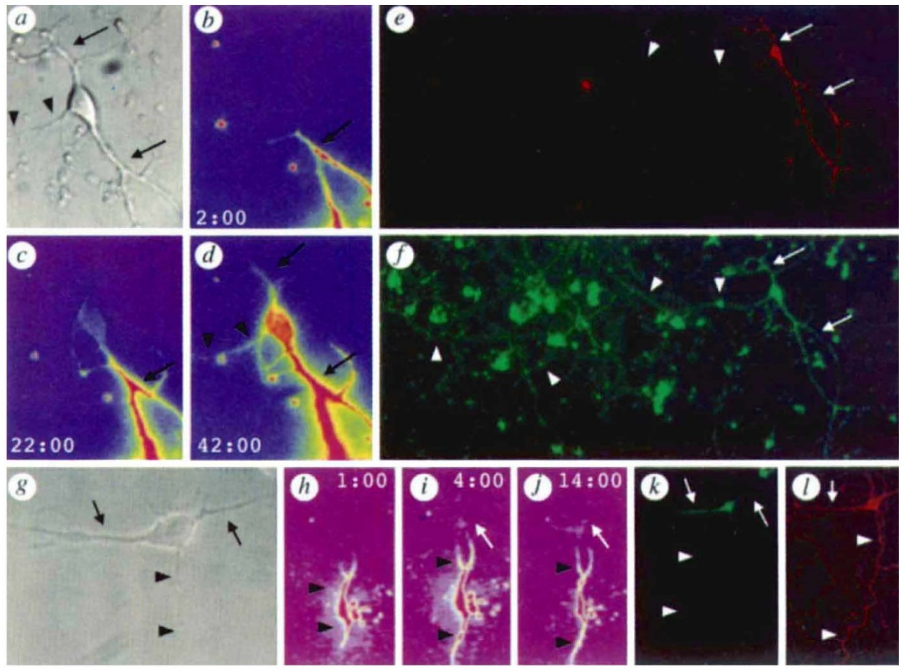
No diffusion barrier at axon hillock

SIR — In epithelial cells, the tight junction poses a barrier preventing diffusional mixing of membrane proteins and lipids between apical and basolateral domains[1,2]. Evidence for a functional equivalent to this tight junction at the axon hillock of neurons has been presented[3]. The viral protein haemagglutinin, which is restricted to axons, was used to induce the fusion of liposomes containing fluorescent phospholipids exclusively along axons. No decrease in staining in the proximal axon and no soma staining was observed, suggesting a diffusion barrier for lipids at the axonal hillock. It has been suggested, however, that the small proportion of label (~10%) that would diffuse onto the cell body during the experiment would not have been detected[4]. Here we present findings that argue against a lipid diffusion barrier at the neuronal axon hillock.

Applying a local perfusion labelling technique[5], we used the fluorescent lipid DiI(C_{12}) to label a short segment of dendritic or axonal membrane of differentiated hippocampal neurons, near the soma, and followed the spread of fluorescence over time. DiI was seen to diffuse across the axon hillock from both somatodendritic (13/13 cases) and axonal sides (22/24 cases) within minutes (*b–d* and *h–j* in the figure). This observation was possible because of the very bright labelling given by DiI and the sensitive fluorescence imaging method used. In addition, labelling of dendrites as well as the axon was possible and so diffusion of label from both sides across the hillock could be followed. Labelling of dendrites resulted in a bright signal whose spread could be detected easily (*d* in the figure) but, as expected, soma labelling was faint after DiI incorporation into the axon (*j*). The fluorescence intensity showed smooth profiles after diffusion, with no abrupt changes at the axon hillock, consistent with free diffusion across this region.

Morphological criteria[6] were used to identify axonal and dendritic processes before DiI labelling. This identity was subsequently confirmed by immunocytochemical staining against either a subtype of the glutamate receptor (GluR1) or microtubule-associated protein 2 (MAP2), both of which are restricted to somatodendritic domains[6,7]. Preferential localization of the membrane protein GluR1 was observed in the somatodendritic domain, whereas a fluorescent lectin, succinylated concanavalin A, uniformly stained the entire neuronal surface (*f* in the figure). Thus, differential membrane protein distribution was maintained in those cells that had shown no diffusion barrier for DiI at the axon hillock. Similar preferential somatodendritic localization of the cytoskeletal protein MAP2 was also observed (*k* in the figure). All nerve processes were revealed by immunostaining against tubulin (*l*).



Diffusion of the fluorescent lipid DiI across the axonal hillock. *a–d*, A 2-week-old neuron was labelled with DiI on a dendrite (axon marked by arrowheads, dendrites by arrows) and the spread of fluorescence followed over time (indicated by numbers in minutes) with a cooled CCD camera. Note the labelling of the soma by 22 min and of the proximal axon by 42 min. Immunocytochemical staining of the neuron showed preferential restriction of GluR1 to the somatodendritic membrane (*e*) and uniform distribution of surface receptors (*f*) for FITC–succinylated concanavalin A along all processes. *g–j*, A 2-week-old hippocampal neuron in culture was labelled with DiI on the axon and the spread of label followed over time. The fluorescence was detected at the soma (white arrow) 4 min after labelling. The axonal identity of the labelled process was confirmed by immunocytochemical staining against MAP2 (*k*) and tubulin (*l*; polyclonal antibody kindly provided by F. Solomon). We note that GluR1 staining showed a tapered gradient into the proximal axon, similar to that observed for MAP2 (ref. 7). Preparation of cultures and fluorescence imaging followed those reported previously[5–7]. The fluorescence intensity was encoded by a continuous scale of pseudocolours. In *b–d* and *h–j*, low intensity is represented by purple/blue, intermediate intensity by yellow and high intensity by red.

Several lines of evidence indicate that the DiI label was properly incorporated into the plasma membrane and that the spread across the axon hillock was due to lateral diffusion within the plasmalemma. The rate of fluorescence spread is consistent with lateral diffusion of membrane lipids (see also ref. 5). The absence of significant lipid internalization is indicated by the ring staining of the soma and the absence of punctate fluorescence within the neuron. The use of a polarized excitation light source[8] confirmed the proper orientation of the majority of DiI fluorophores within the plasma membrane matrix. Finally, in several cases we observed the spread of DiI along a labelled process that was not connected to the nearest soma but turned or grew alongside the soma. No staining of the soma was found in these cases. The faint soma staining observed after local labelling thus did not result from DiI molecules released from the labelled segment or from extracellular DiI crystals that diffused to the soma through the aqueous phase rather than along the labelled process.

Preferential localization of GluR1 in the somatodendritic membrane in the absence of an apparent diffusion barrier at the axonal hillock suggests that other mechanisms are involved in maintaining axonal and somatodendritic domains[9]. The lateral mobility of membrane proteins in these neurons (B.W. and M-m.P., unpublished results) and the tapered gradient of GluR1 staining into the proximal axonal domain (see figure) support the notion that steady-state asymmetry in the distribution of plasma membrane proteins may result from their selective insertion into and/or removal from the plasmalemma.

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