Imaging of Experience-Dependent Structural Plasticity in the Mouse Neocortex in vivo

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Summary. The functionality of adult neocortical circuits can be altered by novel experiences or learning. This functional plasticity appears to rely on changes in the strength of neuronal connections that were established during development. Here we will describe studies in which we have addressed whether structural changes, including the remodeling of axons and dendrites with synapse formation and elimination, could underlie experience-dependent plasticity in the adult neocortex. Using 2-photon laser-scanning microscopy transgenic mice expressing GFP in a subset of pyramidal cells, we have observed that a small subset of dendritic spines continuously appear and disappear on a daily basis, whereas the majority of spines persists for months. Axonal boutons from different neuronal classes displayed similar behavior, although the extent of remodeling varied. Under baseline conditions, new spines in the barrel cortex were mostly transient and rarely survived for more than a week. However, when every other whisker was trimmed (a paradigm known to induce adaptive functional changes in barrel cortex), the generation and loss of persistent spines was enhanced. Ultrastructural reconstruction of previously imaged spines and boutons using serial section electron microscopy showed that new spines slowly form synapses. New spines that persisted for a few days always had synapses, whereas very young spines often lacked synapses. New synapses were predominantly found on large, multisynapse boutons, suggesting that spine growth is followed by synapse formation, preferentially on existing boutons. Altogether our data indicate that novel sensory experience drives the stabilization of new spines on subclasses of cortical neurons and promotes the formation of new synapses. These synaptic changes likely underlie experience-dependent functional remodeling of specific neocortical circuits.

Introduction

During development, the formation of neural circuits in the mammalian neocortex is guided in a stereotypic way by a myriad of intracellular and extracellular molecular cues and is sculpted by spontaneous and sensory-evoked activity (Hua and Smith 2004; Katz and Shatz 1996). Although this so-called activity-dependent plasticity is most robust during development, neuronal circuits remain plastic in the adult brain. For example, cortical sensory maps can change in size and location upon peripheral lesions, including amputations, and changes in experience (Buonomano and Merzenich 1998).
Although this adult plasticity is thought to depend on changes in the strength of established synaptic connections (Hebb 1949), it could also involve structural alterations, including synapse formation and elimination (Antonini and Stryker 1993; Chklovskii et al. 2004; Knott et al. 2002; Lowel and Singer 1992; Ramon y Cajal 1893; Stepanyants et al. 2002; Turner and Greenough 1985; Ziv and Smith 1996). To address this possibility, we have focused our recent studies on dendritic spines and axonal boutons, the sites where the majority of excitatory synapses are found and, therefore, potential substrates for structural plasticity of cortical circuits.

Dendritic spines are tiny protrusions from the dendritic shaft, with volumes that can range from 0.001 to 1 μm³ (Peters and Kaiserman-Abramof 1970; Sorra and Harris 2000). They can undergo fast structural remodeling on time scales of seconds and minutes (Bonhoeffer and Yuste 2002; Dailey and Smith 1996; Fischer et al. 1998; Lendvai et al. 2000; Matus 2000; Yuste and Bonhoeffer 2004), and de novo appearances and complete retractions can occur over hours and days (Dailey and Smith 1996; Engert and Bonhoeffer 1999; Holtmaat et al. 2005; Lendvai et al. 2000; Maletic-Savatic et al. 1999; Trachtenberg et al. 2002). They can emerge (Engert and Bonhoeffer 1999; Maletic-Savatic et al. 1999) or expand (Matsuzaki et al. 2004) in response to synaptic stimulation and make synapses (Knott et al. 2006; Knott et al. 2002; Toni et al. 1999, 2007). Dendritic spines could, therefore, be central to the brain’s capacity to change its connectivity, increasing the dendrites’ ability to connect with axons that are not in direct contact (Chklovskii et al. 2004; Peters and Kaiserman-Abramof 1970; Stepanyants and Chklovskii 2005; Swindale 1981).

Numerous studies that have manipulated sensory experience have shown changes in spine or synapse densities within the brain. These changes have been reported after rearing or extensive training in enriched environments (Beaulieu and Colonnier 1987; Moser 1999; Moser et al. 1994; Turner and Greenough 1985) but also after long-term sensory deprivation (Zuo et al. 2005b). Together with the observations that their dynamics are regulated by activity, these findings have led to the idea that dendritic spines are potential substrates, along with the axons, for activity-dependent plasticity in the adult brain (Bonhoeffer and Yuste 2002; Moser 1999; Segal 2005; Yuste and Bonhoeffer 2004).

Threaded amongst the dendrites and their spines are the sources of their inputs, the axons. Studded along the axonal branches are en passant boutons (EPBs), or bouton terminaux (TBs) at their endings. Like the spine, these structures have also shown the ability to remodel, both in vivo and in vitro. TBs have been proposed to subserve, in part, the formation of dendritic shaft synapses (McGuire et al. 1984), similar to the role of dendritic spines that form synapses with EPBs. Axonal endings and boutons can undergo profound structural changes during development (Antonini and Stryker 1993; Portera-Cailliau et al. 2005; Ruthazer et al. 2003). In the adult cortex, axonal sprouting has been observed after long-lasting peripheral lesions of sensory organs (Darian-Smith and Gilbert 1994; Florence et al. 1998). Many types of axonal boutons display rapid (minutes to hours) activity-dependent structural plasticity in dissociated (Colicos et al. 2001) and organotypic hippocampal cultures (De Paola et al. 2003; Nikonenko et al. 2003). The reorganization of axons at the micrometer level could play a role in circuit plasticity (Stepanyants et al. 2002). Similar to dendritic spines,