

## SYMPOSIUM REVIEW

# Redefining the classification of AMPA-selective ionotropic glutamate receptors

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**Abstract** AMPA-type ionotropic glutamate receptors (iGluRs) represent the major excitatory neurotransmitter receptor in the developing and adult vertebrate CNS. They are crucial for the normal hardwiring of glutamatergic circuits but also fine tune synaptic strength by cycling into and out of synapses during periods of sustained patterned activity or altered homeostasis. AMPARs are grouped into two functionally distinct tetrameric assemblies based on the inclusion or exclusion of the GluA2 receptor subunit. GluA2-containing receptors are thought to be the most abundant AMPAR in the CNS, typified by their small unitary events,  $\text{Ca}^{2+}$  impermeability and insensitivity to polyamine block. In contrast, GluA2-lacking AMPARs exhibit large unitary conductance, marked divalent permeability and nano- to micromolar polyamine affinity. Here, I review evidence for the existence of a third class of AMPAR which, though similarly  $\text{Ca}^{2+}$  permeable, is characterized by its near-insensitivity to internal and external channel block by polyamines. This novel class of AMPAR is most notably found at multivesicular release synapses found in the avian auditory brainstem and mammalian retina. Curiously, these synapses lack NMDA-type iGluRs, which are conventionally associated with controlling AMPAR insertion. The lack of NMDARs suggests that a different set of rules may govern AMPAR cycling at these synapses. AMPARs with similar functional profiles are also found on some glial cells suggesting they may have a more widespread distribution in the mammalian CNS. I conclude by noting that modest changes to the ion-permeation pathway might be sufficient to retain divalent permeability whilst eliminating polyamine sensitivity. Consequently, this emerging AMPAR subclass need not be assembled from novel subunits, yet to be cloned, but could simply occur by varying the stoichiometry of existing proteins.

(Received 2 October 2011; accepted after revision 15 November 2011; first published online 21 November 2011)

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**Derek Bowie** (McGill University, Montreal, Canada) earned his PhD from the University of London with Trevor G. Smart in 1992 after completing an undergraduate degree in Biochemistry and Pharmacology at Strathclyde University in Scotland. He then spent the next 2 years as an Eli-Lilly postdoctoral fellow in Paul Feltz's lab at the Université Louis Pasteur in Strasbourg, France with a short stay in the lab of Jean-Marc Fritschy at the University of Zurich, Switzerland before moving to the National Institutes of Health in the USA in 1994 where he worked with Mark L. Mayer on the biophysics of glutamate receptor ion-channel block. After taking up a faculty position at Emory University in 1998, he turned his attention to gating mechanisms, identifying ion-dependent gating of kainate-type glutamate receptors, before moving to McGill in 2002. He is currently an Associate Professor in the Department of Pharmacology and Therapeutics and recipient of the Canada Research Chair award in Receptor Pharmacology. His research theme has since broadened to also look at the role of glutamate and GABA receptors in neurodevelopment.



This report was presented at the 26th GEPROM Symposium on *Ligand-gated ion channels: from genes to behaviour*, which took place at the University of Montreal, Canada on 14–15 June 2011.

## Introduction

The vast majority of excitatory synaptic transmission in the CNS is mediated by ionotropic glutamate receptors (iGluRs) which are grouped into kainate-, AMPA- and NMDA-selective receptor subclasses (Hollmann *et al.* 1989; Dingledine *et al.* 1999). In addition to their traditional roles in synaptic transmission and plasticity (Kullmann & Siegelbaum, 1995; Isaac *et al.* 2007; Kerchner & Nicoll, 2008), iGluRs have also been implicated in numerous disease states associated with postnatal development (e.g. autism, schizophrenia) (Lisman *et al.* 2008; Krueger & Bear, 2011), cerebral insult (e.g. stroke, epilepsy) (Hamilton & Attwell, 2010; Lai *et al.* 2011) and ageing disorders (e.g. Alzheimer's disease, Parkinsonism) (Bowie, 2008; Proctor *et al.* 2011). In the past, much emphasis has been placed on the role of NMDARs in CNS development, plasticity and pathology since their significant permeability to external  $\text{Ca}^{2+}$  triggers maturation and re-modelling of neuronal circuits as well as cell death (Constantine-Paton, 1990; Dingledine *et al.* 1999; van Zundert *et al.* 2004; Traynelis *et al.* 2010). However, not all neurons express NMDARs; consequently elevations in cytosolic  $\text{Ca}^{2+}$  mediated by glutamatergic signalling must occur via other divalent-permeable iGluRs. In this context, a prime candidate for this role is  $\text{Ca}^{2+}$ -permeable AMPARs (CP-AMPARs) which fulfill various functions in the adult brain such as long-term plasticity at neuronal (Cull-Candy *et al.* 2006; Liu & Zukin, 2007) and neuron–glial (Ge *et al.* 2006) synapses as well as being implicated in ischaemia (Liu *et al.* 2004), epilepsy (Krestel *et al.* 2004) and glioblastoma proliferation (Ishiuchi *et al.* 2002).

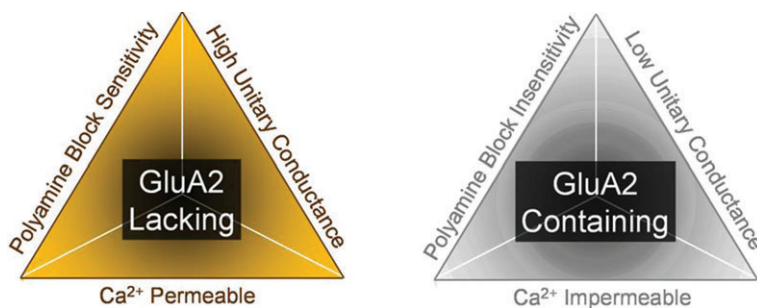
Although AMPARs form tetramers using four possible subunits (i.e. GluA1 to 4), it is assembly with the GluA2 subunit (formerly GluR2 or GluR-B) that is thought to regulate  $\text{Ca}^{2+}$  permeability (Jonas & Burnashev, 1995), external and internal polyamine block (Bowie *et al.* 1999), the pore's unitary conductance (Dingledine *et al.* 1999) as well as subunit assembly and stoichiometry (Greger *et al.* 2007). Thus, GluA2-lacking AMPARs are  $\text{Ca}^{2+}$  permeable, blocked by polyamines and exhibit large single-channel currents (Bowie *et al.* 1999; Dingledine *et al.* 1999) (Fig. 1). In contrast, GluA2-containing receptors are

divalent impermeable, lack polyamine sensitivity and exhibit unitary events that are small in amplitude (Fig. 1). Just how many GluA2 subunits are required to achieve this is a matter of debate as the GluA2 subunit copy number per receptor has been argued to be variable (Washburn *et al.* 1997) or fixed (Mansour *et al.* 2001). To complicate matters, discrimination amongst  $\text{Ca}^{2+}$ -permeable and -impermeable isoforms has often not been employed to directly assess divalent transport rates (Wollmuth & Sakmann, 1998) or by comparing relative ion permeability (Geiger *et al.* 1995). Instead, most studies have relied on using an indirect approach using externally applied polyamines, such as philanthotoxin (PhTX), or channel blockers, such as IEM-1460, as pharmacological markers of CP-AMPARs (Toth & McBain, 1998; Laezza *et al.* 1999; Liu & Cull-Candy, 2000; Thiagarajan *et al.* 2005; Ge *et al.* 2006; Plant *et al.* 2006). This approach is based on the observation that recombinant AMPAR heteromers are rendered  $\text{Ca}^{2+}$  impermeable with fewer GluR2 subunits than required to eliminate polyamine block (Washburn *et al.* 1997). Consequently, AMPARs sensitive to external polyamine block are considered, from a conservative standpoint, to be  $\text{Ca}^{2+}$  permeable whereas the absence of block identifies the  $\text{Ca}^{2+}$ -impermeable isoform.

In this review, I consider the evidence for a third class of AMPAR-type iGluR. This receptor is distinguished by its divalent permeability and weak sensitivity to block by internal and external polyamines. As discussed below, evidence supporting the existence of another functionally distinct AMPAR has been in the literature for more than two decades but its significance has been largely overlooked. With the value of hindsight, however, it is now possible to piece together a common pattern of receptor behaviour amongst these otherwise disparate group of studies that point to the existence of a third type of AMPAR subfamily.

## The emergence of AMPARs with distinct functional and molecular properties

In the 1990s, it became clear that AMPARs have several distinct response characteristics based on a comparison of native and recombinant receptors. In one



**Figure 1. The inter-relationship between the GluA2 subunit, ion permeation and channel block** Left, GluA2-lacking AMPARs are  $\text{Ca}^{2+}$  permeable and strongly blocked by both external and cytoplasmic polyamines. The latter mechanism accounts for the occurrence of inward rectification. Right, in contrast, GluA2-containing AMPARs are thought to be divalent impermeable and insensitive to both external and internal polyamine block with small single channel conductance.

of the first of these investigations, Iino and colleagues reported that most AMPARs expressed by cultured hippocampal neurons were weakly permeable to external  $\text{Ca}^{2+}$  ( $P_{\text{Ca}/\text{Cs}} < 0.18$ ) with near-linear current–voltage ( $I$ – $V$ ) relationships (Iino *et al.* 1990). However, in some cases, AMPAR-mediated membrane currents exhibited appreciable divalent permeability ( $P_{\text{Ca}/\text{Cs}} = 2.3$ ) and profound inward rectification (Iino *et al.* 1990). The occurrence of an inwardly rectifying,  $\text{Ca}^{2+}$ -permeable AMPAR surprised most investigators at the time since it had been assumed that the prime source of  $\text{Ca}^{2+}$  entry into glutamatergic synapses was via the NMDA-type iGluR (Mayer & Westbrook, 1987; Debski *et al.* 1990). However, the occurrence of inward-rectification was consistent with even earlier, and often overlooked, reports describing AMPARs expressed in *Xenopus laevis* oocytes from bovine retina (Parker *et al.* 1985) or rat striatum/cerebellum (Randle *et al.* 1988) polyA<sup>+</sup> mRNA. The molecular identity of the two AMPAR response types was revealed soon afterwards with the cloning and characterization of the GluA1, GluA3 and GluA4 receptor subunits that formed homomeric receptors with inward rectification behaviour which became linear when co-expressed with the GluA2 subunit (Boulter *et al.* 1990; Verdoorn *et al.* 1991).

Over the next years, it was then appreciated that AMPARs with different  $I$ – $V$  characteristics and divalent permeability were preferentially expressed by certain cell types in the CNS. In general terms, excitatory principal cells of neuronal circuits were thought to possess AMPARs with low divalent permeability and linear  $I$ – $V$  characteristics whereas AMPARs of inhibitory interneurons and glial cells exhibited appreciable divalent permeability and inwardly rectifying behaviour (McBain & Dingledine, 1993; Geiger *et al.* 1995; Tempia *et al.* 1996). The situation was nuanced further by data later on showing that different synapses on the same neuron may express different AMPAR types based on their afferent input (Toth & McBain, 1998). It was also understood that recombinant AMPARs could be distinguished by their sensitivity to externally applied polyamine toxins such as argiotoxin and joro spider toxin (Blaschke *et al.* 1993; Herlitze *et al.* 1993; Bowie *et al.* 1999).

The molecular basis of inward rectification of AMPARs vexed the glutamate receptor field for several additional years until it was shown that similar behaviour in  $\text{K}^{+}$  channels was due to voltage-dependent block by cytoplasmic polyamines (Ficker *et al.* 1994; Lopatin *et al.* 1994; Fakler *et al.* 1995). With this information at hand, several labs went on to show that inward rectification of AMPARs (and kainate-type iGluRs) was also caused by channel block via endogenous polyamines, namely spermine, spermidine and putrescine (Bowie & Mayer, 1995; Donevan & Rogawski, 1995; Isa *et al.* 1995; Kamboj *et al.* 1995; Koh *et al.* 1995). In retrospect, it is surprising that the link between inward rectification and

endogenous polyamines was not appreciated sooner given the sensitivity of CP-AMPARs to external polyamine toxin block (Bowie *et al.* 1999). The final piece of the puzzle was the observation that the inclusion of GluA2 into AMPAR heteromers significantly reduced the amplitude of single channel conductance (Swanson *et al.* 1997).

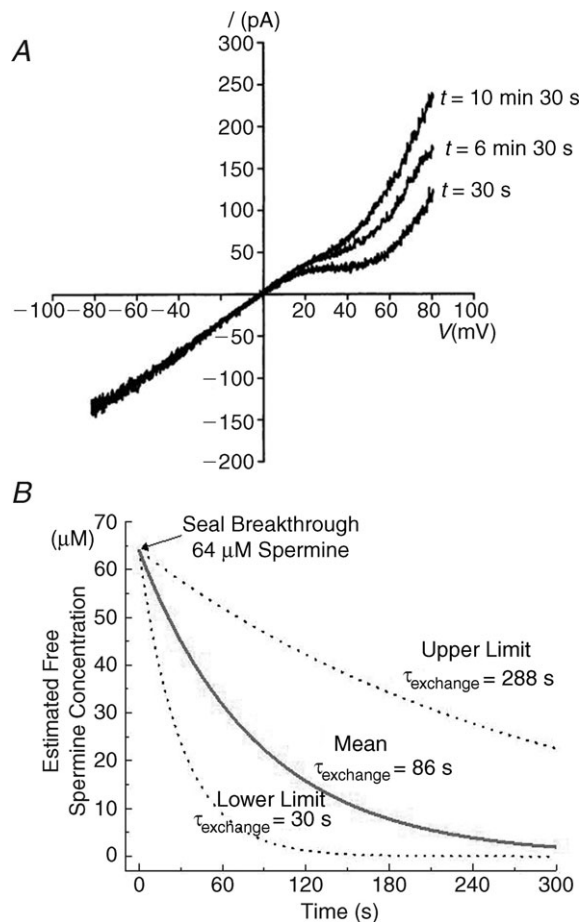
When taken together, the tacit agreement amongst investigators was the existence of two molecularly distinct AMPARs (i.e. GluR2-containing or -lacking) distinguished by their functional properties (i.e.  $I$ – $V$  and divalent permeability), pharmacology (i.e. polyamine toxin block) and unitary conductance (Cull-Candy *et al.* 2006; Isaac *et al.* 2007) (Fig. 1). However, not all data reported in the literature during this time conformed to this dual identity for AMPARs. As explained below, the most contentious issue was, and still is, whether the occurrence of polyamine block really goes hand-in-hand with divalent permeability.

### Ion flow through $\text{Ca}^{2+}$ -permeable AMPA receptors is not always inwardly rectifying

Over the last decades, several studies have emerged reporting receptor behaviour that is inconsistent with the current classification of AMPARs. Two of the studies were published early on as information from cloning studies was beginning to appear and in advance of the work establishing a link between cytoplasmic polyamine block and inward rectification (Gilbertson *et al.* 1991; Otis *et al.* 1995). By studying acutely isolated bipolar cells from the salamander retina, Gilbertson and colleagues reported the existence of calcium-permeable AMPARs ( $P_{\text{Ca}/\text{Na}} \approx 3.2$ ) at about the same time that Iino and colleagues (1990) reported similar findings on cultured hippocampal neurons. An important distinction of these studies is that visual inspection of recordings from retinal cells reveals that the AMPAR  $I$ – $V$  relationships were linear and not inwardly rectifying as reported on hippocampal cells.

From our present understanding of polyamine block, it might be argued that washout of endogenous polyamines occurred during the experiments performed by Gilbertson and colleagues. In keeping with this, washout in other cell types, such as cerebellar granule cells, shows a fairly rapid depletion of channel block over the first 10 min of whole-cell recording (Kamboj *et al.* 1995) (Fig. 2A). However, this is not always the case. For example, in HEK 293 cells, depletion of cytoplasmic polyamines is slower (Bowie & Mayer, 1995) (Fig. 2B), which is more in keeping with the biochemical estimates of unbound polyamines in bovine lymphocytes and rat liver cells (Watanabe *et al.* 1991). Furthermore, most endogenous polyamines appear to be bound within cells to protein and DNA at millimolar concentrations (Watanabe *et al.* 1991) representing a substantial reserve that, in principle, would

be able to maintain the freely diffusible pool at the upper micromolar levels (e.g.  $\sim 60 \mu\text{M}$  for spermine) needed to bring about channel block (Bowie & Mayer, 1995). When fairly rapid washout has been achieved in HEK 293 cells, it required as much as 20 mM ATP in the patch pipette solution for effective chelation to occur (Bowie & Mayer,



**Figure 2. Washout of cytoplasmic polyamines is variable**

**A**, a series of current–voltage relationships of AMPAR responses recorded at different time points of a whole-cell recording from a single cerebellar granule cell. Following seal breakthrough, the initial inward rectification observed at positive membrane potentials dissipated over the next 10 min reflecting washout of endogenous polyamines. Adapted from Kamboj *et al.* (1995) with permission. **B**, in HEK 293 cells expressing GluK2 kainate receptor channels, the lower and upper limits of polyamine washout were estimated using internal solutions that contained 20 mM ATP, to chelate endogenous polyamines, and solutions which lacked it, respectively. By fitting whole-cell membrane currents with a modified Woodhull model of channel block, it was possible to calculate the free concentration of spermine (ordinate axis) from the degree of rectification at different time points (abscissa) after breakthrough (see Bowie & Mayer, 1995 for details). Upper and lower estimates of polyamine exchange time constants ( $\tau_{\text{exchange}}$ ) were calculated to be 30 s and 288 s, respectively. Note that the mean  $\tau_{\text{exchange}}$  is weighted in that 5 out of 8 recordings were obtained with internal solutions containing 20 mM ATP. Adapted from Bowie & Mayer (1995) with permission from Elsevier.

1995) (Fig. 2B). Consequently, the range of washout rates reported in different studies most probably suggests that free endogenous polyamine levels vary between different cell types (Aizenman *et al.* 2002).

Given this, it is not possible to predict the rate at which endogenous polyamines would have been cleared with the 1 mM ATP used by Gilbertson and colleagues in their study. The conundrum, of course, still remains, as Iino and colleagues undoubtedly faced exactly the same problem, but yet observed inward rectification of AMPAR responses in cultured hippocampal neurons (Iino *et al.* 1990). However, if the study by Gilbertson and colleagues uncovered novel properties of extrasynaptic AMPARs, it raises the question of whether their findings could be extended to synaptic receptors.

With this in mind, data described by Otis and colleagues (1995) advances this idea by placing calcium-permeable, polyamine-insensitive AMPARs at glutamatergic synapses. The authors used an ion-substitution approach to study the divalent permeability of AMPARs expressed at synapses of the avian nucleus magnocellularis (nMAG), a homologue of the mammalian ventral cochlear nucleus (Otis *et al.* 1995). The advantage of this preparation is that divalent permeability can be estimated from reversal potentials with some accuracy due to the large and robust membrane currents elicited by AMPARs at nMAG synapses. Like Gilbertson *et al.*, the authors also noted high divalent permeability of AMPARs ( $P_{\text{Ca}/\text{Cs}} = 3.3$ ) in the absence of any noticeable inward rectification (Fig. 3A). Here again, it could be argued that polyamine washout accounts for the absence of inward rectification. However, the Trussell lab was able to address this issue in a later study (Lawrence & Trussell, 2000). Although the authors subsequently observed channel block with high concentrations (1 mM) of spermine, they did not observe inward rectification with much lower and more physiologically relevant concentrations of spermine (i.e.  $60 \mu\text{M}$ , L. Trussell, personal communication). Since then, similar observations have been made from studies of AII amacrine cells in the rat retina where synaptic AMPARs show outward rectification (Fig. 3B and C) (Veruki *et al.* 2003) and appreciable divalent permeability ( $P_{\text{Ca}/\text{Na}} \approx 2$ ) (Morkve *et al.* 2002). Together, these studies suggest that, in some cases, CP-AMPA receptors are insensitive or weakly sensitive to block by cytoplasmic polyamines. As discussed below, this conclusion raises the possibility that some CP-AMPA receptors are similarly insensitive to block by externally applied polyamines.

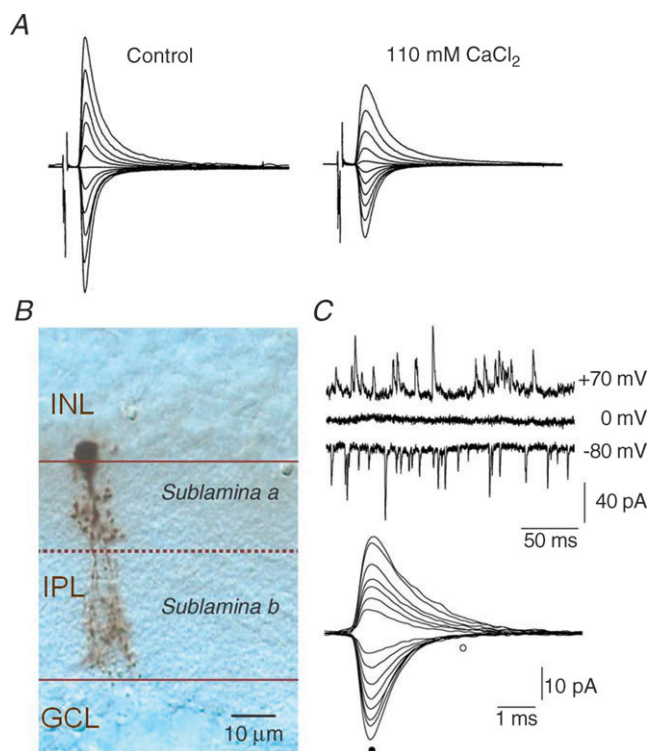
### External polyamine block can be uncoupled from divalent permeability

Two studies from the laboratory of Richard Miller were the first to document inconsistencies in the proposed

relationship between AMPAR divalent permeability and block by externally applied polyamine toxins (Meucci *et al.* 1996; Meucci & Miller, 1998). In the earlier study, Meucci and colleagues (1996) combined  $\text{Ca}^{2+}$  imaging and electrophysiology to compare divalent permeability and toxin sensitivity of native AMPARs expressed by O-2A progenitor cells. Since the inclusion of serum to O-2A cell cultures promotes differentiation into oligodendrocytes or type II astrocytes (Louis *et al.* 1992; Holzwarth *et al.* 1994) as well as GluA2 expression (Holzwarth *et al.* 1994; Patneau *et al.* 1994; Puchalski *et al.* 1994), the authors examined how this process may affect CP-AMPA pharmacology. In undifferentiated cells,  $\text{Ca}^{2+}$  elevations and membrane currents elicited by AMPAR stimulation were blocked by joro spider toxin (JsTX) and argiotoxin 636, consistent with the conventional properties of GluA2-lacking CP-AMPARs expressed by Bergmann glia, for example (Burnashev *et al.*

1992). However, following the addition of serum, neither toxin was able to block the  $\text{Ca}^{2+}$  elevations that arise from ion flow through activated receptors (Meucci *et al.* 1996), suggesting that polyamine block and divalent permeability can be uncoupled in some AMPARs. Consistent with this, Meucci and Miller (1998) were able to recapitulate the same observation, but this time studying the functional properties of recombinant AMPARs assembled from GluA1 and GluA2 subunits. To explain their findings, the authors argued that, in some cases, AMPARs containing the GluA2 subunit are permeable to external divalent ions, suggesting that differentiating progenitor cells express a mosaic of GluA2-containing AMPARs, some of which are  $\text{Ca}^{2+}$  permeable (Meucci & Miller, 1998).

A similar switch in external polyamine sensitivity of CP-AMPARs has also been observed in the neurons of the developing rat retina (Diamond, 2007; Osswald *et al.* 2007). In this case, divalent permeability of AMPARs was assessed using the cobalt ( $\text{Co}^{2+}$ ) staining technique (Pruss *et al.* 1991) in combination with electrophysiological recordings from acutely isolated retinal slices (Fig. 4). During the first two postnatal weeks after birth, the authors observed  $\text{Co}^{2+}$  labelling primarily of horizontal and AII amacrine cell interneurons that was blocked by the polyamine toxin philanthotoxin (PhTX) (Osswald *et al.* 2007). However, around postnatal day 14 when eye opening occurs,  $\text{Co}^{2+}$  staining elicited by AMPAR stimulation was unexpectedly insensitive to a range of known channel blockers (i.e. PhTX, JsTX and IEM-1460) even at concentrations well above those needed to block conventional CP-AMPARs (Fig. 4B). In support of this, Osswald and colleagues observed a similar insensitivity to PhTX in electrophysiological recordings of synaptic AMPARs (Fig. 4A). The most compelling aspect of this work is that sensitivity of AMPARs to external polyamine block was recovered by rearing developing rat pups in the dark, suggesting that light entering the eye during development triggers the surface expression of polyamine-insensitive CP-AMPARs (Osswald *et al.* 2007; Diamond, 2011). Intriguingly, more recent work reveals that overexpression of polyamine-insensitive CP-AMPARs in the adult may be a key factor in the neurotoxic effects of excessive levels of extracellular L-glutamate in models of retinal injury (Lebrun-Julien *et al.* 2009). Here, the surface expression of these novel CP-AMPARs was driven by the release of tumour necrosis factor  $\alpha$  ( $\text{TNF}\alpha$ ) through a  $\text{NF-}\kappa\text{B}$ -dependent pathway in Müller glia (Lebrun-Julien *et al.* 2009). Since glial-derived  $\text{TNF}\alpha$  is more notably associated with regulating CP-AMPARs during homeostatic synaptic scaling (Beattie *et al.* 2002; Stellwagen *et al.* 2005), the observations by Lebrun-Julien and colleagues suggest that an imbalance in AMPAR synaptic scaling may lie at the heart of some chronic retinal diseases such as glaucoma (Almasieh *et al.* 2011).



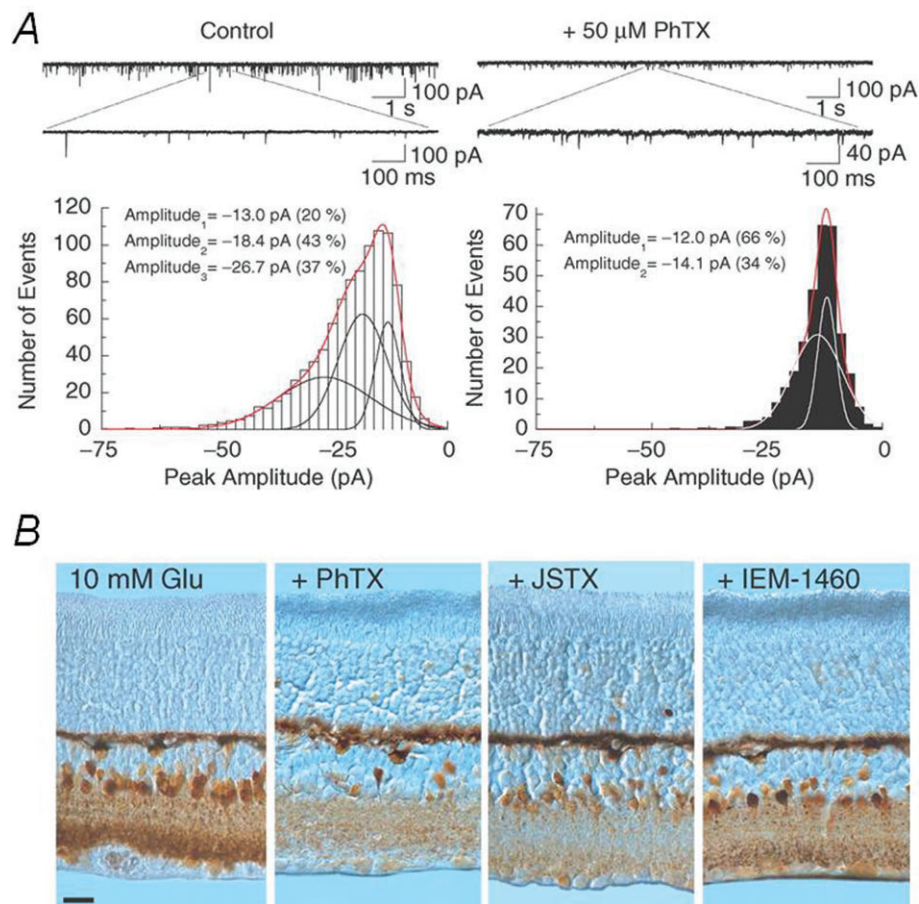
**Figure 3. Not all  $\text{Ca}^{2+}$ -permeable AMPA receptors exhibit inward rectification**

A, nerve-evoked synaptic AMPAR currents elicited by chick nMAG neurons are outwardly rectifying in solutions rich in  $\text{Na}^+$  (left panel) but yet are highly permeable to external  $\text{Ca}^{2+}$  ions (right panel). Traces are adapted from Otis *et al.* (1995) with permission. B and C, likewise, All amacrine cells (B) of the rodent retina express  $\text{Ca}^{2+}$ -permeable AMPARs that also show no sign of inward rectification at positive membrane potentials. Photomicrograph in B is adapted from Osswald *et al.* (2007) and traces in C are adapted from Veruki *et al.* (2003) with permission.

### How might polyamine insensitivity arise?

There are two general ways in which AMPARs may lose polyamine sensitivity whilst retaining the ability to transport calcium ions. The first of these is to *directly* change the physical properties of the ion conductance pathway, for example, by changing the cross-sectional diameter of its narrowest region. The unedited (Q-form) GluA1 AMPAR pore has been estimated to be about 0.78 nm (or 7.8 Å) at its narrowest point (Burnashev *et al.* 1996), which is remarkably similar to the 0.75 nm cross-sectional diameter of the polyamine toxin PhTX (Bähring *et al.* 1997). Since the dimensions of a single calcium ion in the absence of its hydration shells is about 2 Å, it would only take modest changes in the architecture of the pore region to impact polyamine block whilst allowing divalent permeability. On that note, the cross-sectional diameter of the pore of heteromeric AMPARs composed of GluA1 and GluA2 has been estimated to be about 0.70–0.74 nm (Burnashev *et al.* 1996), which may be sufficient to prevent polyamines from entering the pore to bring about

block. This possibility is in keeping with the conclusion of the Meucci and Miller (1998) study that, in some cases, GluA2-containing AMPARs may be divalent permeable but insensitive to block by externally applied polyamines. The authors did not examine whether internal polyamine block was similarly affected. In fact, it is not possible to predict the outcome since biophysical work on homomeric kainate receptors suggests that internal and external polyamine block is non-equivalent (Bähring *et al.* 1997), a finding which is in line with more recent analysis of TARP-bound AMPARs (Jackson *et al.* 2011). Therefore, it remains to be established how GluA2 might affect internal polyamine block. For GluA2-containing AMPARs, the transport of large cations through a narrower pore region would be further complicated by unfavourable changes to the pore's electrostatic environment. The edited GluA2 subunit contains a positively charged arginine residue at the Q/R site which is thought to form the apex of the pore loop region of both AMPA and kainate receptors (Panchenko *et al.* 1999; Sobolevsky *et al.* 2009). In contrast,



**Figure 4. Eternal polyamine block can be uncoupled from AMPA receptor divalent ion permeability**  
 A, typical membrane currents elicited by synaptic AMPARs expressed by All amacrine cells after eye-opening are resistant to supramaximal concentrations of the polyamine toxin, PhTX (right panel). B, likewise,  $\text{Co}^{2+}$  staining elicited by AMPA receptor stimulation reveals that the divalent permeability of these receptors is not blocked by a range of known channel blockers. Data adapted from Osswald *et al.* (2007) with permission.

unedited GluA1, 3 and 4 subunits contain a neutral glutamine residue at the Q/R site. Given this, it would be possible to exert substantial changes to the pore's ability to transport large cations by changing the number of GluA2 subunits per AMPAR tetramer, a process that might be constrained by the emerging set of rules that govern AMPAR heteromerization (Rossmann *et al.* 2011).

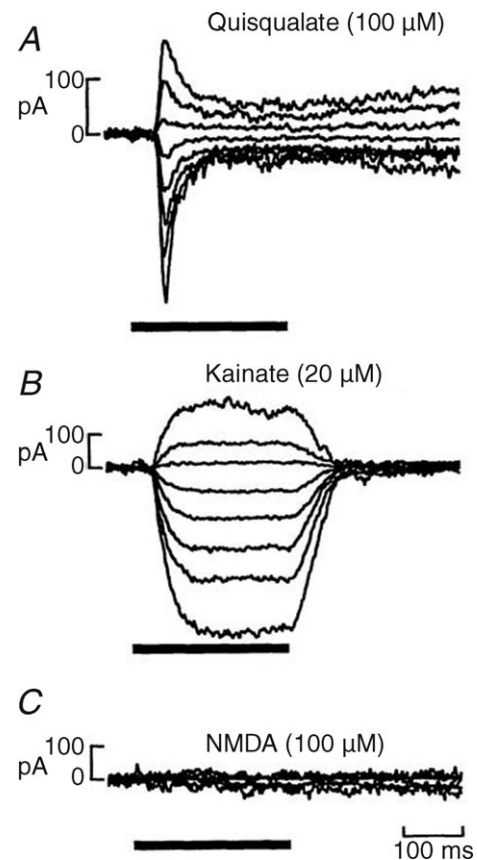
The other way in which to retain divalent permeability but lose polyamine sensitivity is through an *indirect* or allosteric mechanism that affects the pore's permeation properties. This possibility is particularly relevant for AMPARs given the numerous subconductance states that are accessed during activation (Swanson *et al.* 1997; Rosenmund *et al.* 1998; Banke *et al.* 2000; Smith *et al.* 2000). By definition, each conductance state must have different ion permeation properties but, as yet, it is not known if this extends to differences in their divalent permeability or polyamine block. If it were the case, it may be possible to differentially affect channel block characteristics whilst maintaining similar divalent permeability by simply varying the relative contribution of each subconductance state. For example, this could be achieved by AMPAR phosphorylation (by CAM kinase II) (Derkach *et al.* 1999; Kristensen *et al.* 2011) or by varying the concentration of the neurotransmitter, L-glutamate (Smith & Howe, 2000). Another possibility is that allosteric modulation of the pore is achieved through auxiliary protein binding, for example, by the effect of the transmembrane AMPAR regulatory protein, stargazin (Soto *et al.* 2007). In fact, co-expression of GluA1 or A4 AMPARs with stargazin lowers the blocking ability of cytoplasmic spermine whilst having no apparent effect on divalent permeability (Soto *et al.* 2007).

So could stargazin's effect on AMPARs account for the polyamine-insensitive CP-AMPARs described here? Probably not and for several reasons. First, stargazin does not eliminate inward rectification of AMPARs but only reduces it (Soto *et al.* 2007) making it distinct from the near-linear *I-V* properties of native AMPAR responses recorded from nMAG neurons (Otis *et al.* 1995), retinal AII amacrine or bipolar cells (Gilbertson *et al.* 1991; Veruki *et al.* 2003) and O2-A progenitor cells (Meucci *et al.* 1996). Second, stargazin's impact on polyamine block would be most effective on neurons that completely lack the GluA2 subunit which, to my understanding, has only ever been described for Bergmann glia (Burnashev *et al.* 1992). Almost all other neurons in the mammalian CNS are thought to express the GluA2 subunit to some degree which would diminish the role of stargazin (Lambolez *et al.* 1992; Jonas *et al.* 1994; Geiger *et al.* 1995). This issue is, of course, more complicated by the fact that individual synapses of the same neuron may express a mosaic of AMPARs with varying proportions of GluA2 (Toth & McBain, 1998). Third and finally, stargazin does not seem to affect external polyamine block (Jackson *et al.*

2011), making it distinct from the properties of AMPARs expressed by AII amacrine cells and O2-A progenitors (Meucci *et al.* 1996; Osswald *et al.* 2007). It is possible that the molecular basis of polyamine insensitivity in CP-AMPARs is multifactorial in nature, which may allow TARPs or other auxiliary proteins, such as CKAMP44 (von *et al.* 2010) and cornichon proteins (Schwenk *et al.* 2009; Kato *et al.* 2010), to play a role but the details of such a potentially complex mechanism will have to wait for further investigation.

### Diminished role for NMDA receptors at polyamine-insensitive Ca<sup>2+</sup>-permeable AMPA receptor synapses

Given the predominant role assigned to NMDARs at glutamatergic synapses (Mayer & Westbrook, 1987; Dingledine *et al.* 1999), it is curious that



**Figure 5. Cells expressing polyamine-insensitive, Ca<sup>2+</sup>-permeable AMPA receptors often lack NMDA receptors** Whole-cell membrane currents recorded from salamander retinal bipolar cells following a 250 ms application of the AMPAR agonists, quisqualate and kainate (A and B) and the NMDA receptor agonist, NMDA (C) at a range of membrane potentials (−95 mV to +45 mV). Note that although bipolar cells respond robustly to the AMPAR agonists there is no effect of NMDA. Data adapted from Gilbertson *et al.* (1991) and reprinted with permission from AAAS.

**Table 1. Summary table highlighting neuronal and glial cell studies where Ca<sup>2+</sup>-permeable AMPA receptor expression has been found in the apparent absence of synaptic NMDA receptors**

Brain region	Cell type	Synaptic NMDA receptor	I-V relationship	Calcium permeability	External polyamine block	References
Cerebellum	Stellate cell (rat)	<sup>a1</sup> Absent (rat) <sup>a2</sup> Present (mouse)	<sup>b1</sup> Linear <sup>b2</sup> Rectifying	<sup>c</sup> Variable ( $P_{Ca/Na}$ , 0.25 or 1.64)	<sup>d1</sup> Strong (synaptic) <sup>d2</sup> Weak (extrasynaptic)	<sup>a1</sup> (Clark & Cull-Candy, 2002) <sup>a2</sup> (Jackson & Nicoll, 2011) <sup>b1</sup> (Liu & Cull-Candy, 2000, 2002; Jackson & Nicoll, 2011) <sup>b2</sup> (Liu & Cull-Candy, 2000, 2002; Jackson & Nicoll, 2011) <sup>c</sup> (Liu & Cull-Candy, 2002) see also (Goldberg <i>et al.</i> 2003; Soler-Llavina & Sabatini, 2006; Jackson & Nicoll, 2011) <sup>d1</sup> (Liu & Cull-Candy, 2000) <sup>d2</sup> (Liu & Cull-Candy, 2000)
Amygdala	Basolateral interneuron (rat)	<sup>a</sup> Absent	<sup>b</sup> Rectifying	Not determined	<sup>d</sup> Strong	<sup>a</sup> (Mahanty & Sah, 1998) <sup>b</sup> (Mahanty & Sah, 1998; Polepalli <i>et al.</i> 2010) <sup>d</sup> (Mahanty & Sah, 1998; Polepalli <i>et al.</i> 2010)
Retina	Bipolar cell (salamander & cat)	<sup>a</sup> Absent	<sup>b</sup> Linear	<sup>c</sup> Variable ( $P_{Ca/Na}$ , 0.5–3.2)	Not determined	<sup>a</sup> (Gilbertson <i>et al.</i> 1991) <sup>b</sup> (Gilbertson <i>et al.</i> 1991) <sup>c</sup> (Gilbertson <i>et al.</i> 1991; Sasaki & Kaneko, 1996; Pourcho <i>et al.</i> 2002)
	Horizontal cell (rat & perch)	<sup>a</sup> Absent	<sup>b</sup> Linear	<sup>c</sup> Yes (Co <sup>2+</sup> staining)	<sup>d</sup> Weak	<sup>a</sup> (Osswald <i>et al.</i> 2007) <sup>b</sup> (Zhou <i>et al.</i> 1993) <sup>c</sup> (Pourcho <i>et al.</i> 2002; Osswald <i>et al.</i> 2007) <sup>d</sup> (Osswald <i>et al.</i> 2007)
	Aii amacrine cell (rat)	<sup>a</sup> Absent	<sup>b1</sup> Linear <sup>b2</sup> Rectifying	<sup>c</sup> Yes ( $P_{Ca/Na}$ , 1.9–2.1)	<sup>d</sup> Weak	<sup>a</sup> (Osswald <i>et al.</i> 2007) <sup>b1</sup> (Morkve <i>et al.</i> 2002; Veruki <i>et al.</i> 2003) <sup>b2</sup> (Singer & Diamond, 2003) <sup>c</sup> (Morkve <i>et al.</i> 2002; Osswald <i>et al.</i> 2007) <sup>d</sup> (Osswald <i>et al.</i> 2007)
Brainstem	Nucleus magnocellularis neuron (avian)	<sup>a</sup> Absent	<sup>b</sup> Linear	<sup>c</sup> Yes ( $P_{Ca/X}$ , 3.3–5.0)	<sup>d</sup> Weak	<sup>a</sup> (Otis <i>et al.</i> 1995) <sup>b</sup> (Otis <i>et al.</i> 1995) <sup>c</sup> (Otis <i>et al.</i> 1995) <sup>d</sup> (Lawrence & Trussell, 2000)
Glia	Progenitor cells (rat)	<sup>a</sup> Absent	<sup>b1</sup> Linear <sup>b2</sup> Rectifying	<sup>c</sup> Yes (Ca <sup>2+</sup> imaging)	<sup>d1</sup> Weak <sup>d2</sup> Moderate	<sup>a</sup> (Patneau <i>et al.</i> 1994) <sup>b1</sup> (Meucci <i>et al.</i> 1996) <sup>b2</sup> (Zonouzi <i>et al.</i> 2011) <sup>c</sup> (Meucci <i>et al.</i> 1996) <sup>d1</sup> (Meucci <i>et al.</i> 1996) <sup>d2</sup> (Zonouzi <i>et al.</i> 2011)



all of the neurons expressing polyamine-insensitive  $\text{Ca}^{2+}$ -permeable AMPARs seem to entirely lack or have a diminished presence of synaptic NMDARs. The absence of NMDARs was first noted by Gilbertson and colleagues (1991) in their study of cultured retinal bipolar cells (Fig. 5) but has since been an unexpected finding from electrophysiological recordings of a range of neurons and glial cells from diverse brain regions (Table 1). Interestingly, the absence of synaptic NMDARs need not imply that the cell in question fails to express them at all. For example, although retinal AII amacrine cells seem to lack synaptic NMDARs (Osswald *et al.* 2007), they do respond (if somewhat weakly) to bath-applied agonist (Hartveit & Veruki, 1997) suggesting that they can be found in extrasynaptic locales. In the cerebellum, extrasynaptic NMDARs expressed by stellate cells are activated during synaptic transmission only when the level of presynaptic activity leads to neurotransmitter spillover (Clark & Cull-Candy, 2002; Sun & June, 2007). A similar mechanism may be envisaged at the rod bipolar–AII amacrine cell synapse, which is noted for its sustained transmitter release (Snellman *et al.* 2009). Consequently, it remains to be investigated whether NMDARs participate in AII cell signalling if only in a diminished capacity.

The presence of synaptic NMDARs is a more contentious issue at nMAG neurons with some investigators favouring a diminished role during development (Lu & Trussell, 2007) whereas others maintaining they have a functional significance in regulating neuronal firing properties (Pliss *et al.* 2009). Signalling by NMDARs is a critical factor in controlling AMPAR recruitment into developing synapses (Hall & Ghosh, 2008) as well as following periods of sustained patterned activity that gives rise to plasticity mechanisms such as long-term potentiation (Nicoll, 2003). With this in mind, it remains to be established which mechanism(s) controls the recruitment of AMPARs into, for example, developing AII amacrine cell synapses. As mentioned above, it is possible that glial cell signalling may be important by releasing  $\text{TNF}\alpha$ , but other mechanisms may be possible too (e.g. Zonouzi *et al.* 2011). The role of NMDARs is more nuanced in oligodendrocyte progenitor cells since recent work shows that they are not essential for proliferation and maturation, unlike neurons (Debski *et al.* 1990), but hold a key role in negatively regulating the surface expression of CP-AMPARs (De Biase *et al.* 2011). Clearly, a better understanding of the complex interplay between NMDAR signalling and CP-AMPAR surface expression will only be achieved with further study.

## Conclusion

When viewed together, the data from these various studies support the possibility of a third class of AMPA-selective

ionotropic glutamate receptor, that is calcium permeable with little or no sensitivity to block by polyamines. As yet, it is still not clear how polyamine block can be eliminated whilst retaining divalent permeability, though it may only require modest changes to the ion-permeation pathway. Whether this is achieved by varying the proportion of GluA2 subunits per tetramer, as has been proposed (Meucci & Miller, 1998), awaits future investigation. More conservative language is warranted, however, when attributing specific properties to AMPAR responses. For example, the absence of external or internal polyamine block need not imply that the AMPAR under study is  $\text{Ca}^{2+}$  impermeable or, for that matter, GluA2 containing. These characteristics of the AMPAR should only be assigned following empirical determination. It is only then will we be able to appreciate how widespread this putative class of AMPAR-type iGluR is expressed throughout the mammalian CNS.

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### Acknowledgements

This work was supported by operating grants from the Canadian Institutes of Health Research as well as a personal award from the

Canada Research Chair program in Receptor Pharmacology. I wish to thank Drs Larry Trussell (Vollum Institute, OR), June Liu (Louisiana State University), Richard Miller (Northwestern, IL), Olimpia Meucci (Drexel University, PA), Pankaj Sah (University of Queensland) and Stuart Cull-Candy (University College London) for their many thoughtful email exchanges. I am also indebted to members of my own lab, especially Dr David MacLean and Mark Arousseau, for stimulating debate on this topic and comments on the manuscript.