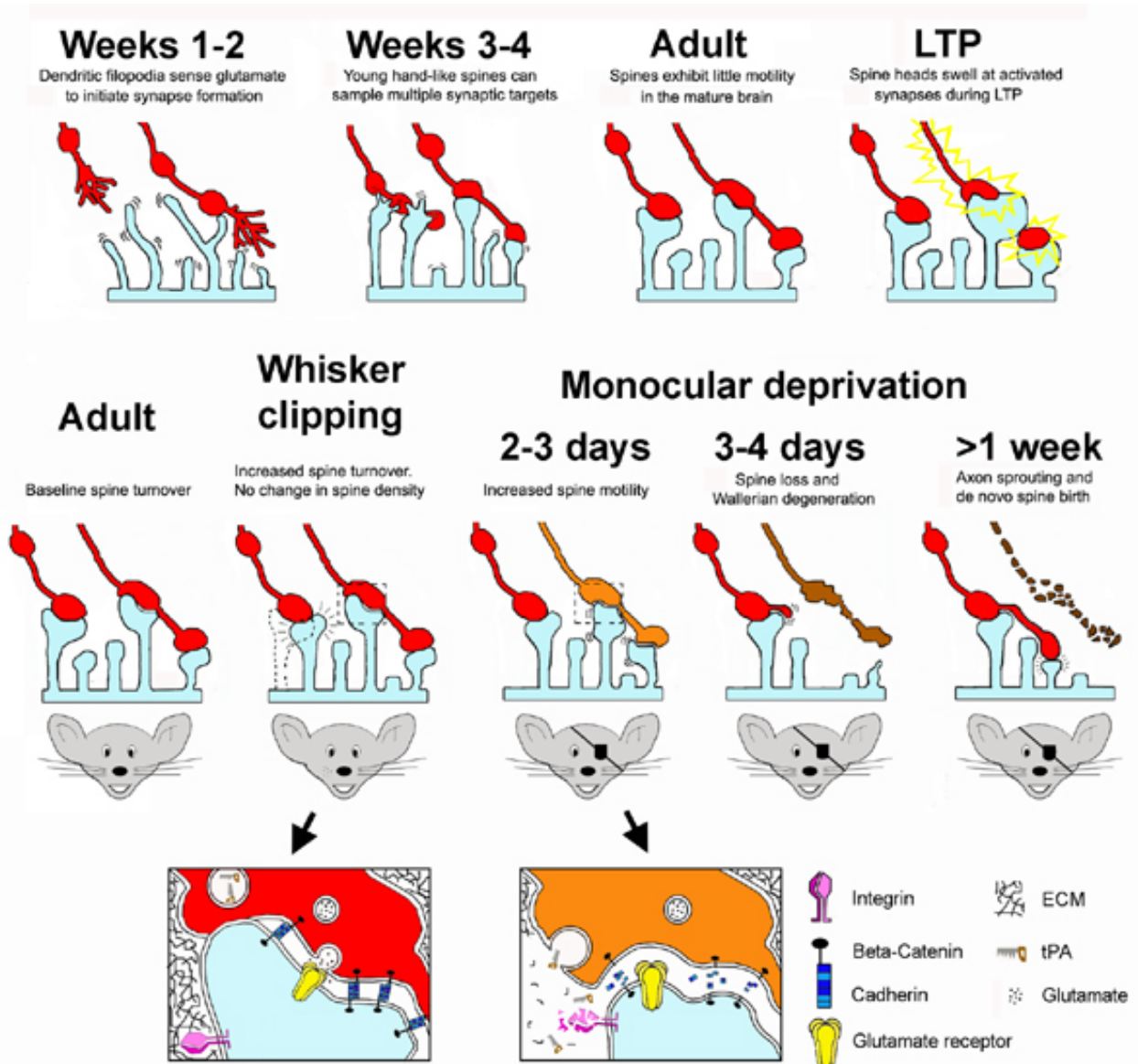
The two papers discussed herein have contributed greatly to our understanding of the mechanisms of ocular dominance plasticity, and the implication of the role of the ECM is particularly interesting. It is important to emphasize that spine motility and turnover in the adult brain are a fact of life. Therefore one should consider that the phenomena described in the two Neuron papers are merely modulations within a range of normal behavior. In other words, spine motility and spine turnover can vary up and down from a baseline level, which

is not zero, and sensory experience (stimulation or deprivation) accentuates those fluctuations in one direction or another. Indeed, the evidence so far points to the notion that the default state of spines is for them to move, but perhaps the ECM holds them in a relatively immobile state. Experience, it seems, dissociates spines from the bond of the ECM leading to increased spine motility and turnover. This model might also explain why spines are more motile during development [13, 20, 31] when the ECM is less compact and changes in experience are more drastic. We are left wondering what happens to the presynaptic terminal. Does it change too with sensory experience? Stay tuned, you can bet the answer will come soon.

Figure 1. Summary cartoon

Figure

1: This



simplified schematic summarizes recent data on spine development, spine motility and spine turnover in the mouse brain, particularly in the context of neuronal activity and experience.

Top row: A: In the first week of postnatal development dendrites (light blue) are covered with long and thin protrusions called filopodia [32]. Dendritic filopodia respond to glutamate presumably released by developing axonal boutons (red; [31]), and this may facilitate early synaptic contacts by filopodia [33]. B: During the second week of postnatal life, immature spines with small heads are refining their connections with axons. Some of these proto-spines possess small filopodia or spinules on their heads (“hands”) which allow them to sample multiple boutons [14]. Note that axon boutons are probably also turning over frequently during development. C: In the adult, spines are less motile. D: LTP induces a rapid and transient growth in some spine heads [34, 35].

Bottom row: A: the enlarged box shows the configuration of the extracellular matrix (ECM) around the synapse. The pre- and post-synaptic elements are held together and to the ECM by a protein “glue” composed of cell adhesion molecules such as laminins, cadherins and integrins. B: Spine turnover persists in the adult however [19, 24, 25], and is increased by sensory deprivation, for instance in the barrel cortex after a checker-board pattern of whisker trimming [19]. Interestingly, however, the density of spines does not change after sensory deprivation in pyramids within primary somatosensory cortex. C: After 2-3 days of monocular deprivation spine motility increases in the apical dendritic stems of layer V pyramids within the binocular region of primary visual cortex [9]. D: Not long after that, spine density decreases, presumably due to pruning of spines deprived of their inputs [8]. The enlarged box shows how monocular deprivation triggers the enzymatic lysis of certain ECM components that leads to observed changes in spine motility and turnover. Note that cadherins and B-catenin are resistant to t-PA, but may later dissociate after prolonged absence of neuronal activity. E: Subsequently, deafferented axons may degenerate and retract some branches [7], while non-deprived axons perhaps sprout to synapse onto new spines.

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