Glutamate receptors regulate actin-based plasticity in dendritic spines

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Dendritic spines at excitatory synapses undergo rapid, actin-dependent shape changes which may contribute to plasticity in brain circuits. Here we show that actin dynamics in spines are potently inhibited by activation of either AMPA or NMDA subtype glutamate receptors. Activation of either receptor type inhibited actin-based protrusive activity from the spine head. This blockade of motility caused spines to round up so that spine morphology became both more stable and more regular. Inhibition of spine motility by AMPA receptors was dependent on postsynaptic membrane depolarization and influx of Ca^{2+} through voltage-activated channels. In combination with previous studies, our results suggest a two-step process in which spines initially formed in response to NMDA receptor activation are subsequently stabilized by AMPA receptors.

The adaptive properties of brain circuitry require that the transient events of synaptic transmission be converted into lasting changes in neuronal connectivity. However, the cellular events underlying this transition—especially those that operate in adult circuitry—are poorly understood. Experience-dependent plasticity is associated particularly with glutamatergic excitatory synapses[1], the vast majority of which are made onto dendritic spines[2]. Abundant evidence indicating that spines can undergo activity-dependent changes in shape and number has raised widespread interest in them as a possible cellular substrate for synaptic plasticity in the brain[3–7]. Dynamic imaging studies on living neurons confirm that spines are motile structures[8–14] and suggest that motile filopodial spine precursors may initiate synapse formation[9]. Changes in spine shape occur in the living brain and, particularly during early postnatal development, levels of spine motility are responsive to alterations in sensory experience[14].

Other studies have begun to suggest how activity in neuronal circuits might influence spine morphology. Experiments with hippocampal slice cultures suggest that maintenance of spine morphology requires continual low-level activation of AMPA receptors by spontaneously released glutamate[15], whereas other studies demonstrate *de novo* spine formation following stimulation protocols that lead to NMDA receptor-dependent long term potentiation (LTP)[11,12,16]. These studies implicate AMPA and NMDA receptors in different aspects of spine plasticity but do not address the nature of the cellular mechanism that converts receptor activation into morphological change. Some observations suggest that actin-based motility may be important in this process. Cytoplasmic actin isoforms, associated with shape changes in motile cells, accumulate at high concentrations in dendritic spines, whereas skeletal muscle actins do not[17]. Also, time-lapse studies of neurons expressing actin tagged with green fluorescent protein (GFP), either alone or fused to actin (GFP–actin), reveal rapid changes in spine shape that are sensitive to blockers of actin dynamics such as cytochalsin D[10,13].

If these events have a meaningful role in synaptic plasticity, they should be regulated by synaptic transmission. Here we examined the influence of AMPA and NMDA glutamate receptors on actin dynamics in dendritic spines of excitatory synapses, where glutamate is the major neurotransmitter. Our results reveal a sequence of events, triggered by glutamate receptor activation, that lead to the inhibition of actin motility and the stabilization of spine morphology.

Results

Glutamate receptor inhibition of spine actin dynamics

We studied the influence of glutamate receptors on spine motility by making video time-lapse recordings of GFP-actin dynamics in dendrites of transfected neurons (Fig. 1a). Dendritic spines in the brain have many morphologies[18,19], and this is reflected in our images of spines of living hippocampal neurons in cell culture. To ensure that our results would incorporate any variations in dynamic behavior associated with this structural heterogeneity, we selected well-separated dendritic segments long enough to contain 30 to over 100 individual spines for recording (Fig. 1b).

To demonstrate the dynamic changes in GFP–actin captured in time-lapse recording, video sequences were processed using a computer algorithm that subtracts gray-scale values between pixels in neighboring video frames and displays the summed differences. Dark areas indicative of high motility were particularly associated with dendritic spines (Fig. 1b). The pixel densities in these difference images also reflect levels of motility. This was used to assess the influence of glutamate receptor activation on spine actin dynamics. The example shown in Fig. 1b is one of many experiments ($n > 50$) in which treating hippocampal neurons with 100 μM glutamate strongly inhibited actin-based dynamics (compare Fig. 1b panels 2 and 4). Glutamate receptor activation exerted a profound effect, completely blocking actin dynamics in all spines on a given segment of dendrite. These effects occurred without gross changes in dendritic morphology (compare panels 1 and 3 of Fig. 1b).

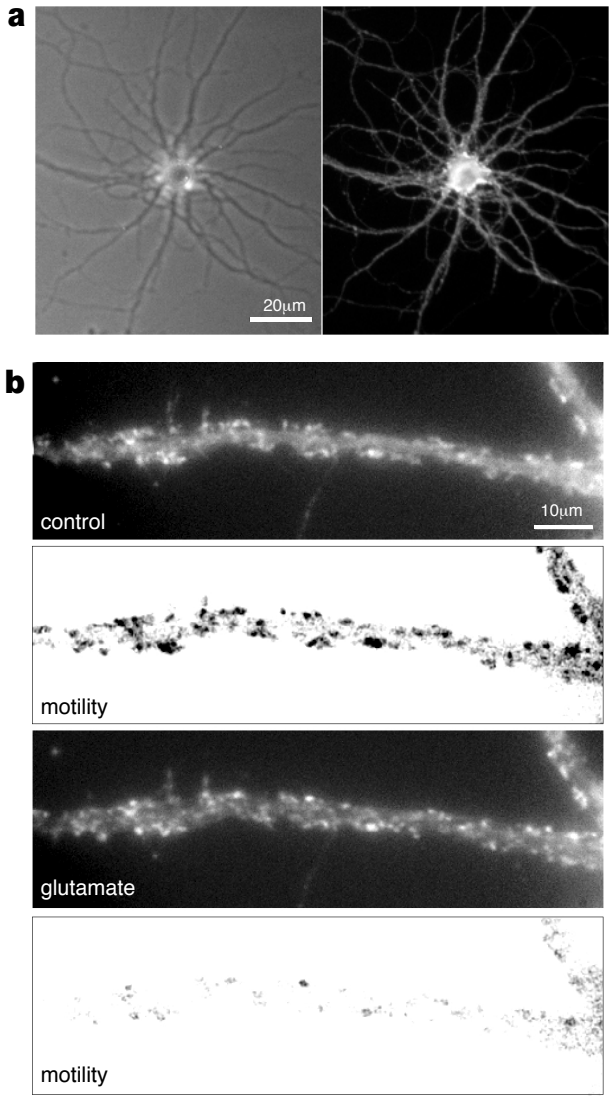

Fig. 1. Glutamate inhibits actin dynamics in dendritic spines. (**a**) Transfected hippocampal neuron expressing GFP–actin throughout the dendritic tree after 3 weeks in low-density culture (phase contrast, left; GFP fluorescence, right). (**b**) A 90 μm segment of dendrite used for time-lapse recording of actin dynamics. Panels 1 and 3, individual frames showing GFP–actin fluorescence recorded under control conditions and after the addition of glutamate (100 μM). The presence of glutamate does not disturb dendritic structure or actin distribution when NMDA receptors are blocked as shown here by 100 μM APV. Panels 2 and 4, corresponding summed difference images showing that actin motility is primarily associated with dendritic spines (panel 2) and is strongly suppressed by the addition of glutamate (panel 4).

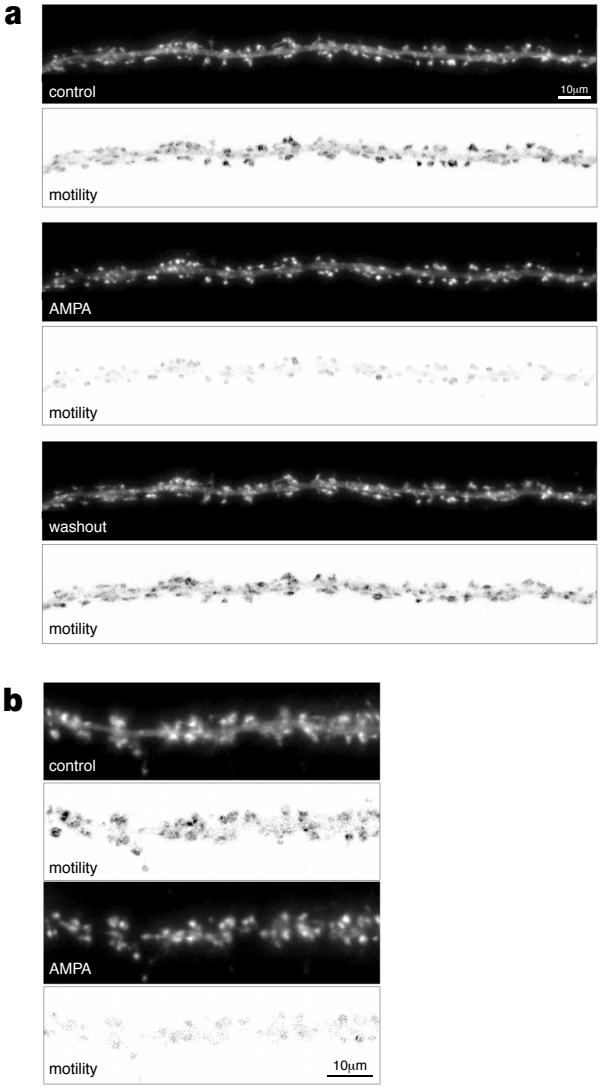

Fig. 2. AMPA receptor stimulation inhibits actin-based dendritic spine motility. (**a**) Individual fluorescence images (upper panels) and summed difference images (lower panels) for the same section of dendrite under control conditions (top), in the presence of 1 μM AMPA (middle) and after AMPA wash-out (bottom). The decrease in the summed image differences in the presence of AMPA demonstrates the inhibition of spine motility, visible in the supplementary video data available on the *Nature Neuroscience* web site. Even at this low magnification, the rounding effect on spine morphology produced by AMPA receptor activation can be seen in many of the spines. (**b**) Another experiment documenting the effect of AMPA (2 μm) on spine motility. In this case, NMDA receptors were blocked with 100 μM APV. See supplementary video data Fig2b.mov for an original time-lapse recording of an experiment with AMPA and APV, available on the *Nature Neuroscience* web site.

Spine motility is sensitive to AMPA receptor activation

To eliminate NMDA receptor-induced excitotoxic effects that occur when hippocampal neurons are exposed to glutamate[20,21], the experiments described above were conducted with the NMDA receptor antagonist APV (100 μM). This indicates that glutamate can block actin-based spine motility independent of NMDA receptors and suggests the involvement of AMPA receptors in regulating actin dynamics in spines. Consequently we examined the effect of exposing cells to AMPA and found that it too had a strong inhibitory effect on spine motility. This effect developed rapidly and was readily reversable when AMPA was removed (Fig. 2). The level of AMPA required to inhibit spine motility was determined by applying increasing concentrations to cultures ($n = 15$). Inhibition was detectable at AMPA concentrations from 500 nM ($n = 4$), with complete blockade of motility occurring between 1 μM ($n = 4$) and 2 μM ($n = 7$). These effects, and similar ones produced by 100 μM kainate ($n = 6$), were completely blocked by the selective AMPA receptor antagonists CNQX (20

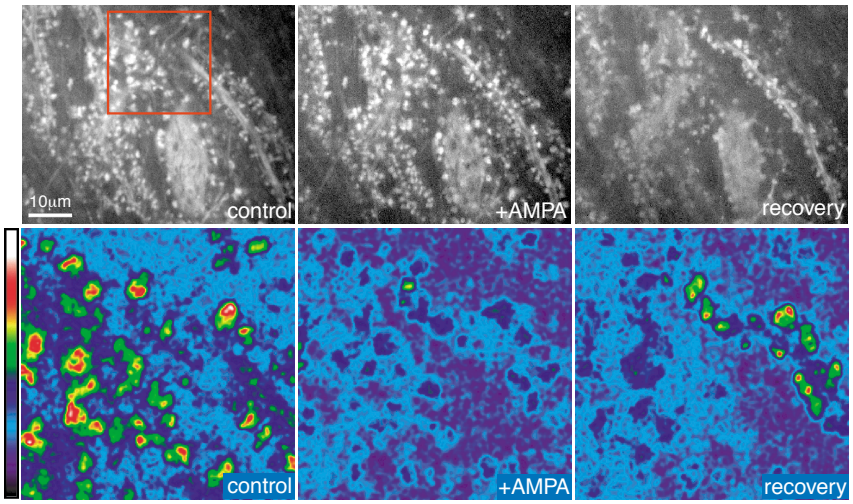

Fig. 3. AMPA inhibits spine motility in organotypic slice cultures from hippocampus. Top panels show individual frames in a time-lapse recording of GFP fluorescence taken by confocal microscopy from a living hippocampal slice established from a transgenic mouse expressing GFP-tagged actin and cultured for four weeks. The labels identify the phase of the experiment from which the frames and their corresponding difference images (bottom) were taken. The difference images are shown using a false color scale (left) and correspond to the area outlined by the red box in the top left panel. Actin dynamics during each phase were recorded in 60 frames over 15 min. AMPA was introduced into the medium 54 min after the beginning of recording and was washed out 34 min before the recording shown in the recovery phase. See the three parts of Fig3_.mov in the supplementary video data for the original time-lapse data for this experiment.

μM, $n = 6$ for AMPA, $n = 3$ for kainate) and AMOA (15 μM, $n = 3$ for AMPA, not determined for kainate).

As a further test for the receptor subtype involved, we recorded the influence of AMPA on spine actin when NMDA receptors were blocked by selective antagonists (Fig. 2b; $n = 17$). Despite the high concentration of NMDA receptor antagonist, AMPA completely blocked spine motility at either 1 μM ($n = 7$) or 2 μM ($n = 10$). Similar results were obtained in experiments in which cells were treated with AMPA in the presence of the open-channel NMDA receptor blocker MK801 at 10 μM ($n = 6$). In all experiments, control recordings were made in the presence of APV or MK801 alone, before the addition of AMPA. Neither drug had any detectable effect on spine shape or motility.

To ensure that these effects were not limited to neurons growing in dispersed cultures, we also examined the influence of AMPA on dendritic spine actin in confocal time-lapse recordings of hippocampal slice cultures established from transgenic mice expressing GFP-tagged actin. A total of 8 experiments with increasing concentrations of AMPA (0.5 μM, $n = 1$; 1 μM, $n = 5$, Fig. 3, left and center; 2 μM, $n = 2$) showed complete inhibition of spine motility. Despite bleaching of the GFP–actin signal (produced by the long periods of imaging necessary to record the effects of drug treatment in slice cultures), recovery of actin dynamics was still observable after AMPA was removed from the perfusing medium (Fig. 3, right).

To assess the possible contribution of NMDA receptors, we recorded actin dynamics in spines exposed to 1 μM NMDA while AMPA receptors were blocked with 100 μM DNQX. In recordings made at physiological salt concentrations, NMDA had no detectable effect on spine motility ($n = 4$; example in Fig. 4, compare panels 2 and 4). This might result from the blockade of NMDA receptor activity by Mg^{2+} that occurs at resting membrane potential[22,23]. To test this possibility, we examined the effect of NMDA on spine motility when the Mg^{2+} concentration in the medium was reduced from 0.5 mM to 0.1 mM ($n = 5$). Under these conditions, NMDA inhibited spine motility in all 5 experiments (Fig. 4, compare panels 4 and 6).

AMPA receptor activation alters spine shape

In addition to blocking spine motility, AMPA receptor activation also produced a distinct change of actin configuration in the spine, in which the irregular profile characteristic of motile spines

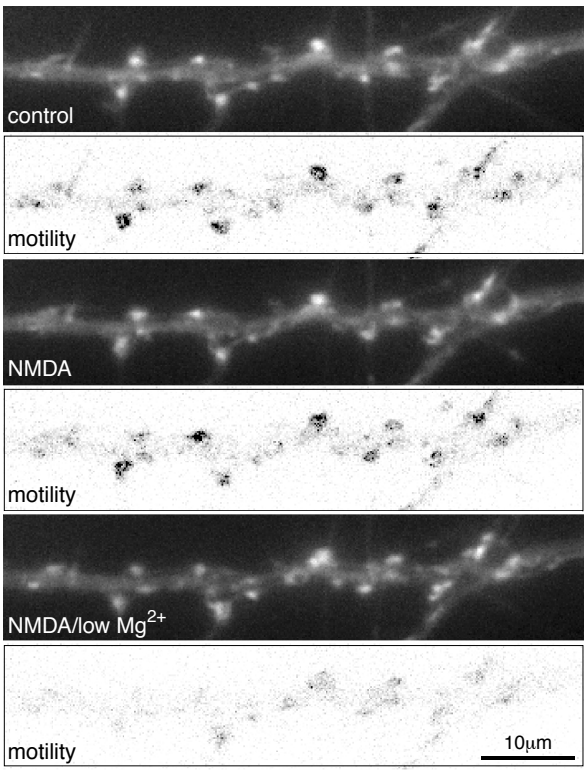

Fig. 4. Inhibition of spine motility by NMDA requires lowering of the external Mg^{2+} concentration. Panels 1, 3 and 5 show individual frames taken from a time-lapse recording of GFP–actin fluorescence under control conditions (panel 1), after the addition of 1 μM NMDA (panel 3) and with 1 μM NMDA in low (0.1 mM) Mg^{2+} (panel 5). The corresponding difference images (motility) show that at normal concentrations of Mg^{2+} (0.5 mM) adding NMDA did not reduce spine motility (compare panels 2 and 4), but when Mg^{2+} was reduced to 0.1 mM, NMDA spine motility was decreased significantly (panel 6). AMPA receptors were blocked with 100 μM DNQX throughout the experiment. Scale bar, 10 μm. See Fig4.mov in the supplementary video data for the original time-lapse data.

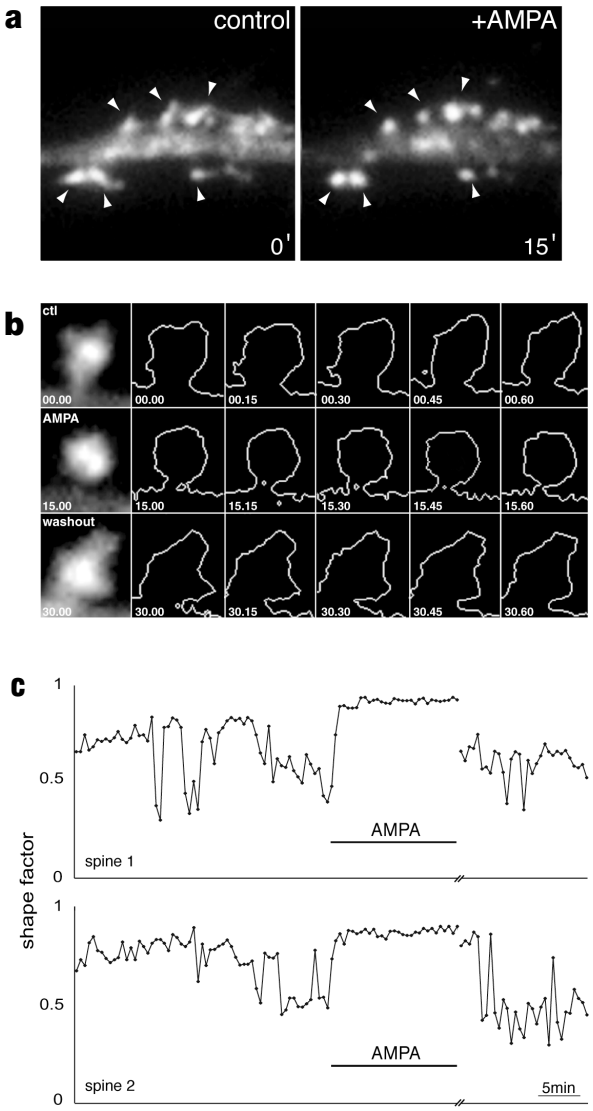

Fig. 5. AMPA receptor activation leads to spine rounding. (**a**) Live cell images of GFP–actin in a dendrite showing the effect of AMPA (1 μM) on spine shape. Motile spines with irregular outlines (arrowheads, left) are round and immobile 15 min after addition of AMPA (right). (**b**) AMPA-induced spine shape change as revealed by profile outlines produced from thresholded images of spine heads. Each row shows a single fluorescence image (left) and derived spine profiles taken 15 s apart. The data are from a continuous recording of a single dendritic spine in control conditions (top row), in the presence of 1 μM AMPA (middle) and after AMPA washout (bottom). The round profile of the spine during AMPA blockade of spine motility is evident. (**c**) Data for two further spines, taken from a separate experiment, displayed using shape factor analysis. The spine rounding and blockade of spine motility induced by AMPA are shown by the increase in value of the shape factor and flattening of the shape factor plots (see text).

was replaced by a more rounded appearance of the spine head (Fig. 5a). To analyze this effect in more detail, we derived outline profiles of individual spines ($n = 9$, Fig. 5b) using data in three separate experiments with independently established cultures. These profiles were used to calculate a shape factor that returns a value of 1 for a perfectly round profile and values below 1 for varying degrees of irregularity (Methods). Changes in spine morphology during a time-lapse recording can then be followed by plotting the shape factor values against time (Fig. 5c).

The example in Fig. 5b shows outline profile data for a single spine in successive frames of a time-lapse recording taken 15 seconds apart. Following an initial control period when the spine changed shape continuously (top row), its morphology became stable after AMPA was added (middle row). When the drug was washed out, shape changes recommenced (bottom row). This sequence of profiles also demonstrates the rounding-up of spines in the presence of AMPA (middle row), an effect that consistently accompanied the inhibition of actin motility by AMPA–receptor activation. Inspection of time-lapse recordings showed that AMPA-induced spine rounding resulted from the cessation of protrusive activity from the surface of the spine head and the apparent collapse of actin into the core of the spine head (Fig. 5a). This effect is most clearly seen in the original video available as supplementary data on the *Nature Neuroscience* web site (http://www.nature.com/neuro/web_specials). The existence of a stable 'core' of actin filaments in the spine head implied by these results is in agreement with previous evidence demonstrating that dendritic spines contain a set of actin filaments that are resistant to actin-depolymerizing drugs[24].

Both the inhibition of motility and the rounding up of spines are strikingly demonstrated in shape factor plots (two examples in Fig. 5c). In both cases, the wide fluctuations in the shape factor that occurred under control conditions were blocked with 2 μM AMPA. At the same time, the shape factor value moved closer to 1, indicating that spine morphology had become more stable and that spine shape had become rounder. When AMPA was subsequently washed out, the shape factor began to fluctuate again, indicating the resumption of spine shape changes (Fig. 5c, right).

Motility inhibition depends on membrane depolarization

AMPA receptor activation causes the influx of Na^+ ions through receptor-associated channels and postsynaptic membrane depolarization. To test whether these events were involved in glutamate receptor-induced inhibition of spine actin dynamics, we examined the effect of AMPA on spine motility when Na^+ was removed from the medium ($n = 4$). All four experiments gave the same result (Fig. 6). Panel 1 shows shape factor data from an initial control recording made to establish the level of spine motility. The medium was then changed to one in which Na^+ ions were removed by replacing NaCl with choline chloride. This had no significant effect on spine motility, which continued unabated (Fig. 6a, panel 2). Next, AMPA (2 μM) was added in the medium lacking Na^+. Under these conditions, AMPA did not inhibit spine motility (Fig. 6a, panel 3). Finally, when Na^+ was re-introduced into the medium together with 2 μM AMPA, spine motility was blocked, and the spines adopted the rounded profile typically found when AMPA receptors are activated (Fig. 6a, panel 4). These results indicate that the effects of AMPA receptor activation on spine motility depend on the influx of Na^+ ions into the spine cytoplasm. Treatment with 500 nM veratridine, which depolarizes the cell membrane by provoking the opening of voltage-dependent Na^+ channels, also blocked spine motility and produced spine rounding ($n = 4$; Fig. 6b). However, blocking voltage-dependent Na^+ channels with 1 μM tetrodotoxin (TTX) did not suppress the inhibitory effect of AMPA on spine motility ($n = 4$, Fig6_TTX.mov, supplementary data on the *Nature Neuroscience* web site). This suggests that Na^+ ions that mediate the effects of AMPA receptor acti-

vation enter the spine cytoplasm through channels associated with the receptor itself.

To determine whether the effects of Na^+ were mediated by depolarizing the cell membrane, we raised extracellular K^+ from 2 to 8 mM ($n = 5$). This caused an immediate blockade of spine motility accompanied by spine rounding (Fig. 7a), which recovered spontaneously as the cell adjusted to the altered external K^+ concentration (Fig. 7b). Increasing external K^+ also inhibited spine motility when AMPA receptors were blocked with 100 µM DNQX ($n = 2$), when NMDA receptors were blocked with 100 µM APV ($n = 2$), or when both blockers were present ($n = 2$). This suggests that membrane depolarization inhibits spine motility downstream of glutamate receptor activation. Altogether these results suggest that depolarization of the postsynaptic membrane is both necessary and sufficient for the inhibitory effect of glutamate receptors on spine actin dynamics.

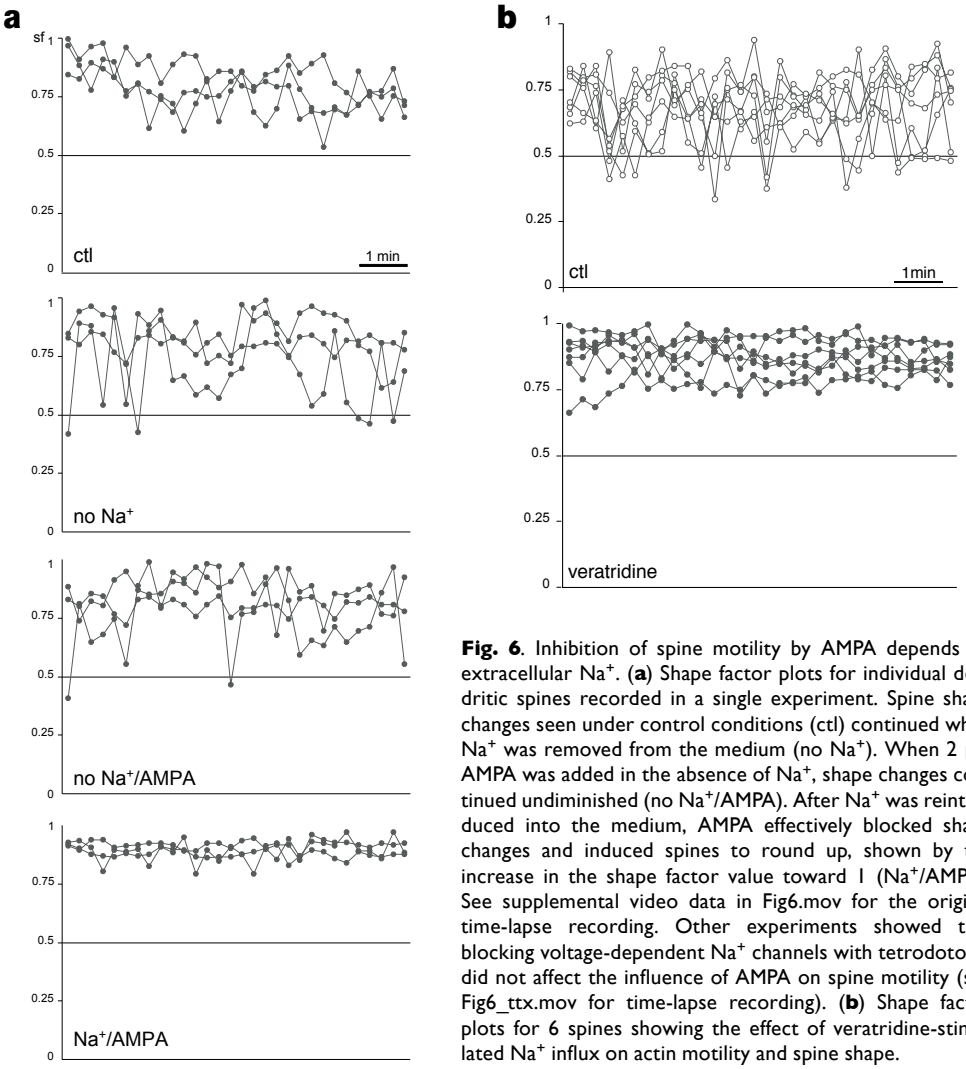

Fig. 6. Inhibition of spine motility by AMPA depends on extracellular Na^+. (**a**) Shape factor plots for individual dendritic spines recorded in a single experiment. Spine shape changes seen under control conditions (ctl) continued when Na^+ was removed from the medium (no Na^+). When 2 µM AMPA was added in the absence of Na^+, shape changes continued undiminished (no Na^+/AMPA). After Na^+ was reintroduced into the medium, AMPA effectively blocked shape changes and induced spines to round up, shown by the increase in the shape factor value toward 1 (Na^+/AMPA). See supplemental video data in Fig6.mov for the original time-lapse recording. Other experiments showed that blocking voltage-dependent Na^+ channels with tetrodotoxin did not affect the influence of AMPA on spine motility (see Fig6_ttx.mov for time-lapse recording). (**b**) Shape factor plots for 6 spines showing the effect of veratridine-stimulated Na^+ influx on actin motility and spine shape.

Calcium mediates the effect of receptor activation

The most likely mechanism by which glutamate receptor-dependent membrane depolarization might influence spine motility is via Ca^{2+}, whose levels can be regulated in individual dendritic spines by synaptic transmission[25–27] and which can influence spine actin through a variety of pathways[21,28,29]. The AMPA receptors of hippocampal pyramidal cells contain GluR2/GluRB subunits, which render their associated ion channels Ca^{2+} impermeable[30,31]. This leaves voltage-activated Ca^{2+} channels (VACs) as the most likely source of glutamate receptor-mediated Ca^{2+} influx into spines, a conclusion consistent with the effectiveness of K^+-induced depolarization in blocking spine actin dynamics. We examined this possibility by testing the ability of different VAC antagonists[32–34] to suppress the inhibitory effect of AMPA on spine motility. The results were quantified by summing pixel densities obtained by subtracting the gray scale values of pixels between frames of time-lapse sequences. Among blockers of high-voltage-activated Ca^{2+} channels, Cd^{2+} (500 µM) suppressed the effects of AMPA by 6 ± 2 % ($n = 4$), nifedipine (20 µM) by 21 ± 10% ($n = 2$) and nimodipine (20 µM) by 24 ± 9% ($n = 3$). We also tested antagonists selective for N-type channels (ω-conotoxin MVIIA at 2 µM, $n = 3$), Q channels (ω-conotoxin MVIIC at 1 µM, $n = 4$) and P/Q channels (ω-agatoxin TK at 50 nM, $n = 1$; 250 nM, $n = 1$; FTX 3.3 at 1 µM, $n = 4$). None of these detectably reduced the inhibition of spine motility by 2 µM AMPA. In contrast, blockers selective for low-voltage-activated Ca^{2+} channels were more effective. Ni^{2+} (10 µM) reduced AMPA inhibition of spine motility by 66 ± 4% ($n = 3$), whereas 10 µM mibefradil produced a 38 ± 9% reduction ($n = 4$). This distinction between the two groups of agents is clearer in the original time-lapse recordings, examples of which are shown in the supplementary video data available on the web site. These results suggest that low- rather than high-voltage-activated Ca^{2+} channels mediate the inhibitory effects of AMPA receptors on postsynaptic actin dynamics.

Discussion

Our experiments show that activation of glutamate receptors can regulate synaptic plasticity by influencing actin dynamics in dendritic spines. The direction of this effect is significant: actin motility was inhibited when glutamate receptors were stimulated, implying that spine morphology is stabilized by signal transmission at glutamatergic synapses. These effects occurred in hippocampal neurons after 3–4 weeks in culture,

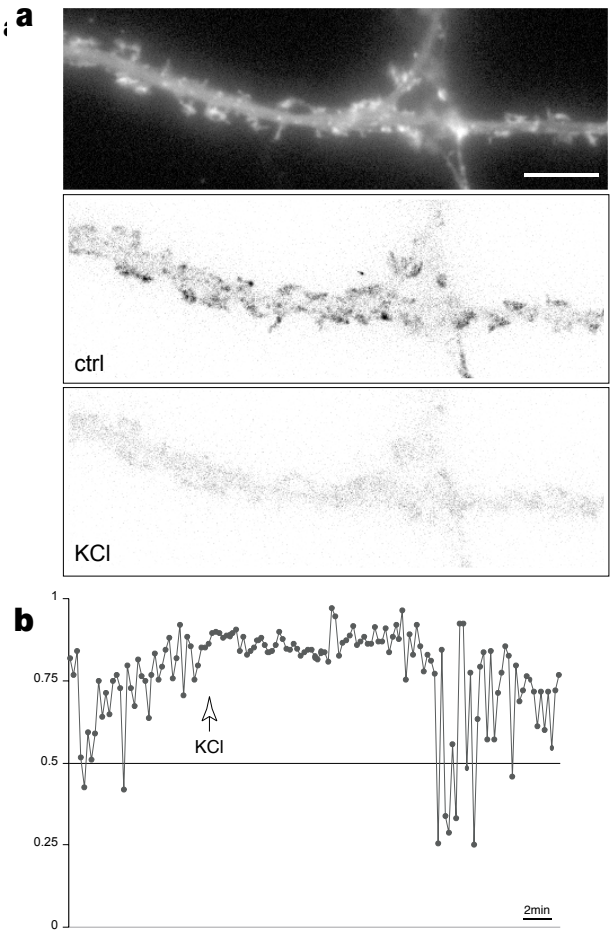

Fig. 7. Membrane depolarization inhibits spine motility. (**a**) A single frame (top panel) and two difference images showing spine motility under control conditions and when K^+ concentration in the medium was raised from 2 mM (control) to 8 mM (KCl). Scale bar, 10 μm. (**b**) Another experiment showing the effect of KCl on spine motility documented using the shape factor plot. At the point indicated by the arrow, medium containing elevated K^+ (8 mM KCl) was introduced into the chamber, producing a transitory inhibition of spine motility accompanied by rounding of the spine profile. This effect reversed spontaneously and fluctuations in spine shape resumed. See Fig7.mov, available as supplementary information, for the original time-lapse recording to Fig. 7b.

when spines are contacted by presynaptic terminals[10] and are part of ultrastructurally intact synaptic contacts[35,36]. The association of this stabilizing influence of glutamate receptors with established synapses is further supported by the persistence of AMPA receptor-regulated spine motility in brain slices after several months in culture, long after spine synapses have been established[37].

Actin dynamics in dendritic spines could be blocked by activation of both AMPA and NMDA receptors. However, AMPA was equally effective when NMDA receptors were blocked by selective antagonists, indicating that AMPA receptors can stabilize spine motility independent of NMDA receptors. In addition to the effects of NMDA receptors shown in previous studies[11,12,16], these results implicate AMPA receptors in the regulation of spine plasticity. The involvement of AMPA receptors is consistent with the failure of AMPA to block spine actin dynamics in the absence of extracellular Na^+, the normal charge carrier for AMPA receptor ion channels, and with the lack of effect of the voltage-dependent Na^+ channel blocker TTX on spine motility. The effects of glutamate receptor activation seem to depend on depolarization of the postsynaptic membrane because they were mimicked by raising extracellular K^+. This treatment was effective when K^+ was increased in the presence of AMPA or NMDA receptor antagonists, indicating that the intermediary depolarization event lies downstream of the receptors. The effects of depolarization in turn seem to be mediated by influx of Ca^{2+} through VACs, known to be activated following membrane depolarization induced by Na^+ influx[38,39]. The comparative effects of selective Ca^{2+} channel antagonists in reducing AMPA-induced inhibition of spine motility favor the involvement of low-voltage T-type channels. However, because we lack specific antagonists for this channel type, a definitive identification is not yet possible.

Distinct roles of AMPA and NMDA receptors

Spine outgrowth from dendrites following NMDA receptor-dependent stimulation protocols[11,12,16] is commonly accompanied by motile activity of the nascent spine[9,11]. In contrast, spines at established synapses require continual activation of AMPA receptors for their maintenance[15]. Taken together with our present data, these results suggest the existence of two distinct modes of morphological plasticity in dendritic spines, one in which the formation of new spines is initiated by LTP-like stimulation operating through NMDA receptors, and a second in which spine morphology at established synapses is stabilized by AMPA receptor activation. This interpretation is consistent with evidence that many newly formed synapses exhibit only NMDA receptor-mediated currents with AMPA receptor-based responses emerging only later by the physical acquisition of AMPA receptors[40–42]. The association of NMDA receptor-dependent LTP with the emergence of new spines[11,12,16] and of AMPA receptor activation with the stabilization of established synapses shown here therefore parallels the physiological maturation of these same glutamatergic synapses.

Glutamatergic transmission leading to an increase of postsynaptic Ca^{2+} levels can thus have opposite effects on spine morphology. A precedent for this seemingly paradoxical situation is shown in growth cones, where Ca^{2+} can promote or suppress actin-based filopodial activity depending on extracellular signals and ancillary circumstances[43–45]. In dendritic spines, the contrasting effects of Ca^{2+} elevation at different stages of synapse formation may be related to the different conditions appropriate for making synapses compared to those required for maintaining them. Forging new connections is appropriate when afferent stimulation is significantly above background, conditions that are represented by the repetitive, high-frequency stimulation needed for NMDA receptor-dependent induction of new spines. Conversely, to be useful for information storage, synapses should be maintained by a mechanism that is relatively stimulus insensitive, conditions that are met by the sensitive AMPA receptor-dependent mechanism for regulating postsynaptic actin dynamics described here.

Methods

Materials. γ-cytoplasmic actin tagged with green fluorescent protein was expressed from a eukaryotic expression plasmid containing a chicken β-actin promoter[10]. Active compounds were obtained from the following sources: amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

(AMPA), D(-)-2-amino-5-phosphonopentanoic acid (APV) , N-methyl-D-aspartate (NMDA), 2-amino-3-[3- (carboxymethoxy)-5-methyl-isoxazol-4-yl]proprionic acid (AMOA), (5R, 10S)-(+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801), 6,7-dinitroquinoxaline-2,3(1h,4h)-dione (DNQX) and kainate from RBI, Natick, Massachusetts; tetrodotoxin (TTX), ω-conotoxin MVIIA, ω-conotoxin MVIIC, ω-agatoxin TK and FTX 3.3 (from Agelenopsis aperta) from Alomone Laboratories, Jerusalem, Israel. Mibefradil was supplied by J. Kemp, Hoffmann–La Roche, Basel, Switzerland.

Cell culture and microscopy. E19 primary hippocampal neurons were transfected with GFP–actin as described[10]. Cultures of dispersed cells[46] were maintained in glia-conditioned, serum-free medium for 3–4 weeks, until the pyramidal cells developed spines of mature appearance. Spines were observed at 37°C in purpose-built observation chambers (Life Imaging Services, Olten, Switzerland) using a GFP-optimized filter set (Chroma Technologies, Brattleboro, Vermont). Illumination intensity was adjusted using neutral-density filters and images taken every 15 s with a 2 s exposure using a MicroMax cooled CCD camera (Princeton Instruments, Trenton, New Jersey) and Metamorph 3.0 imaging software (Universal Imaging Corporation, West Chester, Pennsylvania). Drugs were either added directly to the imaging chamber or introduced by gravity-feed perfusion. Similar results were obtained using glia-conditioned medium or in Tyrode's solution (Life Technologies, Basel, Switzerland) containing glucose (11 mM) and supplemented with 10 mM glycine and 0.2 g/l Na_2HPO_4 adjusted to pH 7.2 to buffer the medium during prolonged imaging without CO_2 gassing. Low Ca^{2+} medium consisted of this supplemented Tyrode's solution containing 0.1 mM instead of 2 mM Ca^{2+} and 2 mM Mg^{2+} instead of 0.5 mM. In some experiments, Mg^{2+} was reduced to 0.1 mM without further changes to the medium. For experiments in Na^+-free conditions, NaCl was replaced by 110 mM choline chloride.

Organotypic cultures from transgenic mice. Transgenic mice were generated using a 5.5 kb *Nde*I/*Eco*R1-fragment containing C-terminal GFP-tagged γ-cytoplasmic actin[10] injected into oocytes of B6CF1 mice. Animals were analyzed by Southern blotting or direct inspection using a Leitz MZ 12 dissection microscope fitted with fluorescence optics. Organotypic slice cultures from hippocampus were prepared according to described procedures[47]. After a minimum of 4 weeks, slice cultures were observed at 37°C under perfusion by gravity feed with artificial cerebrospinal fluid (124 mM NaCl, 2.5 mM KCl, 2.0 mM $MgSO_4$, 1.25 mM KH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 4 mM sucrose, 2.5 mM $CaCl_2$ saturated with 95%O_2/5% CO_2) or Tyrodes buffer (Gibco, Basel, Switzerland). AMPA was added at 1 μM.

Dynamic imaging of actin-GFP in slice cultures were performed using a Leica DM-IRBE inverted microscope fitted with a Yokogawa microlens Nipkow confocal system (Life Science Resources, Cambridge, UK). Summed difference images were derived from time-lapse recordings using MetaMorph image analysis software.

Image analysis. Outlines of spines were created from thresholded images using edge-detection functions of Metamorph 3.0. Changes in spine shape were then assessed using the 'shape factor' routine (sf = 4A/p2) in the Metamorph program, which is calculated from the perimeter p and the area A of the object (spine head). Values close to 0 represent elongated or ruffled shapes, whereas a value of 1 denotes a perfect circle. Data were directly recorded, analyzed and displayed using Microsoft Excel. To visualize drug effects, arithmetical pixel-intensity differences of the same range of consecutive frames before, during and after drug treatment were summed. The resulting images were combined and scaled to display large differences between the original images as dark pixels (gray scale) or using a pseudo-color scale as illustrated.

Note: Supplementary video data can be found on the Nature Neuroscience web site (http://www.nature.com/neuro/web_specials/).

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