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Perisynaptic Schwann Cells at the Neuromuscular Synapse: Adaptable, Multitasking Glial Cells

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The neuromuscular junction (NMJ) is engineered to be a highly reliable synapse to carry the control of the motor commands of the nervous system over the muscles. Its development, organization, and synaptic properties are highly structured and regulated to support such reliability and efficacy. Yet, the NMJ is also highly plastic, able to react to injury and adapt to changes. This balance between structural stability and synaptic efficacy on one hand and structural plasticity and repair on another hand is made possible by the intricate regulation of perisynaptic Schwann cells, glial cells at this synapse. They regulate both the efficacy and structural plasticity of the NMJ in a dynamic, bidirectional manner owing to their ability to decode synaptic transmission and by their interactions via trophic-related factors.

The vertebrate neuromuscular junction (NMJ), arguably the best characterized synapse in the peripheral nervous system (PNS), is composed of three closely associated cellular components: the presynaptic nerve terminal, the postsynaptic specialization, and nonmyelinating Schwann cells. These synapse-associated glial cells are called perisynaptic Schwann cells (PSCs), or terminal Schwann cells (see reviews by Todd and Robitaille 2006; Feng and Ko 2007; Griffin and Thompson 2008; Sugiura and Lin 2011). Multiple roles of PSCs have gained great appreciation since the 1990s and, along with the novel roles of astrocytes in central synapses, have led to the concept of the "tripartite" synapse (Araque et al. 1999, 2014; Volterra et al. 2002; Auld and Robitaille 2003; Kettenmann and Ransom 2013).

Thus, to fully understand synaptic formation and function, it is critical to also consider the active and essential roles of synapse-associated glial cells. We will discuss evidence supporting the existence of a synapse–glia–synapse regulatory loop that helps maintain and restore synaptic efficacy at the NMJ. We will also explore the multiple functions that PSCs exert, functions that are adapted to a given situation at the NMJ (e.g., synapse formation, stability,

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and reinnervation). This will highlight the great adaptability and plasticity of the morphological and functional properties of PSCs.

In this review, we will focus on the multiple roles PSCs play in synaptic formation, maintenance, remodeling, and regeneration, as well as synaptic function and plasticity. Based on the evidence presented, we propose a model in which PSCs, through specific receptor activation, play a prominent role in a continuum of synaptic efficacy, stability, and plasticity at the NMJ. These synaptic-regulated functions allow PSCs to orchestrate the stability and plasticity of the NMJ and, hence, are important for maintaining and adapting synaptic efficacy.

THE TRIPARTITE ORGANIZATION OF THE VERTEBRATE NEUROMUSCULAR JUNCTION

At the vertebrate NMJ, the motor nerve endings are capped by nonmyelinating Schwann cells, in contrast to the motor axons, which are wrapped around by myelinating Schwann cells (Corfas et al. 2004). The existence of PSCs was first suggested by Louis-Antoine Ranvier (1878), who reported clusters of "arborization nuclei," which were distinct from muscle fiber nuclei, and were later identified as nuclei of "teloglia" or terminal Schwann cells at the NMJ (Couteaux 1938, 1960; Tello 1944; Boeke 1949; Ko et al. 2007; Griffin and Thompson 2008). The identity and intimate contacts of Schwann cells with the nerve terminals was further confirmed with transmission, scanning and freeze-fracture electron microscopies (Heuser et al. 1976; Desaki and Uehara 1981; Ko 1981).

With the advance of immunofluorescence microscopy and the availability of fluorescent probes for PSCs, the tripartite nature of the vertebrate NMJ is further appreciated (Fig. 1). For amphibian muscles, two vital probes for PSCs, peanut agglutinin (PNA) (Ko 1987) and the monoclonal antibody (mAb) 2A12 (Astrow et al. 1998), have been particularly useful to reveal the tripartite organization of the NMJ and the dynamic relationship between PSCs and nerve terminals (see below). Figure 1A–D shows an example of a frog NMJ multiple labeled with mAb 2A12 for PSC somata (asterisks) and processes, with antineurofilament antibody for axons and antisynapsin I antibody for nerve terminals, and with α-bungarotoxin (α-BTX) for AChRs on muscle fibers. The merged fluorescent image (Fig. 1D) further reveals the tripartite arrangement, which can also be shown in the electron micrograph of a cross-section of the frog NMJ (Fig. 1I). Unfortunately, neither PNA nor mAb2A12 labels mammalian NMJs.

For mammalian muscles, an antibody to the Ca^{2+} binding protein S100 (Reynolds and Woolf 1992) has been most commonly used for probing mammalian PSCs. Another very useful approach to label mammalian PSCs is the use of transgenic mice that express variants of the green fluorescent protein (GFP) family in axons and Schwann cells to view the dynamic behavior of axons and PSCs in living animals (Kang et al. 2003; Zuo et al. 2004; Li and Thompson 2011). Figure 1 shows an NMJ labeled with α-BTX for AChRs in a mouse that expresses GFP under the control of the S100β promoter in PSC somata (asterisks) and processes (Fig. 1E–H), and CFP in nerve terminal and the preterminal axon (arrow in Fig. 1F). The tripartite organization of the mouse NMJ is further shown in the merged image (Fig. 1H). There are other probes that can also label mammalian PSCs, for example, LNX-1 (an E3 ubiquitin ligase) (Young et al. 2005), NaV1.6 (Musarella et al. 2006), TrkC (Hess et al. 2007) in intact muscles, and antibodies to p75 neurotrophin receptor (Hassan et al. 1994), GAP-43 (Woolf et al. 1992), nestin (Kang et al. 2007), or transcription factor zinc-finger proliferation 1 (Ellerton et al. 2008) in denervated muscles.

It has been shown that there are ~3–5 PSC somata in both frog and mammalian mature NMJs, and that the number of PSCs is correlated with the endplate size (Herrera et al. 1990; Love and Thompson 1998; Lubischer and Bebinger 1999; Jordan and Williams 2001). It is not clear why PSCs are nonmyelinating even though they can be labeled with antibodies to myelinating glial markers, such as protein zero (P_0), myelin-associated glycoprotein (MAG), galactocerebroside, and 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (Georgiou and Charlton

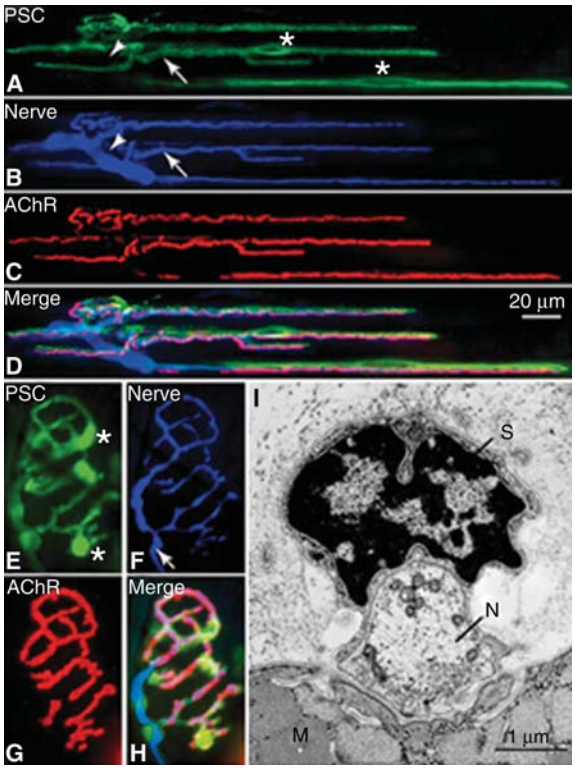

Figure 1. The tripartite organization at the NMJ. (*A–D*) A frog NMJ fluorescently labeled with a monoclonal antibody (2A12) for PSCs (*A*, green), antineurofilament and antisynapsin I antibodies for nerve fibers and nerve terminals (*B*, blue), and α-bungarotoxin (α-BTX) for acetylcholine receptors (AChRs) (*C*, red). The merged picture (*D*) further shows the tripartite arrangement of the frog NMJ. PSC somata (asterisk in *A*) and processes (arrow in *A*) can be labeled with 2A12 antibody, which does not label Schwann cells along the axon (arrowheads in *A* and *B*). Scale bar in *D* also applies to *A–C*. (*E–H*) NMJ in a transgenic mouse that expresses green fluorescent protein (GFP) in Schwann cells (*E*, green) and cyan fluorescence protein (CFP) in nerve terminal (*F*, blue), and labeled with α-BTX for AChRs (*G*, red). Similar to frog NMJ shown in *D*, the merged picture (*H*) further illustrates these three closely associated elements at the mammalian NMJ. PSC somata (asterisk in *E*) and processes, including those associated with the preterminal axon (arrow in *F*), all express GFP. (*I*) Electron micrograph of a frog NMJ in cross section further confirms the tripartite arrangement with the PSC (S), capping the nerve terminal (N), which are in apposition with postjunctional folds on the muscle fiber (M). Scale bar, 1 μm. Glial cells maintain synaptic structure and function and promote development of the NMJ in vivo. (Panels *A–H* from Ko and Thompson 2003; reproduced, with permission, from the authors in conjunction with Springer Science and Business Media. Panel *I* from Reddy et al. 2003; reprinted, with permission, from the authors and Elsevier © 2003.)

1999). It is also not well understood why PSCs cap, but do not enclose entirely, the motor nerve terminal. An otherwise complete enclosure of the nerve terminal would obviously severely compromise synaptic function. Frog PSCs, however, project finger-like processes, which contain L-type calcium channels (Robitaille et al. 1996), into the synaptic cleft and interdigitate with active zones—sites of transmitter release. In contrast, mammalian PSC "fingers" are usually excluded from the cleft, which may be attributed to laminin 11 (α5β2γ1) in the synaptic cleft (Patton et al. 1998). Besides the synaptic cleft and the muscle surface, the basal lamina also covers PSCs (Saito and Zacks 1969; Engel 1994). However, the extracellular matrix molecules associ-

ated with PSC basal lamina are distinct from those in the synaptic cleft and the extrasynaptic muscle surface (Ko 1987; Astrow et al. 1997; Patton et al. 1997; for review, see Patton 2003). It has been suggested that the PSC-associated extracellular matrix may play a role in guiding nerve terminal sprouts at the frog NMJ (Chen and Ko 1994; Ko and Chen 1996; see below). Interestingly, fibroblast-like cells (kranocytes) capping the NMJ have also been shown (Connor and McMahan 1987; Court et al. 2008).

ROLE OF PSCs IN SYNAPTOGENESIS

The intimate arrangement of the tripartite NMJ raises a question as to whether PSCs participate in synaptogenesis. To address this question, one needs to know first if Schwann cells are necessary during the initial navigation of axons to their target muscles (Keynes 1987). It has been shown that motor axons can reach their target muscles and even form the initial nerve–muscle contacts, albeit only transiently, in mutant mice of ErbB2 (Morris et al. 1999; Woldeyesus et al. 1999; Lin et al. 2000), ErbB3 (Riethmacher et al. 1997), and Splotch (Grim et al. 1992) mutant mice, all of which lack Schwann cells in the peripheral nerves. Furthermore, functional nerve–muscle contacts can be formed in cultures without Schwann cells (Kullberg et al. 1977; Chow and Poo 1985). These studies suggest that Schwann cells are not necessary for axonal pathfinding and the initial formation of nerve–muscle contacts.

Although Schwann cells are dispensable for the initial stages of NMJ formation, they play a critical role in promoting subsequent synaptic growth, maturation, and maintenance at developing NMJs. In frog muscles, PSCs appear shortly after the earliest discernible nerve–muscle contacts in tadpoles, and PSCs then quickly extend processes beyond nerve terminals (Herrera et al. 2000). The subsequent growth of nerve terminals appears to follow along the preceding PSC sprouts as shown with repeated in vivo observations of identified developing NMJs in tadpoles (Reddy et al. 2003). Combining repeated in vivo observations with an ablation technique that takes advantage of mAb2A12 and complement-mediated lysis to selectively ablate PSCs in vivo, Reddy et al. (2003) revealed major perturbations in NMJ structure and establishment, which further shows the critical role of PSCs in promoting synaptic growth and maintenance at developing amphibian NMJs in vivo.

PSCs also play an essential role in synaptogenesis in mammalian muscles (Griffin and Thompson 2008). Trachtenberg and Thompson (1996) showed that denervation in neonate, but not in adult, leads to rapid apoptosis of mammalian PSCs, and the apoptosis can be prevented by a glial growth factor, neuregulin 1 (NRG1). In addition, PSC morphology and NMJ structure can be altered by applications of NRG1 or Schwann cell transplants to mammalian muscles (Trachtenberg and Thompson 1997). The observation that partial denervation in neonatal but not in adult rat muscles results in apoptosis of PSCs, and absence of nerve terminal sprouting in neonatal muscles further confirms the importance of PSCs in promoting synaptic growth (Lubischer and Thompson 1999). Moreover, the lack of PSCs may also play a role in the withdrawal of nerve terminals following the initial formation of nerve–muscle contacts in ErbB2 and ErbB3 mutant mice (Riethmacher et al. 1997; Morris et al. 1999; Woldeyesus et al. 1999). Interestingly, Lee et al. (2011) have found that the PSCs are reduced in number and incompletely cover the endplate site in a mutant mouse model of spinal muscular atrophy. The PSC defects may contribute to the abnormal and delayed maturation of NMJs in this neuromuscular disease. Taken together, these studies suggest that PSCs are essential for the growth and maintenance of developing motor nerve terminals at both amphibian and mammalian muscles.

The molecular mechanisms of how PSCs participate in synaptic growth and maintenance at developing NMJs are not well understood. It has been shown that frog Schwann cells express active isoform of agrin and enhance AChR aggregation in muscle culture (Yang et al. 2001). Furthermore, Schwann cell–conditioned medium promotes synaptogenesis in *Xenopus* nerve–muscle cultures (Peng et al. 2003). In particular, Feng and Ko (2008) have shown, using *Xenopus*

tissue culture, that Schwann cell–conditioned medium contains transforming growth factor (TGF)-β1. TGF-β1plays a necessary and sufficient role in promoting NMJ formation, and TGF-β ligands have been implicated in synaptic pruning in the developing visual system (Bialas and Stevens 2013) and synaptic growth in *Drosophila* (Fuentes-Medel et al. 2012). It has also been shown that *Xenopus* Schwann cell–conditioned medium can acutely enhance transmitter release in developing NMJs in culture (Cao and Ko 2007). However, the in vivo role of TGF-β or other Schwann cell–derived factors in synaptogenesis at NMJs remains to be examined.

One hallmark of the mammalian NMJ formation is the innervation of multiple nerve terminals at a single NMJ (polyneuronal innervation) and the subsequent removal of all but one of the nerve endings (synapse elimination) by the second week after birth (Sanes and Lichtman 1999). The potential role of PSCs in pruning excess nerve terminals at multiply innervated NMJs in postnatal muscles has been suggested (Griffin and Thompson 2008). For example, the retraction of nerve terminals and Schwann cell processes from the sites of synapse elimination occur at a similar time course (Culican et al. 1998). Interestingly, retracting nerve terminals shed numerous membrane-bound remnants called axosomes, which are engulfed by Schwann cells during synapse elimination (Bishop et al. 2004). Using time-lapse imaging of labeled single PSCs, Brill et al. (2011) have revealed that young PSCs intermingle dynamically in contrast to the static tile patterns seen in adult NMJs. A recent study using serial electron microscopy has further shown that PSCs participate in synapse elimination by phagocytosis of nerve terminals, although the process involves all axons and PSCs do not seem to select the winner of the competing developing nerve terminals (Smith et al. 2013). However, using simultaneous Ca^{2+} imaging of PSCs and synaptic recording of dually innervated mouse NMJs, Darabid et al. (2013) have shown that activity of single PSCs reflects the synaptic strength of each competing nerve terminal and the state of synaptic competition. Hence, PSCs decode synaptic transmission at a later stage of synaptic competition, allowing them to identify the strongest competing nerve terminal, which is likely to win the ongoing competition. Whether PSCs play an active and necessary role in synapse elimination remain to be further explored.

ROLE OF PSCs IN SYNAPTIC MAINTENANCE, REMODELING, AND REGENERATION AT ADULT NMJs

Maintenance

It is remarkable that the tripartite organization is maintained at the adult vertebrate NMJ despite the continual mechanical disruptions by muscle contractions throughout the animal's life span. To address the question of whether PSCs play a role in synaptic maintenance, Reddy et al. (2003) took advantage of the selective labeling of PSCs with mAb2A12 and combined this with complement-mediated cell lysis to selectively ablate PSCs from frog NMJs in vivo. They observed no significant changes in synaptic structures and function shortly after PSC ablation (within 5 h). At mouse NMJs, Halstead et al. (2004, 2005) have shown that both NMJ morphology and synaptic transmission are also not acutely affected after selective PSC ablation with an autoantibody against disialosyl epitopes of gangliosides seen in Miller Fisher syndrome. This null effect may be caused by the ability of PSCs to both decrease and increase synaptic efficacy (see below; Robitaille 1998; Castonguay and Robitaille 2001; Todd et al. 2010), hence, resulting in no net change in the synaptic output at the NMJ. However, partial or total retraction of some nerve terminals and a ∼50% reduction in transmitter release were seen 1 wk after PSC ablation. These observations suggest that, although PSC may be dispensable for the short-term maintenance, they are essential for the long-term maintenance of frog NMJs. The long-term effect of PSC ablation on the maintenance of the mammalian NMJ remains to be investigated.

Remodeling

Although the tripartite arrangement is maintained at adult NMJs, nerve terminals at frog

NMJs undergo extension and/or retraction throughout adult life (Wernig et al. 1980; Herrera et al. 1990; Chen et al. 1991). To address whether PSCs also undergo similar dynamic remodeling, repeated in vivo observations of identified frog NMJs double-labeled with a vital florescent dye for nerve terminals and PNA for PSCs have been shown (Chen et al. 1991; Chen and Ko 1994; Ko and Chen 1996). These studies have revealed that PSCs and associated extracellular matrix often lead, and may guide, the nerve terminal sprouts. The dynamic relationship between PSCs and nerve terminals has also been confirmed using direct injection of fluorescent dyes into adult frog PSCs and nerve terminals (Macleod et al. 2001; Dickens et al. 2003). These findings suggest that the dynamic behavior of PSCs may contribute to the constant remodeling of nerve terminals seen at amphibian NMJs. In contrast, mammalian NMJs are relatively stable (Lichtman et al. 1987; Wigston 1989). However, there are minor nerve terminal filopodia and lamellipodia adjacent to PSCs (Robbins and Polak 1988), which also protrude short and unstable processes beyond AChR clusters at mammalian NMJs (Zuo et al. 2004). It is still unclear why adult mammalian NMJs show fewer morphological remodeling than amphibian NMJs.

Degeneration and Regeneration

After nerve injury, nerve terminals degenerate and PSCs become phagocytic to remove debris of degenerating nerve terminals at denervated NMJs (Birks et al. 1960). It is interesting to note that Schwann cells at denervated NMJs can release acetylcholine (Dennis and Miledi 1974) although its functional significance is unknown. One seminal work that stimulated our current belief of the novel role of PSCs was the discovery of profuse sprouting of PSC processes shortly after denervation at the mammalian NMJ (Reynolds and Woolf 1992; Astrow et al. 1994; Son and Thompson 1995a,b). Furthermore, on reinnervation, nerve terminals grow along PSC "bridges" formed with PSC sprouts from adjacent denervated junction, and form the so-called "escaped fibers" to innervate the adjacent denervated endplates (Fig. 2A,Ba–d). A similar role of PSC "bridges" in guiding nerve terminal sprouts has also been shown after partial denervation at the adult NMJ (Fig. 2A,Be–g) (Son and Thompson 1995a,b; Love and Thompson 1999). The dynamic relationship between PSC and regenerating nerve terminals after nerve injury at NMJs has been examined with repeated in vivo observations of the same NMJs labeled with vital dyes (O'Malley et al. 1999; Koirala et al. 2000), or in transgenic mice that express GFP in Schwann cells and CYP in axons (Kang et al. 2003). These in vivo studies further confirm that PSC sprouts guide regenerating nerve terminals following nerve injury. It has been suggested that NRG1-ErbB signaling is involved in PSC sprouting, as exogenous application of NRG1 to neonatal muscles or expression of constitutively activated ErbB2 receptors in PSCs induces sprouting and migration of PSCs away from endplate sites (Trachtenberg and Thompson 1997; Hayworth et al. 2006; Moody et al. 2006).

The essential role of PSCs in synaptic repair has also been shown by the absence of nerve terminal sprouting following partial denervation when PSC bridge formation is blocked by direct stimulation or exercise of muscles (Love and Thompson 1999; Love et al. 2003; Tam and Gordon 2003). The importance of PSC sprouts has further been implicated in *mdx* mice (a model for Duchenne muscular dystrophy), in which presynaptic expression of neuronal nitric oxide synthase is decreased and formation of PSC "bridges" is impaired, suggesting that these defects may contribute to the less effective reinnervation and muscle weakness in these mutant muscles (Personius and Sawyer 2005; Marques et al. 2006). PSCs have also been shown to express the chemorepellent Semaphorin 3A in a subset of NMJs that are vulnerable in amyotrophic lateral sclerosis (ALS) (De Winter et al. 2006). Enhanced expression of a cell-surface glycoprotein, CD44, in PSCs in an ALS mouse model further suggests a potential role of PSCs in the motor neuron disease (Gorlewicz et al. 2009). Impaired PSC sprouting seen in aged muscles may also explain the poor reinnervation after nerve injury during aging (Ka-

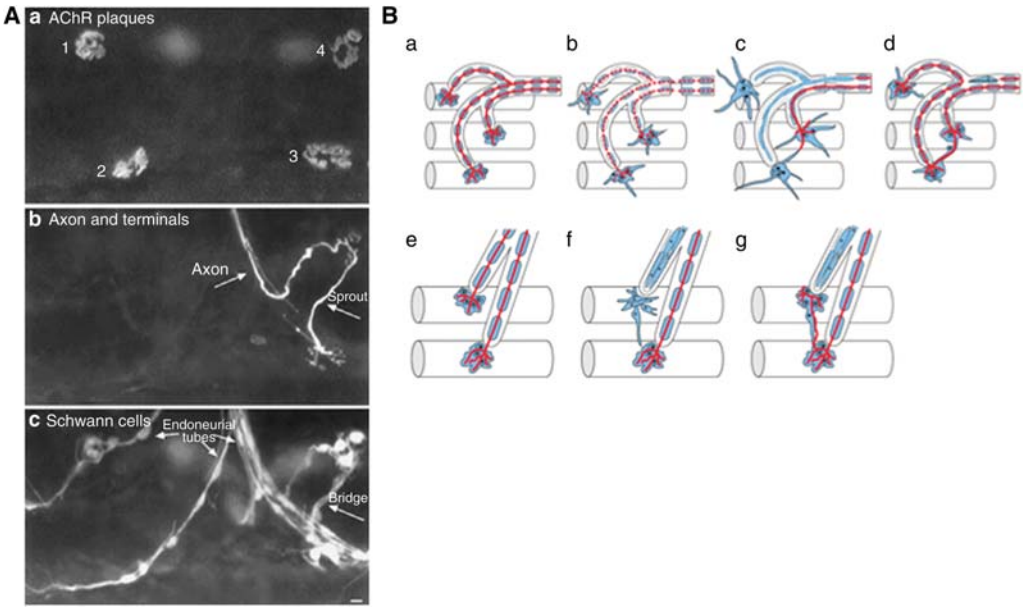

Figure 2. PSCs regulate NMJ repair and remodeling. (*A*) Sprouting after partial denervation. Four endplates are depicted in a rat soleus muscle (3 days after partial denervation) triple labeled with Cy5 conjugated α-BTX for AChRs (*a*), antibodies to neurofilament and synaptic vesicle protein (with an FITC-conjugated secondary antibody) for axons and nerve terminals (*b*), and antibody to S100 (with a rhodamine-conjugated secondary antibody) for PSCs and Schwann cells associated with the endoneurial tubes (*c*). Following partial denervation, Schwann cell processes extend profusely beyond the original endplate sites rich in AChR clusters (compare *a* and *c*). Although endplates 1 and 2 remain denervated, endplate 3 becomes innervated by a nerve sprout growing along a Schwann cell "bridge" linked to endplate 4, which is innervated (compare *a* and *b*). The role of PSCs in guiding nerve terminal sprouting is further depicted in a cartoon in *Be–g*. (From Love and Thompson 1999; reprinted, with permission, from the Society for Neuroscience © 1999.) (*B*) Schematic diagram summarizing the role of PSCs in reinnervation after nerve injury (*a–d*) and in sprouting after partial denervation (*e–g*) at mammalian NMJs. (*a*) Normal muscle fibers with intact NMJs (nerves in red, Schwann cells in blue). (*b*) PSCs sprout after nerve injury. (*c*) Regenerating nerve fibers grow along the endoneurial tubes and reinnervate synaptic sites (the middle muscle fiber). In addition, PSCs protrude processes further to form "bridges" connecting neighboring synaptic sites. (*d*) The PSC "bridges" guide regenerating nerve terminals to innervate adjacent endplates. The regenerating nerve fibers can continue to grow in a retrograde direction along other endoneurial tubes to innervate more endplates. (*e*) Normal muscle fibers with intact NMJs. (*f*) Partial denervation induces PSC sprouting. (*g*) Nerve terminals sprout along PSC bridges. (Panel *B* modified from data in Kang et al. 2003.)

wabuchi et al. 2001). Besides guiding presynaptic nerve terminals, PSCs are thought to play a role in clustering postsynaptic AChRs by expressing neuronal isoforms of agrin at the frog NMJ (Yang et al. 2001). Furthermore, PSCs may play a role in the synthesis of AChRs by expressing neuregulin-2 at the mammalian NMJ (Rimer et al. 2004). Together, these results suggest that PSCs play an important role in synaptic repair following degeneration and disease at the NMJ.

PHYSIOLOGICAL ROLES OF PSCs IN SYNAPTIC FUNCTION

The roles of PSCs in the regulation of maintenance and morphological plasticity of the NMJ underline a large degree of plasticity in PSCs as they must be able to change their properties in various synaptic contexts. Furthermore, these properties imply that PSCs must be able to analyze the synaptic situation to adjust to the changing synaptic environment. To this end,

PSCs decode synaptic properties of the NMJ by the detection of synaptic transmission, and attune to the fine changes that can take place. Hence, PSCs detect synaptic communication, decode the message and, in return, modulate synaptic properties in an intricate way adapted to the synaptic context.

PSCs DETECT SYNAPTIC TRANSMISSION

The development of fluorescent probes to detect free intracellular Ca^{2+} (Tsien 1981) has been a major advance for the study of the dynamic properties of glial cells and PSCs in particular. Indeed, the excitability of PSCs, like other glial cells, does not rely on electrical properties like neurons but rather on a biochemical excitability that largely relies on Ca^{2+}-dependent mechanisms (Auld and Robitaille 2003; Araque et al. 2014). Observations at the vertebrate NMJ were among the first to show that glial cells associated with intact chemical synapses detected synaptic transmission via G protein–coupled receptors (GPCRs) that controlled internal stores of Ca^{2+} (Fig. 3A,C) (Jahromi et al. 1992; Reist and Smith 1992). PSCs at other vertebrate NMJs were also shown to detect the release of neurotransmitters on stimulation of the motor nerve (Fig. 3A,B) (Rochon et al. 2001; Lin and Bennett 2006; Todd et al. 2007, 2010).

PSCs at mature amphibian and mouse NMJs possess muscarinic and purinergic receptors that regulate the release of Ca^{2+} from internal stores (Fig. 3A) (Robitaille 1995; Robitaille et al. 1997; Castonguay and Robitaille 2001; Rochon et al. 2001). At adult NMJs, detection of synaptic transmission by PSCs is mediated by muscarinic receptors (M1, M3, or M5) (Wright et al. 2009) and by purinergic receptors, in particular adenosine A1 receptors (Rochon et al. 2001). Although the characterization of the muscarinic receptor system follows a clear nomenclature, the properties of the purinergic receptor systems still elude a clear classification (Robitaille et al. 1997; Rochon et al. 2001; Rousse et al. 2010).

Consistent with their dynamic involvement in the regulation of the formation and maintenance of the NMJ, PSCs at immature NMJs (at postnatal day 7) also detect the activity of nerve terminals involved in synaptic competition at the mouse NMJ (Fig. 3D,E) (Darabid et al. 2013). Interestingly, the detection of synaptic activity is solely dependent on purinergic receptors, although muscarinic receptors are present and functional. This appears to be dependent upon the localization of the purinergic receptors close to active zones, whereas muscarinic receptors appear more evenly distributed over the PSCs (Darabid et al. 2013).

Interestingly, the biochemical excitability of PSCs, which allows them to detect neurotransmitter release, can be regulated. Indeed, Bourque and Robitaille (1998) showed that the peptide substance P released during sustained and intense synaptic activity at the mature amphibian NMJ caused a reduction in the sensitivity of the muscarinic detection, leading to a reduction in the size of the nerve-evoked Ca^{2+} responses in PSCs (Fig. 3A). Another molecule, nitric oxide (NO), acts in an autocrine manner. Descarries et al. (1998) observed that the synthesizing enzymes for NO are present in PSCs and that NO reduced the efficacy of ATP to elicit Ca^{2+} elevation in PSCs of mature amphibian NMJ.

Three major conclusions can be reached when comparing the properties of PSCs at different NMJs. First, the basic mechanisms are common throughout the different types of NMJ studied. Indeed, the detection of synaptic transmission by PSCs at adult NMJs is always carried by muscarinic and/or purinergic receptors (Robitaille 1995; Robitaille et al. 1997; Rochon et al. 2001; Colomar and Robitaille 2004; Darabid et al. 2013), indicating that fundamental mechanisms are preserved throughout a large sample of NMJs and developmental stages. Second, and somewhat contradictory, PSC properties are also tuned with the properties of the NMJ they are associated with. For instance, PSCs of weaker (e.g., soleus muscle) and stronger (levator auris longus [LAL] muscle) NMJs respond differently to nerve-evoked release of neurotransmitters where the weaker synapses systematically evoked smaller Ca^{2+} elevation in PSCs. These differences are largely the result of the different intrinsic properties of the PSCs at the different synapses (Rousse et al. 2010). Third, the excitability of PSCs can be dynamically modulated either by

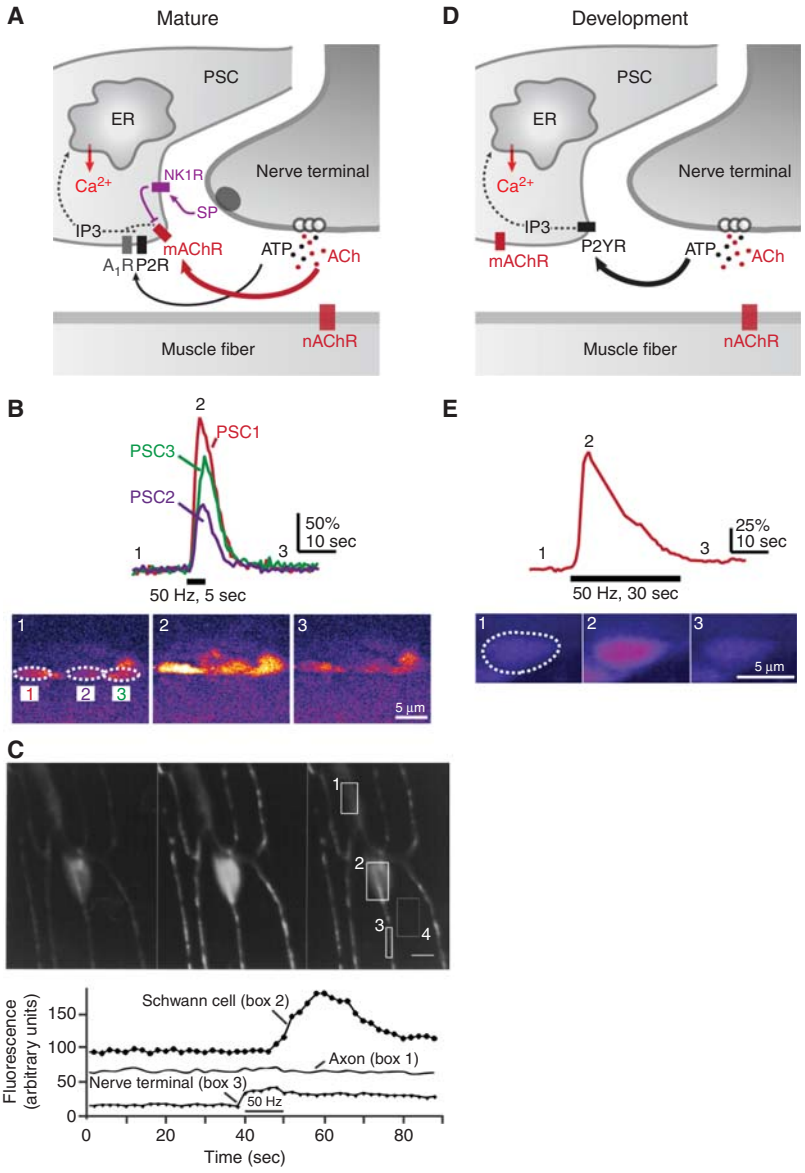

Figure 3. PSCs detect synaptic transmission. (*A*) Diagram depicting the receptors and their actions by which PSCs detect synaptic transmission at mature NMJ and the main regulatory mechanisms. (*B*) (*Top*) Changes in fluorescence of a Ca^{2+} indicator in PSCs of a mature mouse NMJ before, during, and after motor nerve stimulation. (*Bottom*) False color confocal images of the PSCs loaded with a Ca^{2+} indicator and from which the traces have been measured. (*C*) Images of an amphibian neuromuscular preparation showing the changes in fluorescence observed in the axonal compartment (1), the soma of a PSC (2), and the presynaptic terminal area (3) before, during, and after motor nerve stimulation (bar). (*D*) Diagram depicting the receptors and their actions by which PSCs detect synaptic transmission at developing NMJ. (*E*) (*Top*) Changes in fluorescence of a Ca^{2+} indicator in a PSC of an immature (P7) mouse NMJ before, during, and after motor nerve stimulation. (*Bottom*) False color confocal images of the PSCs loaded with a Ca^{2+} indicator and from which the traces have been measured. (Panel *C* from Reist and Smith 1992; reprinted, with permission, from the National Academy of Sciences.)

presynaptic signaling or in an autocrine manner, indicating that the properties of the PSCs and the possible resulting modulation can be adapted (Bélair et al. 2010). Hence, similar to the neuronal elements at the synapse, there are basic, fundamental mechanisms that drive PSCs excitability and responsiveness to synaptic activity, but these properties are in tune with the properties of the synapse they are associated with. This is a fundamental property because it implies that PSCs are adapted to a given synaptic environment and, hence, can participate to the regulation of NMJ properties in a precise and adapted manner.

PSCs DECODE SYNAPTIC PROPERTIES AND ACTIVITY

The different properties of PSCs according to the different synaptic context further suggested that the Ca^{2+}-dependent biochemical excitability of PSCs allowed them to decode synaptic activity. Furthermore, owing to the impacts on cell activity of cytoplasmic changes of Ca^{2+} and the importance of the amplitude and kinetics of such changes, one could argue that such changes represent a code that reflects the level and type of synaptic activity.

There are two recent observations that support this possibility. First, Todd et al. (2010) reported that PSCs at the mouse soleus NMJs detected two different patterns of synaptic activity with different kinetics and amplitude of Ca^{2+} responses (Fig. 4A–C). One pattern was a continuous stimulation (20 Hz, 90 sec) while the other generated a series of burst stimulation (same total duration and number of stimuli). The pattern of continuous stimulation generated a large phasic Ca^{2+} elevation, similar to those previously reported (Fig. 4B,C) (Jahromi et al. 1992; Robitaille 1995; Rochon et al. 2001; Todd et al. 2007, 2010). However, the bursting stimulation generated a sustained Ca^{2+} elevation on which irregular and small Ca^{2+} elevations were observed (Fig. 4B,C). This revealed that the different properties of synaptic signaling was decoded by PSCs and reflected in the differences in the timing, duration, and pattern of Ca^{2+} elevation.

PSCs ability to decode the nature of the synaptic properties was also unraveled during the course of synaptic competition that occurs postnatal at the NMJ (Fig. 4D–G). As indicated above, Darabid et al. (2013) studied PSCs ability to detect transmitter release evoked selectively by two nerve terminals competing for the same postsynaptic site. Ca^{2+} elevations were quite variable and were dependent on the synaptic strength (amount of transmitter release) of each nerve terminal (Fig. 4E,F). Indeed, the stronger nerve terminal (releasing more neurotransmitter) systematically induced larger Ca^{2+} responses than the weak nerve terminal (Fig. 4F) (Darabid et al. 2013). Importantly, PSC Ca^{2+} responses were unaltered in the presence of the K^+ channel blocker (tetraethylamonium [TEA]), which increased transmitter release without affecting directly PSCs excitability (Rousse et al. 2010; Darabid et al. 2013). This indicates that differences in Ca^{2+} kinetics elicited by the two nerve terminals were also determined by intrinsic properties of PSCs. These results indicate that PSCs not only detect the two terminals, but also decode the ongoing competition.

As a whole, these observations indicate that PSCs, through dynamic Ca^{2+} regulation, decode synaptic communication in a given situation. This is particularly important when considering the PSCs as synaptic partners because their properties should be adapted to a given synaptic environment.

PSCs MODULATE SYNAPTIC ACTIVITY AND PLASTICITY

The ability of PSCs to be in tune with the properties of the synapse and decode the pattern of synaptic activity at adult NMJs and ongoing synaptic competition at developing NMJs are strong indicators that PSCs should be able, in return, to talk back to the pre- and postsynaptic elements and modulate the properties of the synaptic communication.

The first observation of synaptic activity modulation by PSCs was made by Robitaille (1998), using the amphibian NMJ. He showed that injection of molecules that increased G-protein activity specifically in PSCs reduced

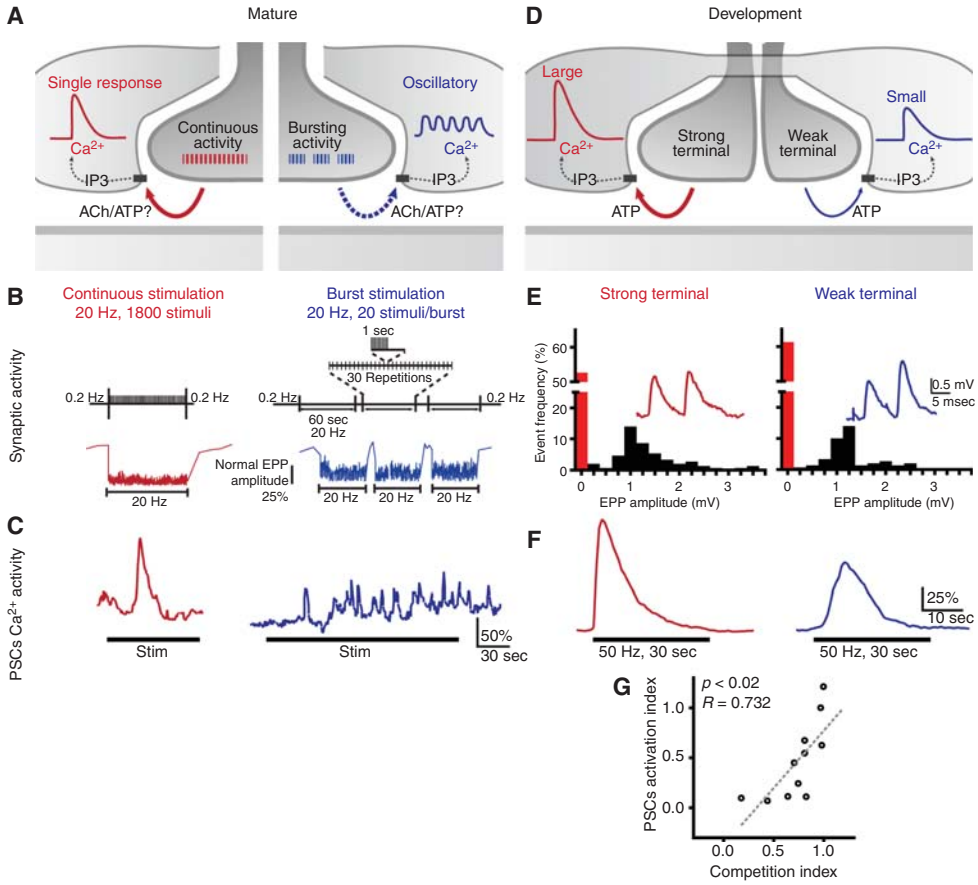

Figure 4. PSCs decode synaptic information. (*A*) Diagram depicting the Ca^{2+} responses in PSCs and the mechanisms involved when motor nerve activity is induced using two different patterns of stimulation (continuous or bursting activity) at mouse mature NMJs. (*B*) The bursting pattern consisting of 30 repetitions of 20 pulses at 20 Hz repeated every 2 sec and a continuous pattern of stimulation at 20 Hz for 90 sec. (*C*) Typical Ca^{2+} responses elicited by the bursting and the continuous motor nerve stimulation illustrated in *B*. Note the difference in the kinetics of the Ca^{2+} responses revealing the ability of PSCs to decode the pattern of synaptic activity. (*D*) Diagram depicting the Ca^{2+} responses in PSCs elicited by independent activity of competing nerve terminals (weak and strong) at NMJs during synapse formation. (*E*) Quantal analysis based on the failure rates of two competing inputs at an immature NMJ. Note the larger percentage of failures of the weak nerve terminal. (*F*) Independent Ca^{2+} responses in the PSC that covers the same two terminals (weak and strong) in *E*. Note the difference in the amplitude of the two responses, the stronger terminal eliciting a larger Ca^{2+} response. (*G*) A PSC activation index as a function of the synaptic strength index showing a continuum in the amplitude of Ca^{2+} responses as a function of the relative strength of competing nerve terminals. These results indicate that a single PSC can decipher the strength of nerve terminals competing for the territory at a same NMJ. (Panels *B* and *C* from data in Todd et al. 2010; and panels *E*–*G* from data in Darabid et al. 2013.)

the amount of transmitter release (Fig. 5A). More importantly, he showed that blocking G-protein activation prevented a large portion of synaptic depression, a short-term synaptic plasticity that occurs at this synapse (Fig. 5B,C). Hence, this was one of the first examples of direct evidence that glial cells at an intact vertebrate synapse were controlling transmitter release and modulating synaptic plasticity. This piece of evidence was a key observation from which the concept of the "tripartite synapse" originated (Araque et al. 1999; Auld and Robi-

taille 2003). This provided a direct demonstration of the dynamic, bidirectional neuron–glia interactions that occur at the NMJ and further emphasizes that PSCs are active and competent synaptic partners at this synapse.

Synaptic plasticity at any synapses is often a balance of reduction (depression) and increase (potentiation) of synaptic efficacy. Hence, it was hypothesized that, if glial cells are indeed competent partners, they would also have the ability to increase synaptic efficacy. This was observed by Castonguay and Robitaille (2001), who showed that selectively chelating Ca^{2+} in PSCs of amphibian NMJs resulted in an increase in synaptic depression, suggesting that a potentiation event was perturbed.

These results indicate that PSCs have the ability to both decrease and increase synaptic

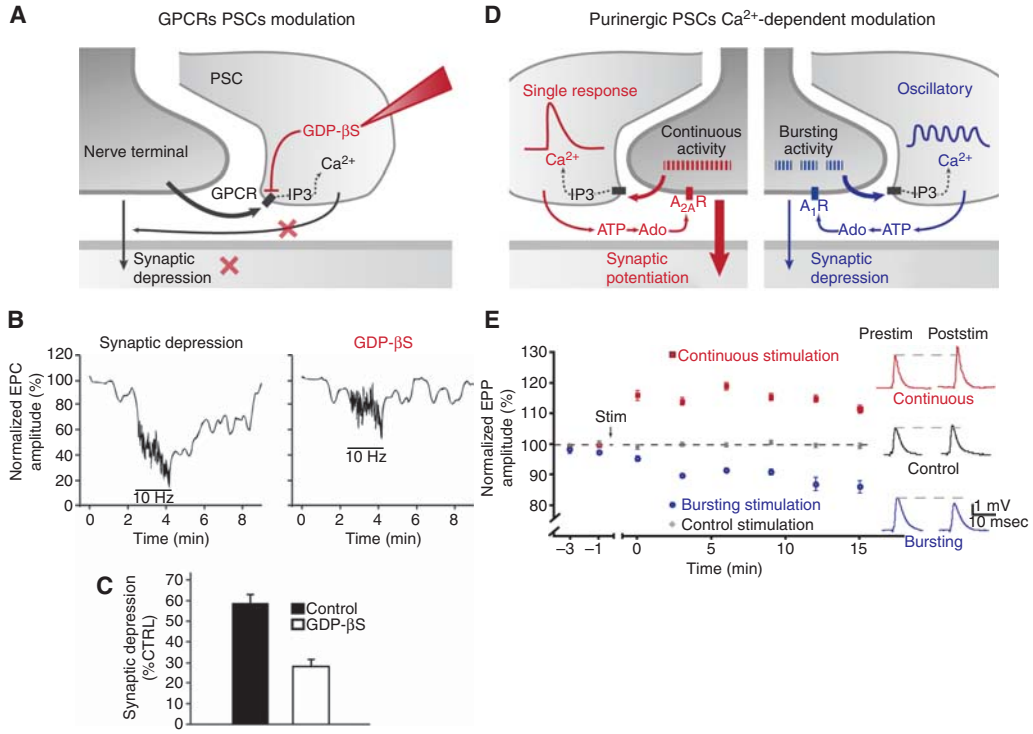

Figure 5. PSCs modulate synaptic transmission and plasticity. (*A*) Diagram of the glial mechanisms involved in synaptic regulation at the mature amphibian NMJ on manipulation of GTP-binding proteins. (*B*) (*Left*) Relative endplate potential amplitude at the frog NMJ before (0.2 Hz), during (10 Hz, 90 sec), and after (0.2 Hz) high-frequency motor nerve stimulation. Note the occurrence of synaptic depression during the high-frequency stimulation. (*Right*) Same protocol performed on the same NMJ as in the *left* panel, but following the injection of GDP-βS in a PSC to block G-protein activity. (*C*) Histogram illustrating the average depression in control and after injection of GDP-βS in PSCs. Note that synaptic depression was significantly reduced following the selective G-protein blockade in PSCs. (*D*) Diagram of the glial mechanisms involved in synaptic regulation at the mature mouse NMJ as a result of the differential activation of PSCs by the two different patterns of stimulation. (*E*) Changes in EPP amplitude at mouse NMJ evoked by the two patterns of stimulation illustrated in Figure 4B. The continuous stimulation induced a long-lasting potentiation that was caused by the phasic and rapid Ca^{2+} elevation in PSC (*inset*), whereas the bursting pattern of stimulation induced a long-lasting depression that was caused by the small and sustained changes in Ca^{2+} in PSCs. Both forms of plasticity were altered when selectively blocking Ca^{2+} elevation in PSCs. These results indicate that, based on their decoding of synaptic activity, PSCs regulate synaptic efficacy and plasticity. Stim, stimulation. (Panels *B* and *C* from data in Robitaille 1998; and panel *E* from data in Todd et al. 2010.)

efficacy, hence, fine tuning the net output at the NMJ regulating muscle functions. However, these results did not indicate that PSCs used this ability to simultaneously regulate synaptic efficacy in a given synaptic context. This was unraveled when studying the decoding ability of PSCs. As indicated above, Todd et al. (2010) observed that different patterns of synaptic activity elicited Ca^{2+} responses with different kinetics and amplitude (Figs. 4 and 5D). Concomitantly, these different patterns of motor nerve stimulation induced different forms of synaptic plasticity, such that the continuous stimulation produced a long-lasting potentiation, whereas the bursting pattern generated a long-lasting depression (Fig. 5D,E). Using selective blockade of Ca^{2+} elevation in PSCs through photo-activation of caged Ca^{2+}, Todd et al. (2010) showed that the different Ca^{2+} signaling in PSCs were responsible for the different synaptic plasticity. This differential modulation was caused by the activation of different types of adenosine receptors (A1 receptors causing depression, A2A receptors causing the potentiation) on hydrolysis of ATP following its release by PSCs (Fig. 5D). Hence, not only do PSCs decode the ongoing synaptic transmission but, as a result, they also react differentially to produce an adapted modulation.

This modulation further illustrates that PSCs, much like other glial cells, release neuromodulatory substances identified as gliotransmitters (Araque et al. 2014). In addition to the involvement of ATP and adenosine in the differential modulation of synaptic transmission, observations from amphibian, lizard, and mouse NMJs indicated that PSCs may also produce and release other potential neuromodulatory substances, such as glutamate, prostaglandins, and nitric oxide (Descarries et al. 1998; Pinard et al. 2003; Pinard and Robitaille 2008; Lindgren et al. 2013). However, it is unclear whether PSCs combine any of these gliotransmitters and whether the same PSC can release them in a differential manner.

PLASTICITY OF PSC PROPERTIES

The results discussed above highlight the fine and efficient regulation of transmitter release by PSCs. In addition, acute modulations in the excitability of PSCs have been discussed, providing evidence that these cells are intrinsically plastic, capable of adapting to a changing synaptic environment. More importantly, it raises the question as to whether PSCs could undergo long term changes in their properties, allowing them to adjust to the changes in the synaptic properties themselves. Two sets of recent evidence supports this possibility.

First, Bélair et al. (2005, 2010) showed that the presynaptic properties of the amphibian NMJ undergo significant adaptation following two different in vivo approaches to alter long-term properties of the presynaptic release of neurotransmitters. Interestingly, PSC properties also underwent a long-term plasticity of their properties. These changes were not directly correlated with the level of transmitter release and instead involved an alteration of the muscarinic- and purinergic-dependent activation of PSCs (Bélair et al. 2010). Hence, this resulted in the alteration of the PSCs decoding ability and possibly of the outcome of their modulation of synaptic transmission. Importantly, these observations reveal that, similar to neurons, glial cells also undergo plastic changes in their properties. Furthermore, it remains to be determined whether the changes in PSCs properties contributed to the changes in the synaptic properties.

The second evidence of long-term plasticity of PSC properties originates from the study of their properties during synapse development. Indeed, it was shown that PSCs detect transmitter release mainly via purinergic receptors during synaptic competition (Darabid et al. 2013) even though muscarinic receptors are present and functional. This is quite different from the situation at mature NMJ in which PSCs detection of synaptic activity heavily relies on the activation of muscarinic receptors. This switch of the type of signaling mechanisms during the maturation of the synapse reflects the adaptation of the function of these cells from the context of synapse formation to a stable and synaptically reliable one.

Hence, not only do PSCs interact dynamically and in a bidirectional manner with the pre- and postsynaptic elements of the NMJ, but also

these interactions are highly plastic indicating that PSCs regulation of the NMJ properties can also be adaptive.

PSCs INTEGRATE SYNAPTIC ACTIVITY TO ESTABLISH SYNAPTIC PROPERTIES

The two main roles of PSCs at the NMJ (i.e., morphological stability/plasticity and synaptic regulation) appear as two independent functions. However, a number of observations indicate that in fact both functions are tightly linked and are essential for the balance between synaptic efficacy and stability and synaptic plasticity and repair. Indeed, the same receptor systems that PSCs use to detect and decode synaptic transmission are also used to regulate a number of genes involved in their reaction on injury (Georgiou et al. 1994, 1999). In fact, using the amphibian NMJ model, these investigators have shown that interruption of synaptic communication is sufficient to trigger an injury-like response in PSCs. This was mediated specifically by the muscarinic receptors, not the purinergic ones, and appears not to depend directly on Ca^{2+}, but rather on CREB-like regulation pathways. Interestingly, Wright et al. (2009) observed that the blockade of muscarinic receptors in vivo induced injury-related changes in PSCs, in particular an abundant level of PSC process sprouting that is normally observed after axonal injury (Son and Thompson 1995b). This suggests that the muscarinic receptor system is particularly important in regulating the PSCs in a mode of maintenance and regulation of synaptic efficacy. Consistent with the data at mature NMJs, it is remarkable that in condition when important changes occur at the NMJ such at developing NMJs during synaptic competition, only purinergic receptors (not the muscarinic ones) are actively recruited by synaptic transmission. Hence, it appears that the contribution of muscarinic receptors is much reduced in situations in which major morphological and functional rearrangement of the NMJs are required (synapse formation or after injury). However, it is unclear whether these changes in receptor activation are caused by the level of receptor expression, the type of receptors, and/or the cellular mechanisms they control. Furthermore, the regulation of PSCs excitability by trophic factors, such as neurotrophin-3 (NT-3), brain-derived neurotophic factor (BDNF), or nerve growth factor (NGF) (Todd et al. 2007), that are also involved in NMJ formation and stabilization also points to the possibility that PSCs two main functions are very interdependent.

We propose a model to integrate the different functions and properties of PSCs according to the different functional states of the NMJ. We propose that activation of PSCs by GPCRs determines the balance between synaptic efficacy/maintenance and remodeling/repair (Fig. 6). At adult NMJ, normal synaptic activity would be detected by a set of muscarinic and purinergic receptors that would regulate the feedback modulation to synaptic functions (modulation, left loop). However, the same receptors also impose a regulation of the expression of a number of genes that allow PSCs to ensure the maintenance and efficacy of the NMJ (maintenance, right loop). On injury or diseases, the balance between muscarinic and purinergic receptor activation would be impaired, altering the gene regulation, thus allowing a change in PSCs phenotype that would allow them to enter into a repair mode (repair, far right loop). Ultimately, this would allow the NMJ to be repaired and synaptic communication reestablished. At this point, PSCs would regain their normal functions (left and right loops).

CONCLUDING REMARKS

PSCs have two main critical functions at the NMJ. First, they control synapse stability. During development, they guide growing nerve terminals and are essential for synaptic growth and maintenance at developing NMJs. Chronic absence of PSCs results in retraction of nerve terminals and reduction in synaptic function, suggesting a long-term maintenance role of PSCs. Similar to developing NMJs, PSCs in adult muscles guide nerve terminal growth during synaptic sprouting and repair after nerve injury. Second, they control synapse plasticity. Owing to their dynamic detection of synaptic trans-

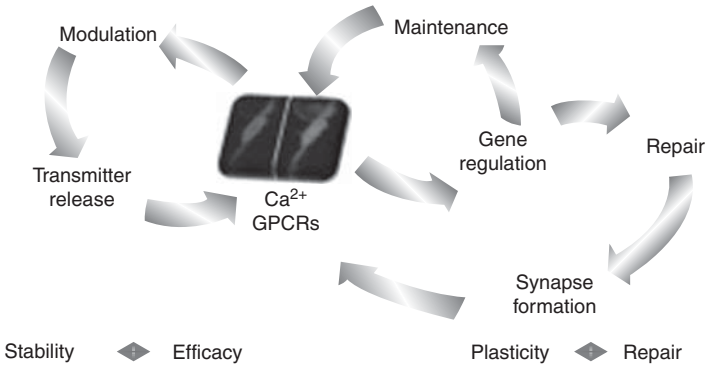

Figure 6. Model of PSCs balanced regulation of NMJ stability and plasticity. PSCs (illustrated as a responding cell) detect synaptic activation through activation of G protein–coupled receptors leading to the activation of Ca^{2+}-dependent events that lead to the modulation of synaptic transmission and plasticity (modulation, *left* loop). The same receptor activation also leads to the regulation of the expression of a number of genes that promote PSCs activity to sustain maintenance and stability of the NMJ (maintenance, *right* loop). However, on dysregulation of transmitter release or following injury, the signaling in PSCs is perturbed, leading to a change in the gene regulation and a switch of PSC phenotype from maintenance to repair (repair, *far right* loop). This repair mode includes removing of remnants of injured nerve terminals and PSC bridging processes to facilitate nerve terminal sprouting toward denervated endplates. Hence, PSCs can integrate both the efficacy and the plasticity of the NMJ to establish the appropriate response according to the state of the NMJ. GPCRs, G protein–coupled receptors.

mission, PSCs can control both the efficacy and the maintenance of the NMJ. This surveillance allows them to alter their properties to allow for synapse repair on injury or other weakening of the synapse. The future challenges would be to unravel the molecular mechanisms of synapse–glia interactions and their likely involvement in neurodegenerative diseases.

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