Neuronal Activity Regulates Diffusion Across the Neck of Dendritic Spines

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In mammalian excitatory neurons, dendritic spines are separated from dendrites by thin necks. Diffusion across the neck limits the chemical and electrical isolation of each spine. We found that spine/dendrite diffusional coupling is heterogeneous and uncovered a class of diffusionally isolated spines. The barrier to diffusion posed by the neck and the number of diffusionally isolated spines is bidirectionally regulated by neuronal activity. Furthermore, coincident synaptic activation and postsynaptic action potentials rapidly restrict diffusion across the neck. The regulation of diffusional coupling provides a possible mechanism for determining the amplitude of postsynaptic potentials and the accumulation of plasticity-inducing molecules within the spine head.

In mammalian excitatory neurons, synaptic stimulation triggers the flow of ions across the dendritic spine membrane, as well as the production of second messengers within the spine head. Buildup of signaling molecules, such as calcium or activated CaMKII (calcium/calmodulin-dependent protein kinase II), within the spine head activates regulatory cascades that lead to the modification of the enclosed synapse (1–4). Furthermore, stimulus-induced transport of proteins across the spine neck, such as CaMKII, protein translation initiation factors, and β-catenin, plays a role in synapse regulation and plasticity (5, 6). Thus, the regulation of diffusion across the spine neck offers a potentially powerful mechanism to control the efficacy and modulatory state of individual synapses.

We examined the regulation of the diffusional barrier posed by spine necks in rat hippocampal pyramidal neurons. Organotypic slice cultures were bidirectionally transfected with the photoactivatable green fluorophore PAGFP (7) and the red fluorophore dsRed. Two-photon laser scanning microscopy (2PLSM) with illumination at 910 nm readily excites dsRed without photoactivation of PAGFP, revealing dendrites and spines that fluoresce in the red spectrum (Fig. 1). Focal illumination with a second laser tuned to 720 nm triggers two-photon activation of PAGFP (8), and the resulting green fluorescence can be subsequently monitored with 910-nm illumination. Photoactivation of PAGFP within individual spines triggers increases in fluorescence within the head that dissipate as activated PAGFP (PAGFP*) diffuses into the dendrite. The decay of the fluorescence transient in the spine head is well fit by a single exponential, yielding a time constant of equilibration (τeq) (9) of PAGFP* across the spine neck (Fig. 1, A to C). Repeated measurements (at 0.1 Hz) in individual spines over ~1.5 min yielded consistent values of τeq (fig. S1) with coefficients of variation (CVs) of ~15 to 20% (Fig. 1D). Conversely, τeq varied over a broad range from spine to spine (Fig. 1E, n = 11/572 cells/spines), with the majority of values ranging from 140 to 350 ms.

In a subset of spines, fluorescence did not decay appreciably in the sampling period of 1.9 s. For these spines, the barrier to PAGFP* movement across the neck was bidirectional, so that PAGFP* within the dendrite is able to diffuse away from the site of photoactivation but does not enter the spine head (Fig. 2, A and B; similar findings in 11 of 11 comparable spine/dendrite pairs). Conversely, PAGFP* diffuses from the dendrite into the heads of spines with less restrictive spine necks (Fig. 2, C and D; similar findings in 8 of 8 comparable spine/dendrite pairs). Thus, the lack of PAGFP* movement in a subset of spines results from a severe diffusional isolation imposed by the spine neck and not from aggregation or cross-linking of PAGFP within the head. Repeated measurements of τeq in these diffusationally isolated spines over prolonged periods revealed that the diffusional barrier is reversible and that large, apparently spontaneous reductions in τeq occur (Fig. 2, E and F; similar findings in 4 of 15 diffusationally isolated spines that were monitored repeatedly for >5 min).

We hypothesized that the heterogeneity of τeq results from active regulation of diffusional coupling in response to variability in neuronal and synaptic activity. Chronic manipulations of activity trigger homeostatic changes in synaptic parameters such as the number and composition of AMPA-type glutamate receptors (AMPA-Rs) at the synapse (10, 11). Consistent with our hypothesis, 24 hours of incubation in the AMPAR antagonist NBQX shifted the distribution of τeq toward faster values (8/367 cells/spines; P < 0.01), whereas block of GABA_A receptors (GABA_ARs) with bicuculline shifted the distribution toward slower values (8/556 cells/spines; P < 0.01) (Fig. 3A). Similar results were obtained with measurements of dsRed diffusion by fluorescence recovery after photobleaching (Fig. S2).

In contrast, block of voltage-sensitive sodium channels (VSSCs) (6/438 cells/spines) or NMDA-type glutamate receptors (NMDARs) (7/449 cells/spines) by incubation in tetrodotoxin (TTX) or carboxypiperezine-4-yl-propyl-1-phosphonic acid (CPP), respectively, had no effect on the cumulative distribution of τeq.
Fig. 1. Measurement of PAGFP* movement across the spine neck reveals heterogeneity of spine/dendrite diffusional coupling. (A) Images of spine/dendrite pairs that demonstrate strong (top), moderate (middle), and weak (bottom) diffusional coupling. In (A) to (C), the arrowhead indicates the site of photoactivation. Scale bar, 1 μm. (B) Fluorescence measured in line scans over the regions indicated by the dashed lines in (A) during photoactivation of PAGFP in the spine head. Scale bar, 200 ms. (C) Quantification of the PAGFP* fluorescence transients in the spine head (black) and dendrite (red) shown in (B). Scale bar, 200 ms. (D) Repeated measurements of \( \tau_{eq} \) in each of nine spines (top). For each spine, the values of \( \tau_{eq} \) obtained from each independent measurement (black points), the average ± SEM (red), and the CV of \( \tau_{eq} \) (bottom) are shown. (E) Cumulative distribution of \( \tau_{eq} \) for spines in control conditions.

Fig. 2. The spine neck is a bidirectional and dynamic barrier to protein movement. (A) Image of spine/dendrite pair (left) demonstrating weak diffusional coupling and fluorescence transients obtained after photoactivation in the spine head (middle) or neighboring dendrite (right). (B) Quantification of the spine (black) and dendrite (red) fluorescence transients from the corresponding panels in (A) (middle and right). (C) Image of spine/dendrite pair (left) demonstrating strong diffusional coupling and fluorescence transients obtained after photoactivation in the spine head (middle) or neighboring dendrite (right). (D) Quantification of the spine (black) and dendrite (red) fluorescence transients from the corresponding panels in (C) (middle and right). (E) Image of spine/dendrite pair that switches from weak to strong diffusional coupling (left). Diffusional coupling was initially weak (middle) but spontaneously switched to strong (right) several minutes later. (F) Quantification of the spine (black) and dendrite (red) fluorescence transients from the corresponding panels in (E) (middle and right). Scale bars, 1 μm (left) and 200 ms (right and middle) for (A), (C), (E), and (F); 10% ΔG/R and 200 ms for (B), (D), and (F).
changes in $\tau_{equ}$. After GABA_A R blockade, $\tau_{equ}$ was significantly larger than for control spines of matched neck length or apparent head width (Fig. 3D). Conversely, after AMPAR blockade, spines tended toward faster $\tau_{equ}$ than control spines with comparable morphology. Furthermore, spine neck lengths were reduced equally after NMDAR or AMPAR blockade (Fig. S3), but only in the latter condition was the distribution of $\tau_{equ}$ shifted to faster values.

To determine whether cell-wide changes in cytoplasmic viscosity account for the changes in $\tau_{equ}$, the diffusion coefficient of PAGFP* ($D_{PAGFP*}$) was measured in aspiny regions of thin (~1 to 2 µm in diameter) dendrites. $D_{PAGFP*}$ (37 ± 10 µm²/s in control conditions) was consistent with previous measurements of green fluorescent protein (GFP) motility (13) and was constant across pharmacological conditions (fig. S4), indicating that the movement of proteins across the neck is specifically regulated in response to the manipulations of activity. Thus, changes in $V$, $L$, or $D_{PAGFP*}$ do not account for the effects of activity on spine/dendrite diffusional coupling, suggesting that the cross-sectional area of the neck is the regulated parameter. This regulation may result from active constriction of the spine neck. Alternatively, the accessible cross-sectional area of the neck may change because of rearrangement of the cytoskeleton or the movement of organelles into the neck (14–17).

Is diffusional equilibration across the spine neck also regulated acutely by the activity of the synapse enclosed in the spine head? The effects of back-propagating action potentials (bAPs), synaptic activity, and the pairing of bAPs with synaptic activity on the spine neck diffusional resistance (Fig. 4) were measured. For these experiments, spine/dendrite diffusional coupling was measured by photoactivation of NPE-HPTS, a caged version of the green-fluorescing, pyramine-based fluorophore HPTS (18). Whole-cell–current clamp recordings were obtained from hippocampal pyramidal neurons that were filled through the patch pipette with NPE-HPTS and Alexa Fluor-594 and bathed in 5 mM MNI-glutamate, a caged version of glutamate (19). Illumination at 720 nm for 0.5 ms was used to photoactivate NPE-HPTS and uncage glutamate, and the laser power was set to generate fluorescence transients of ~20%, a 20% increase in green fluorescence relative to the resting red fluorescence (AG/R) in the spine head. Pairing of uncaging-evoked EPSPs (uEPSPs) with small bursts of bAPs (3 bAPs at 50 Hz) triggered increases in $\tau_{equ}$ that continued after the end of the pairing period (Fig. 4E) ($n = 8/12$ cells/spines, $P < 0.05$). In contrast, bAPs ($n = 8/9$) or uEPSPs ($n = 6/11$) alone, as well as repeated monitoring of $\tau_{equ}$ without stimulation ($n = 6/11$), had no effect on $\tau_{equ}$. For all four experimental conditions (uEPSP/bAP pairing, uEPSPs alone, bAPs alone, and no stimulation), the analyzed spine experienced identical photoactivation and imaging laser exposures. Thus, the restriction of diffusion across the spine neck seen in response to the pairing of bAPs and synaptic stimulation represents a cellular response to the stimulus. Furthermore, because HPTS is a small polar molecule, its diffusion is similar to that of second messengers such as cyclic adenosine monophosphate.

The regulation of spine/dendrite diffusional equilibration may have several functional consequences. First, the susceptibility of individual synapses to plasticity induction
Tissue-Specific TAFs Counteract Polycomb to Turn on Terminal Differentiation

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Polycomb transcriptional silencing machinery is implicated in the maintenance of precursor fates, but how this repression is reversed to allow cell differentiation is unknown. Here we show that testis-specific TAF (TBP-associated factor) homologs required for terminal differentiation of male germ cells may activate target gene expression in part by counteracting repression by Polycomb. Chromatin immunoprecipitation revealed that testis TAFs bind to target promoters, reduce Polycomb binding, and promote local accumulation of H3K4me3, a mark of Trithorax action. Testis TAFs also promoted relocalization of Polycomb Repression Complex 1 components to the nucleolus in spermatocytes, implicating subnuclear architecture in the regulation of terminal differentiation.

Male germ cells differentiate from adult stem cell precursors, first proliferating as spermatogonia, then converting to spermatocytes, which initiate a dramatic, cell type–specific transcription program. In Drosophila, five testis-specific TAF homologs (tTAFs) encoded by the can, sa, mia, nht, and rye genes are required for meiotic cell cycle progression (1,2) and normal levels of expression in spermatocytes of target genes involved in postmeiotic spermatid differentiation (3). Requirement for the tTAFs is gene selective: Many genes are transcribed normally in TAF mutant spermatocytes. Tissue-specific TAFs have also been implicated in gametogenesis and differentiation of specific cell types in mammals (4,5). In addition to action with TBP (TATA box–binding protein) in TFID, certain TAFs associate with HAT (histone acetyltransferase) or Polycomb group (PcG) transcriptional regulatory complexes (6,7). To elucidate how tissue-specific TAFs can regulate gene-selective transcription programs during development, we investigated the mechanism of action of the Drosophila TAFs in vivo.

The ITAF proteins were concentrated in a particular subcompartment of the nucleolus in primary spermatocytes (Fig. 1). Expression of a functional green fluorescence protein (GFP)–tagged genomic sa rescuing transgene revealed that expression of Sa-GFP turned on specifically in male germ cells soon after initiation of spermatocyte differentiation and persisted throughout the remainder of the primary spermatocyte stage, disappearing as cells entered the first meiotic division (Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A). Some Sa-GFP was detected associated with condensing chromatin (arrowheads in Fig. 1A).

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