Bidirectional regulation of dendritic spine dimensions by glutamate receptors

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Introduction

While traditional views of dendritic spines assumed that they serve as the locus of long-term memory storage, and as such they are stable structures, modifiable only by stimuli associated with long-term memory [1,2], more recent studies indicate that spines may vary in size and numbers in a dynamic fashion [3–6]. For example, it has been shown that dendritic spine density varies across the estrus cycle [3], may increase following slicing of the hippocampus [4] and that stimulation which produce long-term potentiation induces formation of novel spines, in a manner that depends on activation of the NMDA receptor [5]. On the other hand, glutamate can produce a fast shrinkage and eventual disappearance of existing dendritic spines [6,7], an effect that is also dependent on activation of the NMDA receptor. Two possibilities can account for these conflicting results. Glutamate can produce elongation of certain types of spines, and shrinkage of others, or that glutamate can produce opposite effects in the same spine, depending on the level of activation of postsynaptic second messenger systems. It is well known that glutamate produces a rise in [Ca\textsuperscript{2+}], and perhaps different amounts or duration of glutamate application cause opposite effects on spine dimensions. The present study was designed to address this issue.

Materials and Methods

Hippocampal cultures were prepared as described previously [8] with slight variations. Briefly, dissociated hippocampi of 19-day old embryos were plated onto poly-L-lysine-coated 12 mm glass coverslips in Eagle’s MEM containing 5% heat-inactivated horse serum and 5% fetal calf serum. Three-week-old cultures were used for the imaging experiments.

Morphological imaging: A coverslip was transferred into the recording chamber in a confocal laser scanning microscope (CLSM, Leica, Heidelberg, Germany), where it was perfused with MEM equilibrated with O\textsubscript{2}/CO\textsubscript{2} gas mixture at about 30°C. Drugs were prepared in the recording medium from frozen stocks before use. Glutamate (0.1 mM) was loaded in a pressure pipette with a tip diameter of 1–2 μm which was placed near the dendrite. The CLSM is equipped with an argon-ion laser for excitation at a wavelength of 488 nm. Individual cells were impaled with micropipettes containing Calcein (Molecular Probes Inc), which is insensitive to intracellular calcium variations, for imaging of dendritic morphology. The dye was iontophoresed into the cell for 0.5–1 min and was allowed to equilibrate for ≥ 30 min before experiments commenced, to assure equal distribution of the dyes in the different cellular...
compartments. For each experiment, a fresh spine/dendritic segment in a new dish was used. The dendrites were imaged with a ×63 water immersion objective. The dendrites were reconstructed in 3D from stacks of successive 10–15 images spaced 0.2–0.3 μm apart. Laser light was reduced to 1–3% of nominal intensity. Using this setting, we were able to image the dendrites repetitively for several hours without significant photodynamic damage. The dendrite was reconstructed at least twice, 30 min apart, before drug application. Glutamate was then applied 3–4 times, each consisting of 1–4 pulses, 6 ms or 30–50 ms in duration. 3D-reconstructed spines/dendrites were imaged immediately, and 15, 30, 60, 120 and 180 min after drug treatment. The cells were still alive and well at the termination of the experiment, as judged by the shape of the dendrites and the somata of the recorded cell. Damaged cells showed blebbed dendrites, and the dye diffused out of the cell. Analysis of spine length and shape was conducted from the 3D reconstructed images, using Adobe Photoshop and NIH image software.

**Calcium imaging:** Cultures were incubated with 5 μM Fura-2AM for 1 h at room temperature, washed and placed on the stage of an inverted Nikon microscope, equipped with a CCCD camera and a filter wheel. Ratio images of selective fields were taken at 340/380 nm illumination, as described previously [9]. Glutamate was applied by 1 or 5 10–20 ms puffs from a micropipette placed 20–40 μm from the imaged cells. Calcium concentrations were calculated using standard equations and software [9].

**Results**

**Calcium imaging:** Cultured neurons respond to pulse application of glutamate with a transient rise in [Ca^{2+}], in a dose-dependent manner. The magnitude and duration of the response to glutamate depend on many factors, including the age of the culture, receptor density, type of cells, composition of the recording medium as well as the ability of the cultured neurons to dispose of the elevated [Ca^{2+}]. These factors were studied extensively elsewhere, and, suffice to say, in our testing conditions, most of the calcium rise produced by glutamate was due to its influx through the NMDA receptor. The responses to glutamate are consistent and reproducible, and a single long (50 ms) or several successive short (10 ms) pulse applications of glutamate produced a corresponding transient increase in [Ca^{2+}]; (Fig. 1).

**Morphological imaging:** Using the same cell type and age as those recorded in the calcium experiments, we imaged individual dendrites at high resolution, to detect changes in spine and dendrite dimensions following exposure to glutamate. In younger cultures (up to 2 weeks in vitro) the dendrites grow extensive arborization of filopodia which express high motility rates even in relatively inactive states. Thus, within the imaging session of 2–3 h, filopodia can extend and retract irrespective of activity states imposed on the culture [8,10]. In the more mature culture (3–4 weeks in vitro) there are few filopodia, and the cells are far less motile. At any rate, the filopodia can be clearly distinguished from dendritic spines, as the latter have spine heads, not seen in filopodia. On the background of non-motile spines in the more mature cultures, any small change in spine shape could be easily detected and quantified. In previous studies we found that exposure to glutamate can reduce spine length by an average of about 30% over 2–3 h in an NMDA-receptor-dependent manner [7]. In the current experiments, short (6 ms) applications of glutamate produced a slow onset, increase in spine length. This could be quickly reversed by a single large application of glutamate, which caused shrinkage of the same spines (Fig. 2), as seen previously [6,7]. While the magnitude of change varied among different spines, the bidirectional trend in spine length was consistent among all the 10 spines examined in two separate experiments.

![FIG. 1. Averaged responses of cultured hippocampal neurons to pulse application of glutamate. Image pairs were taken once every 10 s, before and immediately after application of glutamate via a pressure pipette. The two traces are averages of the same four cells responding to glutamate, applied in one or five puffs. The responses are larger and longer lasting following the five than the one puff. In addition, the short response leads to an immediate recovery back to baseline, whereas the large response did not recover.](image-url)
Glutamate regulates dendritic spine length

Discussion

The present results illustrate that the same family of spines can be made to elongate or shrink with the same stimulation, depending on the intensity of the stimulation, and the subsequent change it produces in [Ca\(^{2+}\)]. Earlier studies, by us and others, have shown that glutamate, or afferent stimulation, can promote formation of novel spines [5], elongation of existing growth cones and spines [11], as well as shrinkage or elimination of spines [7]. With the exception of one study showing that novel spines can be formed on one dendritic segment, while another segment can lose its spines following a stimulation protocol that promotes long-term potentiation [5], none addressed the possibility that the same stimulation can cause both formation and elimination of the same population of spines. Assuming that glutamate can activate different postsynaptic second messenger cascades, depending on the magnitude or duration of the rise of [Ca\(^{2+}\)], produced by it, we set out to test this possibility. We now found that the same spines can switch from elongation to shrinkage, if the stimulus properties are changed, so that the [Ca\(^{2+}\)] rise is higher in response to glutamate. While we have as yet no evidence that different second messenger cascades lead to the different responses, it is likely that an enhanced and prolonged rise in [Ca\(^{2+}\)], will affect actin polymerization [12] differently from short rises in [Ca\(^{2+}\)], and further experiments are required to address these possibilities.

Regardless of the molecular mechanisms, the functional consequences of a change in spine length are of importance for the understanding the tremendous heterogeneity of spine dimensions in central neurons. In a recent study addressing this issue [13], we found that longer spines are more independent from the parent dendrites than short spines, in that their calcium transients are longer lasting than those of short spines, and their calcium stores are less likely to be repleted upon use than those of the shorter spines. Taken together, these results indicate that dendritic spines may undergo a dynamic adaptation to incoming signals, so that both the properties of the stimulus and the physical dimensions of the receiving spine will determine the impact of the afferent stimulation, and its ability to change the morphology of the spine, and its interaction with the parent dendrite. These observations are of major functional consequences for the understanding of information processing in central neurons.

Conclusion

The same dendritic spines in cultured hippocampal neurons can either expand or shrink in response to topical application of glutamate, depending on the intensity/duration of the glutamate stimulation, which produces different changes in [Ca\(^{2+}\)] in the affected neurons.

References


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