Activity-Dependent Synaptic Plasticity in Retinal Ganglion Cells

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At many excitatory synapses, AMPA-type receptors (AMPA) are not statically situated in the membrane, but undergo continuous rounds of endocytosis and exocytosis, referred to as rapid cycling. AMPAR cycling is believed to play a role in certain forms of synaptic plasticity, but the link between cycling and synaptic function is not well understood. We have previously demonstrated that AMPARs cycle in neurons of the inner retina, including amacrine and ganglion cells, and that cycling is inhibited by synaptic activity. Recording from cultured neurons and ON ganglion cells in the flat-mount retina, we now show that rapid cycling is primarily, perhaps exclusively, restricted to AMPARs that contain the GluR2 subunit, and that cycle is confined to extrasynaptic receptors. We also demonstrate a form of plasticity at the ON bipolar cell–ON ganglion cell synapse, whereby synaptic quiescence drives a change in the composition of AMPARs from predominantly GluR2-containing to GluR2-lacking. Finally, we provide evidence linking synaptic receptor composition and cycling, showing that disruption of cycling leads to increases in the number of GluR2-containing receptors in the ON bipolar–ON ganglion cell synapse. We propose that cycling lowers the number of GluR2-containing receptors at the surface and, consequently, within the synapse. After increased levels of synaptic activity, cycling ceases, and all GluR2-containing receptors are free to go to the surface, where they can be delivered to synapses. Our results suggest that by regulating the cycling of AMPARs, ambient light can modulate the composition of synaptic receptors in ON ganglion cells.

Key words: retina; AMPA receptor; trafficking; PICK1; GRIP; activity; ganglion cell; light; SVKI; dynamin; mouse

Introduction

The expression of AMPA-type glutamate receptors (AMPA) is dynamically modulated at the membrane surface through multiple trafficking mechanisms (Malinow and Malenka, 2002). One of the least well understood of these is the rapid, constitutive cycling of AMPARs into and out of the synaptic membrane. Cycling AMPARs appear to be targets of modulation during synaptic plasticity, allowing in part for the rapid delivery or removal of surface receptors. In hippocampal CA1 pyramidal neurons, depletion of the cycling pool of AMPARs prevents the expression of long-term depression (Luscher et al., 1999; Luthi et al., 1999), whereas the insertion of AMPARs from recycling endosomes has been reported to contribute to the expression of long-term potentiation (LTP) (Shi et al., 2001; Park et al., 2004; Plant et al., 2006).

AMPA cycling may modulate synaptic receptor composition as well as numbers during plasticity. Hippocampal LTP has been reported to involve the synaptic insertion of GluR2-lacking, Ca^{2+} -permeable AMPARs, which are rapidly replaced by GluR2-

containing, Ca^{2+} -impermeable AMPARs in the cycling pool (Shi et al., 2001; Plant et al., 2006) (but see Adesnik and Nicoll, 2007). In cerebellar stellate cells, high-frequency stimulation triggers a longer-lasting change in receptor composition, with GluR2-lacking AMPARs being replaced by GluR2-containing receptors (Liu and Cull-Candy, 2000, 2002). Two proteins linked to the endocytosis and cycling of AMPARs, the GluR2/3-binding proteins protein interacting with C kinase 1 (PICK1) and *N*-ethylmaleimide-sensitive factor (NSF), are necessary for the synaptic insertion of GluR2-containing receptors during this form of LTP (Gardner et al., 2005). An increasing number of studies now suggest that activity can modify the composition of synaptic receptors through trafficking of AMPARs (Bellone and Luscher, 2006; Clem and Barth, 2006; Goel et al., 2006).

We have previously found that light deprivation for at least 8 h induces AMPAR cycling as a result of inhibiting light-dependent synaptic activity (Xia et al., 2006). We therefore examined the possibility that cycling might be associated with a form of synaptic plasticity at the ON bipolar cell–ON ganglion cell synapse. In support of this idea, we report here a light-dependent change in the composition of surface AMPARs from primarily GluR2-containing in the light-adapted state to GluR2-lacking in the absence of light. Our data provide evidence that light-stimulated synaptic activity in the ON pathway drives a change in the composition of AMPARs at the ganglion cell synapse, converting the synapse from a Ca^{2+} -permeable to a Ca^{2+} -impermeable state.

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Materials and Methods

Whole-mount retina preparation. Whole-mount retinas were prepared from 3- to 5-week-old mice (C57BL/6; Charles River, Cambridge, MA), a preparation in which we have previously characterized light-mediated changes in AMPAR trafficking (Xia et al., 2006). Mice were anesthetized with halothane (Sigma-Aldrich, St. Louis, MO) and killed by cervical dislocation. A piece of retina $\sim 4 \text{ mm}^2$ was dissected from the area close to the optic nerve, peeled off from the sclera and pigment epithelium in a dish containing Ames media (Sigma-Aldrich) bubbled with 95% O_2 and 5% CO_2 . The retina was transferred to the recording chamber and placed flat, ganglion cell layer up, over a 25 mm^2 cellulose acetate/nitrate membrane filter (Millipore, Bedford, MA) with a hole in the center to allow light to pass through. The filter paper was attached with vacuum grease to the chamber. Retinas were bathed in Ames media at a flow rate of 3–5 ml/min at room temperature. Access to ganglion cells was achieved by severing the Müller cell endfeet with a pipette mounted on a second manipulator. All manipulations were performed under dim red illumination. For light-deprivation experiments, animals were maintained in absolute darkness for 12 h overlapping with their normal presumptive night period before isolation of the retina. Control animals were dark adapted for between 30 and 60 min to allow for the acquisition of light responses. We found previously that this brief period of dark adaptation is not sufficient to induce cycling or changes in AMPAR composition subunit in control animals.

Ganglion cells were viewed through a $40\times$ objective under infrared illumination with a CCD camera attached to a Nikon Eclipse E600FN microscope. Light stimulation was provided by a 20 W halogen lamp focused through the $40\times$ objective via a camera port equipped with a diaphragm to control the diameter of light spots. An interference filter (peak transmittance at 500 nm) and neutral density filters were inserted in the light path to control the intensity and wavelength of light stimulation, and a shutter (Uniblitz; Vincent Associates, Rochester, NY) was used to control the duration of the stimulation. The intensity of the unattenuated light stimulus was measured to be $2.3 \times 10^8 \text{ photons} \cdot \mu\text{m}^{-2} \cdot \text{sec}^{-1}$ at 500 nm. AMPA ($100 \mu\text{M}$) was applied from a second pipette using positive pressure (2–4 psi) for 50–200 ms using a computer-controlled solenoid valve (Picospritzer; General Valve, Fairfield, NJ).

Preparation of cell cultures. Retinas were isolated from newborn (postnatal day 0) rats after cryoanesthesia and were incubated for 45 min at 37°C in DMEM with HEPES (Mediatech, Manassas, VA), supplemented with 6 U/ml papain (Worthington, Freehold, NJ) and 0.2 mg/ml cysteine. Papain was then inactivated by replacing the enzyme solution with complete medium composed of DMEM, 5 mM HEPES, 0.1% Mito⁺ serum extender (Collaborative Research, Bedford, MA), 5% heat-inactivated fetal calf serum, 0.75% penicillin–streptomycin–glutamine mix (Invitrogen, Carlsbad, CA). Osmolarity was adjusted to 300 mOsm by addition of distilled water. Retinas were triturated through a fire-polished Pasteur pipette, plated onto glass coverslips pretreated with poly-D-lysine (0.1 mg/ml), and maintained in complete medium supplemented with 15 mM KCl. At 72 h after plating, cells were treated with the antimitotics 5-fluoro-2-deoxyuridine (0.01 mg/ml) and uridine (0.026 mg/ml) for 24 h. Subsequently, every second day, 50% of the culture medium was exchanged for fresh medium. Cells were used for recording or immunohistochemistry at 8–21 d *in vitro*.

Solutions and recording. Extracellular solution for flat-mount retina was Ames Ringer, bubbled with 95% O_2 /5% CO_2 . The composition of the external solution for cultured cell recording was (in mM) 147 NaCl, 2 KCl, 2 CaCl_2 , 1.5 MgCl_2 , and 10 HEPES, pH 7.4. To isolate AMPA receptor-mediated responses in flat-mount retina, cells were bathed with a mixture of antagonists including $50 \mu\text{M}$ tetrahydrofuran (Sigma-Aldrich) to block GABA_C receptors, $1 \mu\text{M}$ strychnine (Sigma-Aldrich) to block glycine receptors, $100 \mu\text{M}$ picrotoxin (Sigma-Aldrich) to block GABA_A receptors, and $50 \mu\text{M}$ APV (Tocris Cookson, Ellisville, MO), to block NMDA receptors. For cultured cells, only picrotoxin was used. Activity was blocked in cultures by adding CNQX (Tocris Cookson) for 12–24 h before experiments. 1-Naphthylacetylspermine (NASPM; Sigma-Aldrich) and philanthotoxin (PhTX; Sigma-Aldrich) were used to block

Ca^{2+} -permeable AMPARs. We found that both agents cause a similar blockade of AMPAR currents in cultured CNQX-treated neurons: $25 \mu\text{M}$ NASPM reduced the puff response to $46.9 \pm 3.6\%$ of baseline (see Fig. 2A), whereas PhTX ($2 \mu\text{M}$) reduced the response to $42 \pm 5\%$ of baseline (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). PhTX was used in experiments performed in the flat-mount retina, because it was easier to obtain whole-cell recordings with PhTX in the bath.

For both culture and flat-mount retinas, recording pipettes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) by a two-stage vertical puller (Narishige, Tokyo, Japan) and filled with a K^+ gluconate-based solution (125 mM) that also contained 10 mM KCl, 10 mM EGTA, 10 mM HEPES, 4 mM ATP, and 1 mM GTP (pH 7.4 by KOH). In flat-mount retina recordings, the internal solution also contained $50 \mu\text{M}$ QX-314 (Tocris Cookson) to block Na^+ currents. Dynamin inhibitory peptide (DIP) and Pep2-SVKI were both purchased from Tocris Cookson. Spermine was purchased from Sigma-Aldrich. Compounds were dissolved in water, aliquoted, and diluted into the internal solution immediately before experiments. Pipette resistances were typically 3.5–5 M Ω . Unless specified, cells were held at E_{Cl^-} , calculated to be -65 mV . Holding potentials were corrected for a 10 mV junction potential, but series resistance, typically measuring 15–20 M Ω , was not compensated for.

Data analysis. Recordings were obtained with an Axopatch 200B using Axograph acquisition software and digitized with a Digidata 1322A interface (Molecular Devices, Sunnyvale, CA). Analysis was performed using Axograph X and Kaleidagraph (Synergy Software, Reading, PA) software. Rectification ratios were measured in the following way: I – V relations of responses (either light or AMPA puffs) were measured for each cell over a voltage range of -80 to $+60 \text{ mV}$. Responses were first normalized to responses obtained at -60 mV (which were usually more reliable than the responses obtained at -80 mV). A line was then fit to the region of the I – V between -60 and -20 mV , which was generally quite linear. For each cell, a rectification ratio was calculated as the ratio of the measured and extrapolated currents at $+40 \text{ mV}$. This method does not rely on any assumption about reversal potentials, as do two-point measurements of rectification ratio, because this method essentially normalizes the size of the measured current to the predicted linear current, regardless of the specific reversal potential for that cell. Statistical significance was determined using Student's t test. Error bars represent the SEM.

Immunocytochemistry. An antibody recognizing the extracellular N terminus of GluR2 (mouse monoclonal, Millipore) or GluR1 (rabbit polyclonal, Calbiochem, San Diego, CA) subunit was applied to live cells for 30 min at 37°C in complete medium. Antibody was removed and cells were returned to the incubator. Cells were then fixed at indicated times (0–30 min) with 4% paraformaldehyde in PBS for 10 min. After TBS wash, cells were blocked with 2% BSA in TBS and incubated with donkey anti-mouse or rabbit Cy3 ($5 \mu\text{g/ml}$; Jackson ImmunoResearch Laboratories, West Grove, PA) for 45 min. For experiments measuring internalized receptors, cells were permeabilized in TBS/BSA with 0.1% Triton X-100. Cells were then incubated with donkey anti-mouse or -rabbit FITC ($5 \mu\text{g/ml}$). After several washes, slips were mounted in Vectashield (Vector Laboratories, Burlingame, CA).

Clusters of AMPARs were identified using a Nikon $60\times$ objective, standard fluorescence, and Cy3 and FITC filter sets (Omega, Brattleboro, VT). Fluorescent images of microscopic randomly chosen fields containing labeled neurons were acquired using a cooled CCD camera (Hamamatsu, Bridgewater, NJ). Images were analyzed using MetaMorph software (Molecular Devices). Images were background subtracted and thresholded to include only signals at least twofold greater than the diffuse labeling in dendritic shafts. This process highlighted punctate labeling of surface AMPARs in dendrites. Out-of-focus and extended, nondiscrete regions of staining were excluded from the quantitation. Integrated signal intensity values of surface AMPARs were determined for dendrites, normalized to area, and graphed as a ratio of labeling intensity of 0 min controls for each treatment condition. For all immunocytochemical analysis, n refers to the number of experiments

Figure 1. Rapid cycling of GluR2- but not GluR1-containing AMPARs in cultured retinal neurons. **A**, **B**, GluR1- or 2-containing receptors were labeled in live retinal neurons with an antibody against an extracellular N-terminal epitope. After antibody removal, cells were fixed at 0, 15, or 30 min and processed to label remaining surface (Surf.) or internalized (Intern.) AMPARs. **A**, Images of surface-labeled AMPARs demonstrate decreased surface GluR2 but not GluR1 AMPARs over time. **B**, Graphs of surface or internalized AMPAR immunofluorescence over time in control and CNQX-treated neurons. There was a marked decrease in surface and increase in internalized GluR2 (left graph), but not GluR1 (right graph), staining over 30 min when activity was blocked for 12 h with CNQX ($n = 4$ for each condition), indicating enhanced subunit-specific constitutive AMPAR endocytosis. Norm., Normalized.

(with at least 10 cells per experiment). Figure images were digitally processed using Adobe (San Jose, CA) Photoshop.

Results

Synaptic activity specifically regulates the cycling of GluR2-containing AMPARs

We have previously reported that prolonged deprivation of excitatory synaptic input retinal in neurons induces the rapid cycling of AMPARs into and out of the membrane surface. This form of cycling, in which membrane receptors exhibit a turnover rate in the tens of minutes, is observed both in cultured retinal neurons treated overnight with CNQX and in ON ganglion cells from mice or rats maintained in the dark for at least 8 h (Xia et al., 2006). To better characterize this phenomenon and its functional consequences, we first investigated whether AMPAR cycling in retinal neurons is dependent on receptor subunit composition, as in hippocampal neurons, in which GluR2/3, but not GluR1/2, heteromers are thought to undergo cycling (Sheng and Lee, 2001; Malinow and Malenka, 2002). We therefore measured constitutive GluR1 or GluR2 AMPAR endocytosis to estimate the extent of AMPAR cycling for each subunit. Surface GluR1 or GluR2 subunits were antibody labeled in live retinal neurons grown in culture for 8–21 d. After wash-off of the antibody, cells were returned to 37°C for various durations. Over time, if receptors are cycling, antibody-bound surface AMPARs are internalized and replaced by the insertion of unbound receptors. Constitutive endocytosis was measured by immunocytochemically detecting both the loss of antibody bound receptors at the surface and the intracellular accumulation of AMPARs internalized from the surface (see Materials and Methods). As reported previously (Xia et al., 2006), we found that GluR2 surface levels remained constant over time in cells receiving excitatory input (Fig. 1A,B). However, after 12 h of blockade of activity with CNQX, there was a large time-dependent decrease in surface and an increase in

intracellular GluR2 levels observed over 30 min as a result of constitutive AMPAR endocytosis (Fig. 1A,B). The GluR1 receptor, in contrast, showed no evidence of constitutive internalization in either the control or CNQX-treated conditions. These data demonstrate that GluR2- but not GluR1-containing AMPARs undergo rapid constitutive cycling after prolonged reductions in synaptic activity.

Cycling of GluR2-containing AMPARs alters the composition of surface AMPARs

Retinal neurons express significant levels of GluR2-lacking AMPARs (Morkve et al., 2002; Singer and Diamond, 2003), which exhibit the unique property among AMPARs of passing Ca^{2+} (Dingledine et al., 1999). Our observation of cycling of GluR2- but not GluR1-containing AMPARs suggests that Ca^{2+} -impermeable AMPARs are induced to cycle after inactivity, whereas Ca^{2+} -permeable GluR2-lacking AMPARs remain stable in the membrane surface. To test this possibility, we used the subunit-specific antagonist NASPM, which selectively blocks Ca^{2+} -permeable AMPARs (Iino et al., 1996; Washburn and Dingledine, 1996; Toth and McBain, 1998). Responses to pulses of AMPA (100 μM) were recorded in retinal neurons that had been maintained in CNQX for 12 h to induce AMPAR cycling. NASPM (25 μM) reduced current amplitude to $46.9 \pm 3.6\%$ of baseline, indicating that approximately one-half of the surface receptors contain the GluR2 subunit (Fig. 2A). In a second set of experiments, a dynamin inhibiting peptide (DIP) was included in the recording pipette to block constitutive endocytosis. This resulted in a run-up of AMPAR responses to $169 \pm 17\%$ of the initial amplitude within 10 min, as cycling AMPARs were constitutively delivered to and trapped at the membrane surface (Fig. 2B). After the insertion of AMPARs from the cycling pool into the surface, the response was reduced by NASPM to $71 \pm 4\%$ of the run-up amplitude (Fig. 2B,C). This value is in good agreement with the predicted value of 68.7%, which assumes that all of the receptors added to the surface contain the GluR2 subunit. This demonstrates that unlike the overall population of surface AMPARs, the cycling population of receptors consists of mostly or all (Ca^{2+} -impermeable) GluR2-containing receptors.

Activity modulates the subunit composition of surface AMPARs in retinal neurons

As the delivery of mobile AMPARs to the membrane surface by disrupting cycling results in a change in the surface composition of AMPARs, it is possible that activity-induced inhibition of cycling might also alter the surface receptor makeup. If after cessation of cycling, GluR2-containing receptors are maintained intracellularly, the proportion of these receptors at the membrane would be reduced. If the receptors instead become localized to the membrane surface, the levels of GluR2-containing AMPARs at the surface would increase. We therefore measured blockade by NASPM of surface AMPARs in cultured cells exposed to ongoing synaptic activity, a condition in which AMPARs do not rapidly

Figure 2. Inhibition of cycling GluR2 AMPARs results in a net increase of surface GluR2 subunits. **A**, Summary of the effect of bath-applied NASPM on AMPAR-elicited currents in cultured retinal neurons maintained overnight in 50 μ M CNQX to induce AMPAR cycling ($n = 11$). Right, Example of a responses to pulses of AMPA before (black) and during (gray) NASPM application. **B**, Top, Diagram depicts experimental paradigm to study the relative composition of cycling AMPARs. Neurons were maintained overnight in CNQX to induce AMPAR cycling. While recording AMPA-mediated currents, cells were dialyzed with a DIP to inhibit endocytosis of AMPARs and deliver cycling receptors to the surface. Changes in the composition of AMPARs could then be analyzed

cycle in retinal neurons (Xia et al., 2006). In these neurons, currents were reduced to $68.2 \pm 2.6\%$ of baseline, a proportion of block comparable with that observed in cells in which cycling AMPARs had been allowed to accumulate at the surface after endocytosis blockade (Fig. 2C). Our results suggest that rapidly cycling AMPARs become localized to the membrane after the activity-induced cessation of cycling, resulting in a large increase of proportion surface GluR2-containing AMPARs.

Light deprivation alters the surface AMPAR composition in ON ganglion cells

We next investigated whether physiological activity also causes a shift in the surface AMPAR composition in neurons of the flat-mount retina. To avoid possible complications posed by limited access of NASPM into tissue, we took advantage of a second assay for measuring surface GluR2-containing AMPARs levels. In the presence of intracellular spermine, GluR2-lacking receptors exhibit strong inward rectification, whereas receptors that contain the GluR2 subunit exhibit a linear current–voltage (I – V) relationship (Washburn et al., 1997). Because we previously have shown that ~ 8 or more hours of light deprivation can switch AMPA receptors of ON ganglion cells into a cycling mode (Xia et al., 2006), we compared the I – V relationship of AMPA-mediated responses of ON ganglion cells from control and light-deprived animals (maintained for 12 h in the dark). AMPA-elicited currents were measured at potentials from -80 mV to $+60$ mV in cells recorded with 300 μ M spermine in the pipette solution, and the rectification ratio was calculated (see Materials and Methods). In control animals, AMPA-elicited currents showed slight inward rectification (rectification ratio of 0.78 ± 0.09 , where 1.0 represents no rectification) indicating that with normal levels of light-driven activity, most re-

by application of NASPM. Bottom, Summary of effect of DIP and subsequent NASPM addition on total AMPA-elicited responses. Response amplitudes were renormalized to the response before NASPM application (y -axis on the right side; $n = 6$). Right, Representative traces depict currents after dynamin blockade and after NASPM application. **C**, Summary of the fraction of NASPM-mediated blockade as a function of cycling condition. Block of AMPA current by NASPM in the absence of GluR2-containing AMPAR cycling (untreated, $n = 18$) is shown. For comparison, data from **A** and **B** are replotted. Note that NASPM blocks a similar fraction of total AMPAR under conditions when AMPARs are not cycling and when cycling AMPARs have been trapped at the surface.

Figure 3. Light deprivation changes surface AMPAR subunit composition. Top, AMPA responses at +40 mV and -60 mV from ON ganglion cells of control and light-deprived mice. Bottom, *I*-*V* plot of AMPA-mediated currents from ON ganglion cells in retinas isolated from control mice and animals maintained in darkness for >8 h (light-deprived). AMPA-elicited responses were recorded from ON ganglion cells at a series of holding potentials from -80 to +60 mV. Spermine (300 μ M) was included in the recording pipette. Values were normalized to the response at -60 mV, and the mean $\pm$ SE at each voltage was plotted (control: $n = 8$, light-deprived; $n = 7$). Dashed line is the linear fit from -80 mV to -20 mV in control cells.

ceptors contain a GluR2 subunit (Fig. 3). On the other hand, AMPA currents from light-deprived animals exhibited a substantially stronger inward rectification (rectification ratio of 0.40 ± 0.08). A similar rectification ratio was obtained from cultured neurons lacking excitatory synaptic input (0.50 ± 0.09 , $n = 5$; data not shown). The increased inward rectification of the AMPA response of ON ganglion from light-deprived animals suggests that, as in cultured neurons, in the absence of activity there is a reduction in the relative number of GluR2-containing receptors.

Extrasynaptic but not synaptic AMPARs cycle in ON ganglion cells

Our studies of AMPAR composition and trafficking to this point have focused on the total population of surface-expressed AMPARs that are either detected immunocytochemically or activated by exogenous AMPA. We next wished to establish the impact of AMPAR cycling specifically at synapses. We first investigated whether synaptic AMPARs undergo rapid cycling in retinal neurons, as studies of hippocampal neurons are conflicting regarding the mobility of AMPARs at synapses (Lüscher et al., 1999; Luthi et al., 1999; Adesnik et al., 2005). Taking advantage of the fact that the light response is mediated primarily by AMPARs (Chen and Diamond, 2002), we recorded both light- and AMPA-elicited responses in the same ON ganglion cells from animals

that were light deprived overnight. Dialyzing the dynamin inhibitory peptide for 20 min induced a 1.33 ± 0.07 -fold run-up of AMPA responses (Fig. 4A). On the other hand, the light responses from the same cells were stable during this same period of time (1.04 ± 0.09) (Fig. 4A). We conclude that in ON ganglion cells, extrasynaptic AMPARs are selectively mobilized into a cycling mode by decreasing synaptic activity.

We confirmed this observation in cultures of retinal neurons. Cultures were incubated overnight in CNQX to initiate cycling (Xia et al., 2006). Once coverslips were transferred to the recording chamber, they were thoroughly rinsed to remove CNQX. We took advantage of the fact that spontaneous EPSCs (sEPSCs) are mediated by synaptic AMPA receptors (Taylor et al., 1995; Tian et al., 1998) and compared sEPSCs with AMPA-elicited responses. After 20 min of dialysis with the dynamin peptide, the AMPA response increased to 1.63 ± 0.19 -fold of its original amplitude, whereas the mean amplitude of sEPSCs actually ran down (0.78 ± 0.04 of original size) (Fig. 4B,C).

PICK1/GRIP binding inhibits the entry of GluR2-containing receptors into synapses

One explanation for the absence of observed AMPAR cycling at synapses is that GluR2-containing AMPARs constitutively inserted into the extrasynaptic membrane are actively prevented from entering the synapse. We therefore tested whether GluR2-interacting proteins influence the ability of AMPARs to enter the synapse in the intact, flat-mount retina. Pep-SVKI, a peptide that prevents binding of PICK1 and GRIP to GluR2/3 AMPARs (Daw et al., 2000; Xia et al., 2000; Kim et al., 2001), was dialyzed into ON ganglion cells from control animals, in which cycling of GluR2-containing AMPARs is absent. Under these conditions, disruption of the interaction between PICK1/GRIP and GluR2/3 had no significant effect on the amplitude of the AMPA-elicited response (0.92 ± 0.06 of baseline after 20 min) (Fig. 5A, top, B). This is to be expected if, in the absence of rapid cycling, PICK1/GRIP and GluR2-containing AMPARs do not interact. However, after light deprivation, pep-SVKI now increased the amplitude of the AMPA-evoked response by 1.19 ± 0.05 -fold ($p < 0.01$ after 20 min) (Fig. 5A, middle, B), indicating that disruption of PICK1/GRIP-GluR2 binding allowed additional AMPARs to accumulate at the surface.

In these same cells, we tested whether the binding of GluR2 to PICK1 or GRIP contributed to the exclusion of cycling AMPARs from the synapse. In control ON ganglion cells dialyzed with pep-SVKI, light responses ran down slightly (0.86 ± 0.05), likely because of a slight bleaching of the cone photopigment (Fig. 5C). In contrast, in retinas from light-deprived animals, the light response of ON ganglion cells ran up to 1.17 ± 0.03 of the original size ($p < 0.01$ after 20 min) (Fig. 5C). Thus, when the influence of PICK1 and/or GRIP was removed, AMPARs receptors readily entered the synaptic compartment.

The amount of potentiation of the light response was significant but relatively small. However, if our hypothesis is correct that only GluR2-containing AMPARs are held away from the synapse, then pharmacologically removing GluR2-lacking receptors should reveal a greater proportional run-up of synaptic current. In agreement with our hypothesis, we observed that introduction of the pep-SVKI peptide in the presence of PhTX, another blocker of Ca^{2+} -permeable AMPARs (Toth and McBain, 1998; Plant et al., 2006), induced a $51 \pm 12\%$ increase in synaptic currents (Fig. 5A, bottom, C). These results are consis-

tent with the idea that GluR2-containing AMPARs that are free of PICK/GRIP binding can enter the synapse. On the other hand, GluR2-containing AMPARs that are still bound to PICK/GRIP but are forced to accumulate at the surface (e.g., after treatment with DIP) must remain in the extrasynaptic compartment and are excluded from the synapse.

Light deprivation alters the composition of synaptic AMPARs in ON ganglion cells

Several studies suggest that AMPARs diffuse readily between extrasynaptic and synaptic compartments (Tardin et al., 2003; Groc et al., 2004). Therefore, under light-adapted conditions in ON ganglion cells, previously cycling GluR2-containing receptors localized to the membrane surface and not bound to PICK1/GRIP might be available to diffuse into the synaptic compartment. To test this, the *I*–*V* relation of the light response in cells dialyzed with spermine was measured (Fig. 6*A*, bottom). In control animals, the rectification ratio of the light response was 0.63 ± 0.07 , indicating an abundance of GluR2-containing AMPARs. The rectification ratio obtained from light-deprived animals, however, was 0.34 ± 0.06 . Thus, in the absence of activity, when AMPARs containing the GluR2 subunit begin to cycle, there is a large reduction in the expression of GluR2-containing AMPARs at the synapse.

We finally investigated whether the change in synaptic composition can be accounted for simply by the removal of GluR2-containing AMPARs from the membrane surface and synapses during prolonged inactivity. If so, a reduction in the overall amplitude of synaptic responses would be predicted after light deprivation. The average size of light responses from control mice was 470.18 ± 130.99 pA, not significantly different from that of light-deprived mice (474.85 ± 101 pA, $p = 0.99$) (Fig. 6*B*). To avoid complications of presynaptic changes such as bleaching of photoreceptors or differences in the release probability of presynaptic ON bipolar cell terminals, we also measured the amplitude of spontaneous activity (sEPSC) in ON cells from light-deprived and control retinas as above. Once again, no significant difference was observed between the mean amplitude of sEPSCs in ganglion cells from control (20.09 ± 2.89 pA) and light-deprived mice (19.04 ± 2.71 pA, $p = 0.80$) (Fig. 6*B*). Together our data demonstrate that activity can significantly affect the subunit composition, but not the number of synaptic AMPARs in ON ganglion cells, providing evidence of a novel form of synaptic plasticity at these synapses.

Figure 4. Rapid AMPA receptor cycling is confined to the extrasynaptic compartment of retinal neurons. **A**, Mice were light deprived over 8 h to induce rapid cycling of AMPA receptors in ON cells (Xia et al., 2006). Cells were dialyzed with dynamin inhibitory peptide to interrupt endocytosis of AMPA receptors. Left, Dynamin caused a 1.33 ± 0.07 -fold increase in puff responses, but light responses were stable (1.04 ± 0.09) over 20 min ($n = 6$, $p = 0.001$). Right, Representative traces showing the response to a 1 s flash to full field stimulation and a 200 ms puff of AMPA immediately after break in and at 20 min. **B**, Responses to puffs of AMPA and sEPSCs were recorded from retinal neurons after 14 d *in vitro* when all cells had detectable sEPSCs. Cycling of AMPA receptors was induced by incubating cultures with 50 μ M CNQX to block activity. Amplitudes of AMPA-elicited responses and sEPSCs were averaged every 5 min and normalized to initial responses. Left, Summary of the puff response (1.63 ± 0.19 -fold change, $n = 6$) and the mean amplitude of sEPSCs from the same cells (0.78 ± 0.04 -fold change, $n = 6$). Right, Recording of AMPA-elicited responses (top) and sEPSCs (bottom) from the same cell. **C**, Probability (left) and amplitude (right) histograms for sEPSCs from a representative cell obtained during the first and second 5 min period of recording. Both histograms indicate a run-down of the amplitude of spontaneous events. Norm freq, Normalized frequency.

Discussion

Activity triggers a switch of AMPAR subtypes in retinal neurons

Previously we found that AMPAR cycling in retinal neurons is induced by a prolonged deprivation of synaptic activity (Xia et al., 2006). Here we report that it is specifically a GluR2-containing subpopulation of AMPARs that cycle into and out of the extra-

Figure 5. Interaction of GluR2 AMPARs with PICK1/GRIP proteins maintains AMPARs out of the synapse in neurons from light-deprived retina. **A**, Left, Recordings of light- and AMPA-evoked responses from representative ON ganglion cells dialyzed with pep-SVKI to disrupt binding of AMPARs to PICK1/GRIP. Adaptation conditions are as indicated. For the cell depicted in the bottom trace, the media contained 2 μ M PhTX during the entire recording. **B**, Summary of the effect of pep-SVKI on AMPA-elicited responses in light-deprived (1.19 ± 0.05 , $n = 4$) and control (0.92 ± 0.06 , $n = 6$) mice. **C**, Summary of the effect of pep-SVKI on light responses in control ($n = 6$) and light-deprived mice ($n = 6$) and cells from light-deprived mice in which 2 μ M PhTX was included in the extracellular solution to block GluR2-lacking AMPARs ($n = 5$).

synaptic membrane. The increased cycling of GluR2-containing receptors is associated with a decrease in the overall proportion of these receptors at the membrane surface. Under conditions in which AMPARs cycle, GluR2-containing AMPARs bound to PICK1/GRIP are sequestered away from synapses. However, with light-induced blockade of AMPAR cycling, the proportion of synaptic GluR2-containing AMPARs in the ON ganglion cells is significantly increased. Thus, extrasynaptic cycling appears to modulate the composition of synaptic receptors by retaining GluR2-containing receptors away from synapses, instead favoring incorporation of GluR2-lacking receptors. This demonstrates for the first time that the ON bipolar–ON ganglion cell synapse exhibits activity-dependent synaptic plasticity. An emerging theme, supported by our findings and by studies in cerebellum and visual and barrel cortices, is that the composition of AMPARs is not fixed, but rather subject to activity-dependent regulation.

Cycling of AMPARs can account for changes in surface AMPAR composition

The decrease in the proportion of surface GluR2-containing AMPARs in darkness occurs on the same time scale as the induction of extrasynaptic AMPAR cycling, suggesting a possible link between these processes. In fact, when we allowed cycling AMPARs to enter the membrane surface by blocking AMPAR endocytosis, the composition of AMPARs was returned to the same state as found in neurons not exhibiting cycling. This indicates that the cycling population of receptors, unlike most of those at the membrane surface, is made up of GluR2-containing AMPARs. Additionally, it provides a demonstration that the induction of subunit-specific AMPAR cycling alone can dramatically change the surface AMPAR composition by reducing the number of

Figure 6. Light-deprivation alters the composition, but not the amplitude of synaptic AMPAR currents. **A**, Left, Mean amplitude of ON cell light responses from control (470.18 ± 130.00 pA, $n = 8$) and light-deprived (474.85 ± 131 pA, $n = 8$, $p = 0.99$) mice. Right, Mean amplitude of ON cell sEPSCs from control (20.09 ± 2.89 pA, $n = 7$) and light-deprived mice (19.04 ± 2.71 , $n = 6$, $p = 0.8$). **B**, Top, Representative light responses at -60 and $+40$ mV from two cells, one from a control and one from a light-deprived retina. Pipette contained 300 μ M spermine. Bottom, Summary of the normalized I – V relation of the light response obtained from control ($n = 6$) and light-deprived ($n = 7$) retinas. Dashed line is the extrapolated linear fit to the data from -60 to -20 mV.

GluR2-containing AMPARs stabilized at the membrane surface at any one time.

The induction of AMPAR cycling and subsequent AMPAR subunit changes in retinal ganglion cells occurs over a slow time

scale (>8 h). The requirement of at least 8 h of darkness suggests a protein synthesis-dependent process. It is not clear what factors may be regulated to induce the increased cycling of AMPARs. A number of synaptic regulatory proteins have been found to be actively regulated by changes in activity in neurons (Ehlers, 2003). One possible candidate is suggested by recent studies that demonstrate that the expression of Arc/Arg3.1 is regulated in response to chronic changes in activity in hippocampal neurons. Elevated Arc/Arg3.1 expression, in turn, was found to enhance the endocytosis of AMPARs, particularly GluR2/3 receptors (Rial Verde et al., 2006) (but see Shepherd et al., 2006) and thereby reduce surface receptor levels. However, whether Arc/Arg3.1 only enhances endocytosis rates or whether it may also increase overall cycling rates, as seen in our study, has not been reported. Additionally, in the hippocampus, inactivity leads to decreased Arc/Arg3.1 expression and reduced AMPAR endocytosis (Shepherd et al., 2006), opposite to what we observe in the retina. Other likely candidates that might regulate the cycling of AMPARs are the GluR2-binding proteins PICK1 and GRIP themselves, whose function we find to be elevated in light-deprived ON ganglion cells (Xia et al., 2006). Additional study will be necessary to establish the exact molecular mechanism by which cycling is induced.

AMPA cycle outside of retinal synapses

In the present study, we found that interrupting endocytosis did not affect either synaptic light responses or sEPSCs, but did elicit a significant run-up of the AMPA-elicited response on the same cells. Thus, AMPAR cycling appears to be localized to nonsynaptic sites in the retina. Other studies also support the possibility that the trafficking of synaptic and extrasynaptic AMPARs has different properties. Although original reports in the hippocampus suggested that synaptic receptors cycle (Lüscher et al., 1999), there is recent evidence that AMPARs may turn over rapidly only in the extrasynaptic domain. A study using a photochemical method of labeling AMPARs found that the insertion of AMPARs into synapses occurs over hours, probably through slow metabolic trafficking (Adesnik et al., 2005). In contrast, AMPARs were found to be delivered to extrasynaptic somatic sites in minutes, implying rapid constitutive cycling. Our data suggest that in the light-deprived retina, AMPARs similarly only show this high rate of turnover in the extrasynaptic region. Evidence from cerebellar stellate cells also suggests differential trafficking of AMPARs to the synaptic and extrasynaptic regions: AMPARs at extrasynaptic sites have a distinct receptor composition from those at synapses (Liu and Cull-Candy, 2000).

It is clear that the extrasynaptic AMPAR pool is not completely isolated from synapses. Optical monitoring of the movement of AMPARs in cultured hippocampal neurons suggested that extrasynaptic AMPARs have a high mobility of lateral diffusion (Borgdorff and Choquet, 2002; Tardin et al., 2003). The mobility can be bidirectionally regulated by spontaneous neuronal activity (Tardin et al., 2003; Groc et al., 2004). In the hippocampus, the endocytosis of extrasynaptic AMPARs precedes the removal of synaptic AMPARs (Ashby et al., 2004), raising the possibility that extrasynaptic trafficking could affect synaptic AMPAR expression through lateral movement. In cerebellar stellate cells, AMPARs in extrasynaptic receptors appear to be mobilized to enter synapses during synaptic plasticity, resulting in a change in AMPAR composition. Therefore, changes in the trafficking of AMPARs in the extrasynaptic region appear to be able to modulate a pool of receptors that are then available to alter the number or makeup of synaptic AMPARs through lateral move-

ments. Our data suggest that lateral exchange of AMPARs is likely to be quite slow in neurons of light-deprived retina, because we saw no evidence of diffusion into synapses over a 30 min period of recording.

Differences in both the cycling properties and AMPAR makeup of extrasynaptic and synaptic pools imply that molecular mechanisms exist that can segregate the expression of AMPARs at these sites. One molecule that seems to modulate this process is NSF. Studies have demonstrated that binding of NSF to the GluR2-containing receptor is necessary to enable it to enter the synapse (Gardner et al., 2005). As NSF functions to dissociate PICK1 from the GluR2 subunit, the removal of PICK1 may be the key step in allowing the GluR2-containing receptors to enter the synapse. Our data in the retina support this possibility. Although delivery of cycling receptors to the membrane surface using the dynamin inhibitory peptide only allows receptors to enter the extrasynaptic membrane, introduction of pep-SVKI caused an increase in both synaptic and extrasynaptic receptors (Fig. 6), possibly by dissociating GluR2 from PICK1. This, together with our other findings, suggests a model in which activity in the retina drives a cessation of AMPAR cycling and decreases the interaction of PICK1 with GluR2-containing AMPARs, thereby allowing the receptors to move into the membrane surfaces and synapses (see supplemental Fig. 2, available at www.jneurosci.org as supplemental material). It remains possible that GluR2-containing AMPARs are regulated at synaptic and extrasynaptic sites by exchange with different intracellular pools of receptors. However, the similarities in the composition of AMPARs in the synaptic and extrasynaptic regions under light and dark conditions suggest a shared mechanism of regulation.

Physiological implications

Our experiments demonstrate that light modulates AMPARs of retinal ON ganglion cells, providing a significant shift of subunit composition, a plasticity newly identified in the retina. As the final stop of visual signaling processing in retina, ganglion cells summate important spatial, temporal, and contrast information and convey it to the higher levels of the visual system. Thus, a subtype switch of AMPARs may significantly shape visual signaling. We observe a change in the subunit content of synaptic AMPARs in the ON pathway with darkness, suggesting it may be a component of retinal light adaptation. Because we do not observe any detectable change in the number of AMPARs in retinal synapses with the induction of cycling, it appears that any effects on synaptic transmission through these neurons resulting from a switch in AMPAR composition are likely to be linked to changes in receptor signaling properties, not synaptic strength. In darkness, the Ca^{2+} -permeable GluR2-lacking receptors replace the Ca^{2+} -impermeable GluR2-containing receptor, potentially allowing for an increased Ca^{2+} influx into ganglion cells. Calcium could increase sensitivity in dim light through activation of downstream targets. One such calcium-activated target is the transcription factor CREB, whose activity has been found previously to enhance neuronal excitability in neurons of the nucleus accumbens after cocaine administration (Dong et al., 2006). On a more rapid timescale, CaMKII activation has been found to increase the backpropagation and decrease the threshold for sodium channel action potentials in the dendrites of hippocampal neurons (Tsubokawa et al., 2000; Xu et al., 2005). Similarly, enhanced calcium-dependent excitability in ganglion cells might also contribute to increased visual sensitivity, in addition to that mediated by light adaptation in photoreceptors and the interneuron network.

References

- Adesnik H, Nicoll RA (2007) Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation. *J Neurosci* 27:4598–4602.
- Adesnik H, Nicoll RA, England PM (2005) Photoinactivation of native AMPA receptors reveals their real-time trafficking. *Neuron* 48:977–985.
- Ashby MC, De La Rue SA, Ralph GS, Uney J, Collingridge GL, Henley JM (2004) Removal of AMPA receptors (AMPA) from synapses is preceded by transient endocytosis of extrasynaptic AMPARs. *J Neurosci* 24:5172–5176.
- Bellone C, Luscher C (2006) Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nat Neurosci* 9:636–641.
- Borgdorff AJ, Choquet D (2002) Regulation of AMPA receptor lateral movements. *Nature* 417:649–653.
- Chen S, Diamond JS (2002) Synaptically released glutamate activates extrasynaptic NMDA receptors on cells in the ganglion cell layer of rat retina. *J Neurosci* 22:2165–2173.
- Clem RL, Barth A (2006) Pathway-specific trafficking of native AMPARs by in vivo experience. *Neuron* 49:663–670.
- Daw MI, Chittajallu R, Bortolotto ZA, Dev KK, Duprat F, Henley JM, Collingridge GL, Isaac JT (2000) PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. *Neuron* 28:873–886.
- Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. *Pharmacol Rev* 51:7–61.
- Dong Y, Green T, Saal D, Marie H, Neve R, Nestler EJ, Malenka RC (2006) CREB modulates excitability of nucleus accumbens neurons. *Nat Neurosci* 9:475–477.
- Ehlers MD (2003) Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. *Nat Neurosci* 6:231–242.
- Gardner SM, Takamiya K, Xia J, Suh JG, Johnson R, Yu S, Hugarir RL (2005) Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron* 45:903–915.
- Goel A, Jiang B, Xu LW, Song L, Kirkwood A, Lee HK (2006) Cross-modal regulation of synaptic AMPA receptors in primary sensory cortices by visual experience. *Nat Neurosci* 9:1001–1003.
- Groc L, Heine M, Cognet L, Brickley K, Stephenson FA, Lounis B, Choquet D (2004) Differential activity-dependent regulation of the lateral mobilities of AMPA and NMDA receptors. *Nat Neurosci* 7:695–696.
- Iino M, Koike M, Isa T, Ozawa S (1996) Voltage-dependent blockage of Ca^{2+} -permeable AMPA receptors by joro spider toxin in cultured rat hippocampal neurons. *J Physiol (Lond)* 496:431–437.
- Kim CH, Chung HJ, Lee HK, Hugarir RL (2001) Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. *Proc Natl Acad Sci USA* 98:11725–11730.
- Liu SJ, Cull-Candy SG (2002) Activity-dependent change in AMPA receptor properties in cerebellar stellate cells. *J Neurosci* 22:3881–3889.
- Liu SQ, Cull-Candy SG (2000) Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* 405:454–458.
- Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA (1999) Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron* 24:649–658.
- Luthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, Henley JM, Isaac JT, Collingridge GL (1999) Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. *Neuron* 24:389–399.
- Malinow R, Malenka RC (2002) AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* 25:103–126.
- Morkve SH, Veruki ML, Hartveit E (2002) Functional characteristics of non-NMDA-type ionotropic glutamate receptor channels in AII amacrine cells in rat retina. *J Physiol (Lond)* 542:147–165.
- Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD (2004) Recycling endosomes supply AMPA receptors for LTP. *Science* 305:1972–1975.
- Plant K, Pelkey KA, Bortolotto ZA, Morita D, Terashima A, McBain CJ, Collingridge GL, Isaac JT (2006) Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. *Nat Neurosci* 9:602–604.
- Rial Verde EM, Lee-Obourne J, Worley PF, Malinow R, Cline HT (2006) Increased expression of the immediate-early gene *arc/arg3.1* reduces AMPA receptor-mediated synaptic transmission. *Neuron* 52:461–474.
- Sheng M, Lee SH (2001) AMPA receptor trafficking and the control of synaptic transmission. *Cell* 105:825–828.
- Shepherd JD, Rumbaugh G, Wu J, Chowdhury S, Plath N, Kuhl D, Hugarir RL, Worley PF (2006) *Arc/Arg3.1* mediates homeostatic synaptic scaling of AMPA receptors. *Neuron* 52:475–484.
- Shi S, Hayashi Y, Esteban JA, Malinow R (2001) Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* 105:331–343.
- Singer JH, Diamond JS (2003) Sustained Ca^{2+} entry elicits transient postsynaptic currents at a retinal ribbon synapse. *J Neurosci* 23:10923–10933.
- Tardin C, Cognet L, Bats C, Lounis B, Choquet D (2003) Direct imaging of lateral movements of AMPA receptors inside synapses. *EMBO J* 22:4656–4665.
- Taylor WR, Chen E, Copenhagen DR (1995) Characterization of spontaneous excitatory synaptic currents in salamander retinal ganglion cells. *J Physiol (Lond)* 486:207–221.
- Tian N, Hwang TN, Copenhagen DR (1998) Analysis of excitatory and inhibitory spontaneous synaptic activity in mouse retinal ganglion cells. *J Neurophysiol* 80:1327–1340.
- Toth K, McBain CJ (1998) Afferent-specific innervation of two distinct AMPA receptor subtypes on single hippocampal interneurons. *Nat Neurosci* 1:572–578.
- Tsubokawa H, Offermans S, Simon M, Kano M (2000) Calcium-dependent persistent facilitation of spike backpropagation in the CA1 pyramidal neurons. *J Neurosci* 20:4878–4884.
- Washburn MS, Dingledine R (1996) Block of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. *J Pharmacol Exp Ther* 278:669–678.
- Washburn MS, Numberger M, Zhang S, Dingledine R (1997) Differential dependence on GluR2 expression of three characteristic features of AMPA receptors. *J Neurosci* 17:9393–9406.
- Xia J, Chung HJ, Wihler C, Hugarir RL, Linden DJ (2000) Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. *Neuron* 28:499–510.
- Xia Y, Carroll RC, Nawy S (2006) State-dependent AMPA receptor trafficking in the mammalian retina. *J Neurosci* 26:5028–5036.
- Xu J, Kang N, Jiang L, Nedergaard M, Kang, J, (2005) Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *J Neurosci* 25:1750–1760.