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Running to Stand Still

Ionotropic Receptor Dynamics at Central and Peripheral Synapses

Emile G. Bruneau and Mohammed Akaaboune*

Department of Molecular, Cellular and Developmental Biology and Program in Neuroscience, University of Michigan, Ann Arbor, MI

Abstract

For synapses to form and function, neurotransmitter receptors must be recruited to a location on the postsynaptic cell in direct apposition to presynaptic neurotransmitter release. However, once receptors are inserted into the postsynaptic membrane, they are not fixed in place but are continually exchanged between synaptic and extrasynaptic regions, and they cycle between the surface and intracellular compartments. This article highlights and compares the current knowledge about the dynamics of acetylcholine receptors at the vertebrate peripheral neuromuscular junction and AMPA, *N*-methyl-D-aspartate, and γ-aminobutyric acid receptors in central synapses.

Index Entries: Acetylcholine receptor; AMPA receptor; NMDA receptor; GABA receptor; neuromuscular junction; cycling; dynamics; recycling; turnover; half-life.

Introduction

Much of our understanding about rapid changes in receptor trafficking in response to activity has come from studies on the AMPA receptor (AMPAR) *(1–3)*. Using primary neuronal cultures and organotypic hippocampal slice preparations, a large body of work has found that AMPARs are inserted and removed rapidly from the synapse in response to different stimulation protocols. These changes in receptor density are involved in the processes of long-term potentiation (LTP) and long-term depression (LTD), which have long been believed to be cellular mechanisms of learning and memory *(4,5)*. Interestingly, many of the characteristic behaviors of AMPARs have been found to apply to *N*-methyl-D-aspartate (NMDA) and γ-aminobutyric acid (GABA)

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*Author to whom correspondence and reprint requests should be addressed. E-mail: makaabou@umich.edu.

receptors in the central nervous system (CNS) and acetylcholine receptors (AChRs) in peripheral neuromuscular junctions (NMJs).

Just as findings in the CNS have directed investigation in peripheral synapses, work in the NMJ has provided great insights into the processes of presynaptic vesicular release, synaptic pruning, receptor clustering, and surface mobility that have proved to be applicable to synapses in the CNS. This is largely because of the accessibility of the NMJ and the availability of the highly specific AChR ligand bungarotoxin, which has allowed the analysis of in vivo synapse formation and maintenance in living animals. More recently, much work has been done to understand the trafficking of AChRs at the NMJ. The NMJ is dramatically larger than a central synapse and has the extremely high synaptic receptor density of more than 10,000 receptors/μm^2. Building on research started more than three decades ago regarding the dynamics of AChRs, more recent studies using conjugated bungarotoxin, fluorescent labeling, and time-lapse imaging in the living mouse have provided great insights into synaptic receptor dynamics in vivo.

Although each receptor has its own complement of accessory proteins and regulatory elements to enable receptor trafficking and activity-dependent changes in trafficking to occur, some interesting commonalities between excitatory and inhibitory synapses of the CNS and cholinergic synapses in the peripheral nervous system have emerged, and these are explored in this article as well.

Regulation of AMPA Receptor Dynamics at Excitatory Central Synapses

The AMPAR is a tetrameric ionotropic glutamate receptor composed of Glu1-4 subunits *(6)*, which are expressed as GluR1/2 and GluR2/3 heteromers in most brain regions *(7)*. Numerous studies in recent years have used both direct and indirect methods to measure basal dynamics (removal, insertion, recycling, and lateral mobility) of AMPARs (Fig. 1A). These studies have shown that the half-life of AMPARs varies widely, depending on cell type and technique used (optical, biochemical, or electrophysiological), ranging from minutes to days. For example, $[S]^{35}$ uptake experiments have estimated the turnover of AMPARs in cerebellar granule cells to be 18 to 23 h for GluR2/3 and GluR4 subunits *(8)* and 48 h for the GluR1 subunit *(9)*, whereas biotinilation assays have estimated surface GluR1 expression to have a half-life of 30 h on cultured cerebellar granule cells *(9)*, 11 to 43 h on spinal cord cultures (depending on culture age; refs. *10* and *11*), and approx 1 to 2 h on cultured hippocampal neurons *(12–14)*. However, using fluorescent bungarotoxin to bind to recombinant AMPARs and fluorescent AMPAR antibodies, the insertion and removal of AMPARs under basal conditions has been shown to occur very rapidly, on the order of minutes *(15,16)*. Similar results were obtained when electrophysiological recordings were used: the run-down or run-up of synaptic AMPAR excitatory postsynaptic currents (EPSCs) in response to pharmacological blockade of either exocytosis or endocytosis was found to occur rapidly over a time-course of minutes *(17–20)*.

One limitation of most of these studies is that they are not able to resolve synaptic and extrasynaptic receptor pools. Most recently, an elegant study using photo-inactivation of AMPARs with the compound ANQX allowed a more complete analysis of AMPAR dynamics *(21)*. This technique has the power to specifically determine AMPAR turnover at distinct neuronal regions (synaptic, extrasynaptic, and soma). Interestingly, this study found that AMPAR insertion at the soma was rapid (half-life < 15 min), whereas AMPAR recovery at extrasynaptic and synaptic sites on the dendrites was much slower, on the order of hours. Although the average half-life on the neuronal surface is reasonably consistent with the average surface half-life estimated in previous work, this study still contradicts several previ-

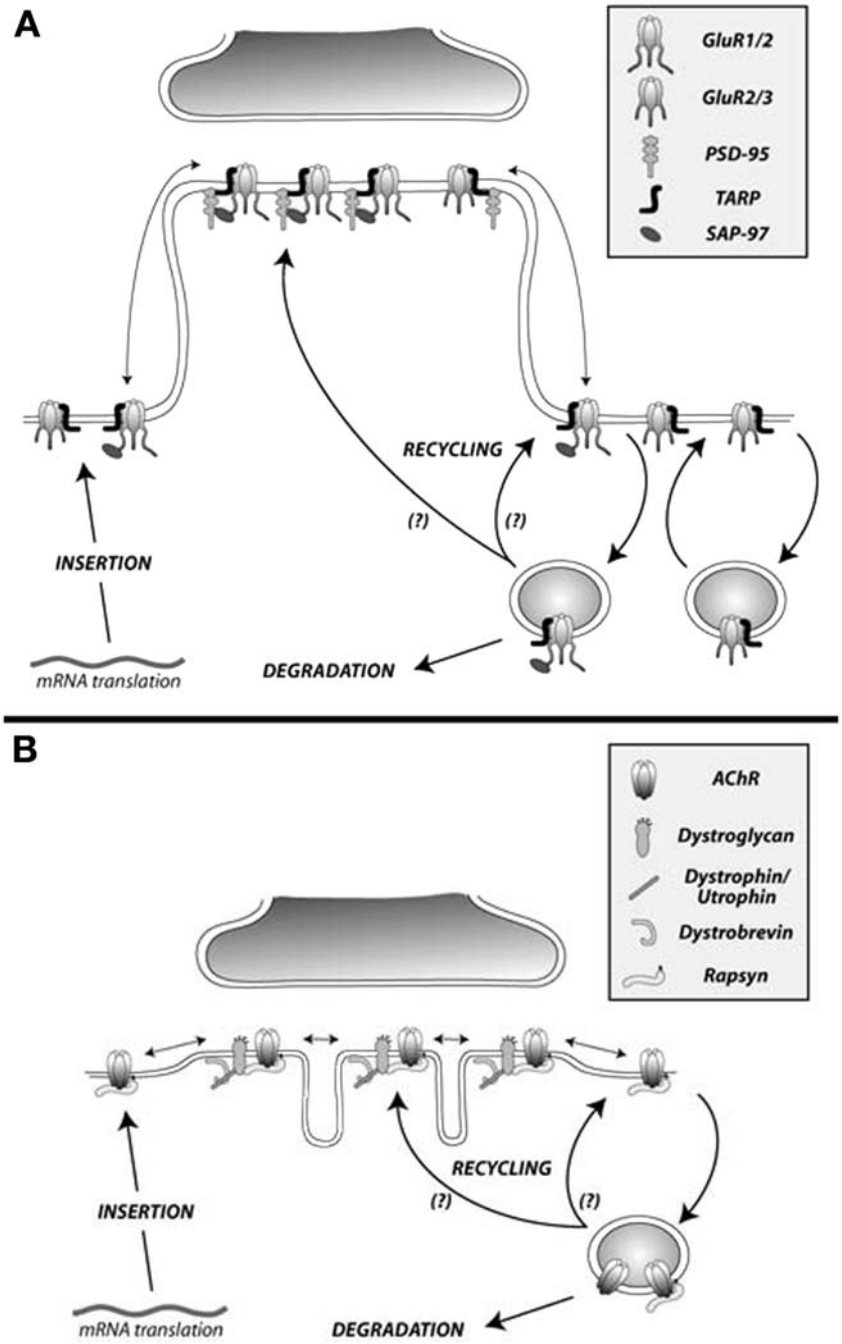

Fig. 1. A model of postsynaptic receptor dynamics at glutamatergic and cholinergic synapses. **(A)** Receptor dynamics at excitatory glutamatergic synapses in the central nervous system (CNS). At glutamatergic synapses, the density of AMPARs at synaptic sites is controlled by the insertion, lateral migration, degradation, and recycling of receptors. These dynamics can be altered by a number of different events, including activity and phosphorylation, and are regulated by intracellular scaffolding proteins. **(B)** Receptor dynamics at excitatory cholinergic synapses in the peripheral neuromuscular junction (NMJ). Similar to receptors in the CNS, the density of acetylcholine receptors (AChRs) at synaptic sites in the NMJ is controlled by the insertion, lateral migration, degradation, and recycling of receptors. AChR dynamics are also controlled by activity and phosphorylation, as well as by proteins in the intracellular scaffold.

ous studies showing fast synaptic insertion and removal of AMPARs.

Although the aforementioned estimates of AMPAR half-life under basal conditions vary widely from minutes to days, it is almost universally accepted that rapid and significant changes in AMPAR number can occur within seconds at synaptic sites in response to activity. For example, during LTD or LTP, the endocytosis, exocytosis, or recycling of receptors can be altered by the regulated association of AMPARs with numerous intracellular proteins that increase or decrease synaptic AMPAR expression *(1–3,22,23)*. LTP and LTD, which have been most extensively studied in the hippocampus, have been shown to involve changes in the GluR1/2 receptors that are expressed at a low basal level synaptically and are then inserted into the synapse rapidly in response to LTP induction protocols *(12,14, 19,24–27)*. Conversely, GluR1/2 receptors are removed rapidly in response to LTD or depotentiation *(12,15,28,29)*, and while GluR2 and GluR2/3 knockout mice show no deficits in LTP or LTP-dependent learning *(30,31)*, the targeted deletion of GluR1 significantly impairs LTP *(32)* and hippocampus-dependent learning *(33,34)*, which can be rescued by the delivery of GluR1 into the hippocampus *(35)*. Furthermore, rapid experience-dependent insertion of GluR1/2 receptors has been shown to occur in vivo after fear conditioning in the amygdala *(36)*, and the rapid removal of GluR1/2 receptors has been observed in vivo after monocular deprivation in the visual cortex *(37)* or activity deprivation in the barrel cortex *(38)*.

AMPAR dynamics are regulated by a host of accessory proteins that facilitate or inhibit the insertion and removal of AMPARs. For example, the TARP family of proteins (γ-3, γ-4, γ-8 and Stargazin (γ-2) bind AMPARs to the postsynaptic density protein PSD-95 to mediate AMPAR synaptic clustering *(39–44)*. TARP-AMPAR association is activity-dependent, because glutamate binding has been shown to dissociate TARPs from AMPARs *(45)*, and Stargazin phosphorylation has been shown to mediate the expression of both LTP and LTD *(46)*. It has been suggested that TARP-AMPAR dissociation might facilitate lateral diffusion of AMPARs out of synapses and into the extrasynaptic space, where they could be internalized by clathrin-coated pits *(44,47)*. In fact, a mechanism of receptor delivery and removal from synapses by extrasynaptic endo- and exocytosis and lateral diffusion was first described for AChRs in the NMJ *(48)* and has since been found to occur similarly for AMPARs. In glutamatergic synapses, endocytic zones have been identified in the extrasynaptic area close to the postsynaptic density (PSD) *(49)*, and pH-sensitive green fluorescent protein conjugates have shown that internalization occurs in this area in response to NMDA receptor (NMDAR) activation *(50)*. The lateral movement of AMPARs between synaptic and extrasynaptic spaces is regulated by calcium *(51)*, and single-particle imaging shows that receptor mobility within the PSD and extrasynaptic area is increased with treatments that stimulate neuronal activity *(52,53)*. Finally, transient internalization of extracellular receptors appears to precede synaptic loss of receptors *(50)*, and diffuse extrasynaptic staining of inserted AMPARs has also been observed to precede AMPAR aggregation at the PSD *(14)*, indicating that the extrasynaptic area may be involved both in endo- and exocytotic events.

Several proteins have also been demonstrated to be involved in subunit-specific regulation of AMPAR dynamics. The "short-tailed" AMPAR subunits, including GluR2 and GluR3, are stabilized at synapses by the binding of glutamate receptor-interacting protein (GRIP) or its homolog AMPA receptor-binding protein, and GluR2 subunits are additionally stabilized by *N*-ethylmaleimide-sensitive factor (NSF) *(28,54–57)*. Disruption of GRIP/ABP binding by protein interacting with C-kinase-1 (PICK1) and the replacement of NSF with the clathrin adaptor protein AP-2 induce the endocytosis of GluR2/3 AMPARs *(54,58–64)*. The "long-tailed" AMPAR subunits, including GluR1 and GluR4, have quite different intracellular binding domains, which allow another complement of proteins to regulate

their dynamics. For example, SAP-97 links GluR1 to PSD-95, a protein that localizes specifically to synapses *(65–70)*. The AMPAR–SAP-97 interaction has previously been shown to be essential for activity-dependent delivery of GluR1 into spines (refs. *71*,*72*; however, *see* ref. *73*), and PSD-95 has been shown to modulate AMPAR incorporation during LTP in vivo *(74)*.

Regulation of NMDA Receptor Dynamics at Excitatory Central Synapses

The NMDAR is a heterotetramer composed of NR1, NR2(A-D), and NR3(A-B) subunits, all of which have endo- and exocytosis motifs that allow binding of regulatory proteins. In the mammalian forebrain, NMDARs are predominantly expressed as NR1/2A and NR1/2B diheteromers *(75–78)*. Although the discovery that AMPARs undergo rapid changes in response to activity has driven much of the research on receptor dynamics at the glutamatergic synapse toward the AMPAR, focus has more recently turned toward the trafficking of the NMDAR *(79–82)*. Similarly to the AMPAR, NMDAR half-life estimates vary depending on experimental method (ranging from hours to days), and NMDAR half-life also changes significantly through development. For example, in cultures from early stage neurons, a surface biotinilation assay found that over 20% of NMDARs are internalized after 30 min (half-life < 2 h) *(83)*, which is comparable to 15% internalization of surface AMPARs after 30 min *(12)*. In more mature neurons, the turnover of NMDARs slows dramatically *(83)* to a rate that is two- to threefold slower than AMPARs *(8,12,15,84,85)*.

As with AMPARs, the synaptic delivery and removal of NMDARs in response to activity can be much more rapid with significant changes in NMDAR number occurring within minutes. For example, glycine/glutamate treatment causes an approx 25% decrease in surface NMDAR expression after 15 min in cultured hippocampal cells *(85)*, and LTP induction in adult hippocampal cells results in an approx 20% increase in NMDARs in the same time-period *(86)*. Similarly, dark-reared neonatal rats show rapid and persistent increases in NR2A subunit expression in the visual cortex in vivo within an hour of light exposure *(87)*. Similarly to the AMPAR, the dynamics and resulting changes in surface expression of the NMDAR can be controlled at the level of insertion, removal, or recycling.

Several studies have shown that the insertion of the NMDAR is strongly regulated by the NR1 subunit. NMDA export is first regulated at the level of translation, because the NR1 subunit can be spliced to produce alternate C-terminal tails, with activity blockade resulting in a C´-cassette that allows rapid export *(88–91)*. An endoplasmic reticulum retention signal on the NR1 C-terminal region also serves as a control point for exocytosis and surface expression and has been shown to be regulated by protein kinase C (PKC) *(92)*. PKC, a kinase that is also implicated in the dynamics of AMPARs *(61,63,93)* and GABARs *(94)*, has been shown to increase NMDAR surface expression *(95)* and is essential for LTP expression in hippocampal slices from older mice *(86)*. Because the NR1 subunit is obligatory for the surface expression of NMDARs, the activity-dependent alternate splicing and PKC masking of a retention signal allow NMDAR exocytosis to be altered by activity to control surface expression.

Although the NR1 subunit also contains signals that are potentially involved in internalization *(96)*, NMDAR endocytosis appears to be controlled largely by signals on the intracellular tails of NR2A and NR2B. The NR2 subunits bind to the homologous PDZ-containing proteins PSD-95 and SAP-102 *(97–102)*, which play a role in anchoring or stabilizing NMDARs, rather than facilitating their delivery to synapses. For example, the delivery of NR2B to neonatal synapses precedes PSD-95 *(103)*, and PSD-95 knockouts still express surface NMDARs *(104)*. Rather,

the association of PSD-95 with the NR2 subunit of the NMDAR appears to regulate internalization, because activity-dependent dephosphorylation of NR2 results in the replacement of PSD-95 with the clathrin adaptor protein AP-2, which initiates endocytosis *(83, 102,105,106)*. This interaction is mediated by several different factors. For example, the NMDAR co-agonist glycine is able to recruit AP-2 to synapses, and subsequent binding of both glycine and NMDA/glutamate initiates rapid internalization of NMDARs, which results in LTD at synapses *(85)*.

After endocytosis, NR2A and NR2B receptors are trafficked along different pathways: the NR2A-containing receptors appear mostly in late endosomes on the way to lysosomal degradation, whereas the NR2B-containing receptors are found in recycling vesicles, which are able to deliver approx 15% of internalized receptors back to the surface after 1 h *(96,107)*. Although changes in NMDAR expression have traditionally been believed to mediate homeostatic plasticity changes through slow cycling mechanisms *(81)*, the rapid cycling of NMDARs observed more recently suggests a method by which very rapid changes in NMDAR surface expression could be modulated. Indeed, although AMPAR dynamics have been shown to control much of the activity-dependent changes observed in early postnatal neurons, changes in NMDAR number have been associated with LTP in older cells *(86)*. Additionally, in older cultures of rat hippocampal neurons, the internalization of NMDAR subunits occurs just as rapidly as AMPAR subunits after the stimulation of mGluRs and the initiation of LTD *(108)*.

Between exocytosis and endocytosis lies a robust mechanism that allows for the surface exchange of receptors between the synaptic and extrasynaptic spaces *(47)*. Elegant single-particle tracking experiments have shown that the NMDAR mobility is high outside of synapses and decreases markedly once it enters the synapse *(53,109)*. This mobility has also been shown to be altered by activity, which could be because of the activity-dependent upregulation of synaptic PSD-95 *(74)*.

Regulation of γ-Aminobutyric Acid Receptor Dynamics at Inhibitory Central Synapses

The $GABA_A$ receptor is a pentameric ionotropic receptor constructed from seven subunit classes. However, it appears likely that the majority of GABARs in vivo are composed of two α-, two β-, and one γ-subunit *(110,111)* and are anchored to the intracellular cytoskeleton by the GABAR-associated protein and gephyrin *(112–114)*. The $GABA_A$ receptor is responsible for the majority of inhibitory transmission in the adult brain, and GABAR surface expression is regulated in vivo by numerous endogenous and exogenous factors, including ethanol, insulin, and agonist binding *(115–117)*. The lifetime of GABAR has been shown to have two phases: one rapid with a half-life of approx 4 h and one more slow with a half-life of approx 32 h *(118)*. More recently, GABAR lifetime was estimated to be approx 12 h *(119)*. The surface dwell time has been measured with biotinylation assays, which have shown 25% internalization after 30 min (half-life: approx 1 h) *(119)*. However, this assay was not able to resolve recycled receptors or to distinguish between synaptic and extrasynaptic zones and, therefore, may have underestimated the lifetime of GABARs.

Although comparatively little is known about GABAR dynamics, much work shows that GABARs share characteristics with both AMPA/NMDARs and AChRs. For example, similarly to the ionotropic glutamate receptors, constitutive endocytosis of surface GABARs is initiated by clathrin-mediated endocytosis and is regulated by the phosphorylation state of the adaptor protein AP-2 *(119–121)*. After internalization, GABARs are either targeted to lysosomes for degradation or recycled back into the plasma membrane *(94,122,123)*. This recycling mechanism is able to insert 50% of internalized receptors back to the surface after internalization *(119)*. The ubiquitin-like molecule, Plic-1, is also potentially involved in the cycling of internalized GABARs and prevents

proteosomal degradation and potentially increases the size of the available intracellular pool of GABARs *(124)*. Recently, the proteins NSF and GRIP were also found at GABAergic postsynaptic sites *(120,125,126)*, raising the possibility that these two proteins, which regulate ionotropic receptor trafficking in glutamatergic synapses, may play a key role in GABAR dynamics. Finally, although GABARs share a number of trafficking proteins with glutamatergic receptors, they also share a common cytoskeletal anchoring framework with AChRs—namely, the intracellular cytoskeleton complex to which both of these receptors bind is the dystrophin–glycoprotein complex (DGC). The role of the DGC in regulated trafficking of GABARs is poorly understood, although disruption of several DGC proteins results in a reduction both in the size of GABAR clusters and the overall number of surface GABARs *(127)*.

Regulation of AChR Dynamics at Excitatory Peripheral Neuromuscular Synapses

At the adult NMJ, the AChR is composed of two α-, one β-, one δ- and one ε- subunit *(128–130)*. Early in development, the γ-subunit is highly expressed and is capable of forming functional channels but is replaced completely by ε-subunit-containing receptors in the adult *(129)*. Because of the availability of a snake toxin α-bungarotoxin (BTX) that selectively and irreversibly labels AChRs, the turnover of AChRs at developing, mature, and denervated NMJs has been studied extensively using radioactively labeled BTX. These studies primarily used I^{125}-BTX to determine the loss of receptors from muscle cells. Using this approach, the time-course of AChR loss was estimated by comparing radioactivity between muscles from different animals that were examined at varying times after applying the radioactive toxin. These studies found that the half-life of receptors on mature, innervated muscle cells ranged from approx 7 to 13 d *(131–133)*.

Although the I^{125}-BTX approach forged the first inroads in estimating ionotropic receptor turnover in vivo, it had some drawbacks. First, by summing radiolabeled ligands from the entire muscle, it could not reveal the receptor dynamics at individual NMJs. For example, muscles with different populations of muscle fiber types may have different turnover rates. Second, the inactivation of AChRs with BTX could greatly affect the way that AChRs are removed from synapses. Finally, the density of postsynaptic molecules can change enormously when the junction expands or shrinks during growth or denervation, which could provide erroneous estimates when counting radiolabeling on muscle cross-sections.

The subsequent development of fluorescently conjugated BTX and quantitative fluorescence microscopy largely circumvented these limitations. Studies using this tool have been able to study the turnover of AChRs more directly by assaying the intensity of fluorescently tagged BTX that is irreversibly bound to AChRs at individual identified junctions viewed more than one time in living mice *(134,135)*. This approach has led to a substantially different view of AChR dynamics than was provided by previous studies (Fig. 1B). For example, they demonstrated that when neuromuscular transmission was functional, receptor lifetime in the junctional membrane was quite long (approx 2 wk). However, when all AChRs were inactivated by BTX or another AChR inhibitor (curare), receptor lifetime decreased substantially to a half-life of several hours *(136)*.

Recently, we devised a novel labeling method, which revealed that AChRs at the NMJ are not simply degraded on internalization, as previously believed *(137)*. A significant number of receptors, rather than being metabolized on internalization, recycle back into the postsynaptic membrane *(138)*. The unexpectedly high contribution of recycled AChRs at synapses implies that the classical concept of "metabolic stability" of junctional AChRs *(131)*

must be revised. Specifically, earlier work did not consider the possibility that receptors continuously recycle back to the postsynaptic membrane with their BTX tag to contribute to the junctional receptor population. This work has led to the idea that previous experiments using fluorescently labeled BTX must be reinterpreted *(131,136,139–141)*. This also argues that junctional AChRs are being internalized from the surface at a significantly faster rate (half-life: approx 4 d after a one-time blockade) than previously believed. Because recycled and pre-existing AChRs can be labeled with different fluorophores, we also monitored the lifetime of recycled and pre-existing AChRs from the same postsynaptic membrane and found that recycled AChRs turn over approximately three times more rapidly than pre-existing AChRs (Bruneau and Akaaboune, 2006, unpublished data).

In sharp contrast to innervated synapses, AChRs on mouse-derived C2C12 myotubes do not recycle *(138)*, despite the short half-life of AChRs in the postsynaptic membrane *(142)*. This suggests that a nerve factor(s) is required for triggering the recycling of AChRs and indicates that the insertion of newly synthesized AChRs is the main source for maintaining normal receptor density at any time in aneural muscle cells. We are currently exploring the nature of the nerve factors that may be involved in the initiation of receptor recycling.

Similarly to other ionotropic receptors in central synapses, the turnover rate of AChRs at the NMJ is also subject to synaptic activity. For example, following muscle denervation, AChR lifetime decreases substantially to a half-life of approx 1 to 3 d *(129,143,144)*. Interestingly, the blockade of synaptic transmission not only increases the turnover rate of AChRs but also depresses the insertion of recycled AChRs into the postsynaptic membrane *(138)*. However, blocking synaptic potentials with TTX does not affect AChR dynamics, suggesting that spontaneous release of neurotransmitter is sufficient to maintain normal AChR turnover *(136)*. In support of this idea, the loss of AChRs can be prevented in denervated muscle by the restoration of synaptic activity or direct muscle stimulation *(136,140,144,145)*.

Previous studies have shown that the nerve clustering factor agrin not only causes aggregation of AChRs but may also increase the stability of AChRs at denervated NMJs *(146)*. However, work with mouse-derived C2C12 aneural myotubes shows that agrin does not alter receptor turnover *(142)*, suggesting that other factors present at the mature NMJ in conjunction with agrin may be implicated in AChR stability. The lifetime of AChRs can also be controlled by postsynaptic scaffolding proteins. For example, in mice lacking α-dystrobrevin, a protein of the DGC, dramatic changes occur in the NMJ, including a severe reduction in the number and the density of postsynaptic receptors as well as a rapid rate of removal (half-life: approx 2.7 d vs approx 2 wk in control) *(139)*. Similar reductions in AChR number and density were observed in mice lacking α-syntrophin *(147)*, although the lifetime of AChRs was not assessed in this study. Recent work by Phillips et al. *(148)* has further implicated the receptor-associated protein rapsyn in controlling the lifetime of AChRs: electroporation of rapsyn into mouse muscle in vivo decreased the loss of AChRs . However, from our unpublished results, it appears that rapsyn does not play an important role in AChR dynamics at clusters on the surface of aneural myotubes (Bruneau and Akaaboune, 2006, unpublished results). The investigation of the role of other scaffolding proteins on AChR dynamics is underway.

Future Directions

Although a large body of work exists on ionotropic receptor dynamics in the CNS, this work is limited by the inaccessibility of these synapses and, therefore, has been performed almost exclusively in culture or organotypic slice preparations outside of the intact system. On the other hand, the peripheral NMJ is highly accessible in the living animal. Therefore, the fundamental question is whether the NMJ is similar enough to provide insights into synapses in the

CNS. In the past 30 yr, the NMJ has proved its utility on this score by driving the understanding of synapse elimination, presynaptic quantal release, extrasynaptic endo- and exocytosis, and lateral migration of receptors. In the field of receptor dynamics, however, the applicability of information gained about AChRs from in vivo studies appeared to be of limited applicability to ionotropic receptors in the CNS because of a few key apparent differences—namely, a synaptic AChR turnover at least two orders of magnitude slower than AMPARs and a lack of AChR recycling.

Over the last few years, these apparent differences have been slowly dismantled as estimates of AMPAR lifetime have decreased with new methods, whereas the estimates of AChR dynamics have increased. The discovery that receptor recycling, which is a hallmark of receptor dynamics in central synapses, also occurs at the NMJ has further brought central and peripheral receptor dynamics into closer relation (Fig. 1). Finally, as the components of the intracellular scaffolds of each of the ionotropic receptors have become resolved, structural homologies between AMPA/NMDAR and GABARs, as well as GABARs and AChRs, have begun to appear. It will be interesting to see if understanding the functional role of these proteins at the accessible NMJ can help to continue the trend of in vivo investigation at peripheral synapses informing our understanding and directing research into processes that occur at central synapses in vivo. It is a productive tradition that will hopefully continue to further our understanding of ionotropic receptor dynamics in the intact, living animal.

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