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Glial Modulation of Synaptic Transmission at the Neuromuscular Junction

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ABSTRACT The neuromuscular junction (NMJ) is a cholinergic synapse that controls muscle contraction. Glial cells, called perisynaptic Schwann cells, surround nerve terminals at the NMJ. Transmitter release induced by repetitive nerve stimulation, elicit a frequency-dependent activation of G-protein-coupled receptors on perisynaptic Schwann cells and the release of calcium from internal stores. In return, perisynaptic Schwann cells modulate synaptic activity during and following high-frequency stimulation through short-term plasticity. In the present review, we discuss evidence of glial involvement in the short-term plasticity at the NMJ and the potential impact of such modulation on synaptic efficacy. © 2004 Wiley-Liss, Inc.

INTRODUCTION

The neuromuscular junction (NMJ) is the site of information transmission from the motor neuron to a muscle fiber. In the adult, motor neuron innervation of muscle fibers is monosynaptic, under most conditions. Its accessibility, large size, and simplicity have contributed to making it one of the most studied synapses and one of the best models for the study of synaptic transmission through the nervous system. At the NMJ, the entire synaptic structure is covered with specialized glial cells. These cells are termed perisynaptic Schwann cells or terminal Schwann cells. Unlike myelinating Schwann cells which surround motor neuron axons, perisynaptic Schwann cells are nonmyelinating cells. They play a role in the recovery of synaptic integrity following injury, as well as helping to establish synaptic efficacy (Reddy et al., 2003; for a review of synaptic recovery and Schwann cells, see Son et al., 1996). In this review, we will focus our attention on the short-term modulation that perisynaptic Schwann cells can exert on synaptic activity at the NMJ.

First we will briefly describe the synaptic transmission at the NMJ. Then we will discuss studies showing that perisynaptic Schwann cells are sensitive to synaptic activity and how these glial cells modulate synaptic transmission. Finally, we will discuss the potential impact of a glial modulation of NMJ activity under physiological or pathological conditions.

SYNAPTIC TRANSMISSION AND PLASTICITY AT THE NMJ

At the NMJ, synaptic transmission is mediated by acetylcholine (ACh; Dale et al., 1936; Langley, 1905). An action potential arrival at the nerve terminal induces the opening of N-type calcium channels (N-type in frog, mammals and/or P/Q types in mammals) that are clustered at active zones (Robitaille et al., 1990; Westenbroek et al., 1998). The subsequent massive calcium entry in the nerve terminal induces exocytosis of synaptic vesicles and the release of ACh, which activates postsynaptic muscular nicotinic receptors (Takeuchi and Takeuchi, 1960). The opening of nicotinic receptors generates membrane depolarization, the end-plate potential (EPP), which further opens voltage-dependent sodium channels and initiates the onset of the muscular action potential, leading to mus-

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cle contraction. Unlike most synapses of the central nervous system, neurotransmitter release at the NMJ corresponds to the release of a large number of synaptic vesicles (20–200, depending on species) (Del Castillo and Katz, 1954; Slater et al., 1992). Under physiological conditions, the number of ACh quanta associated with each action potential is always higher than is necessary to establish a muscular action potential. This excess of quanta released establishes the safety factor of the NMJ (for review, see Wood and Slater, 2001), making this synapse extremely reliable.

To control muscle contractions, the release of ACh occurs in high-frequency bursts of activity, since motor neurons fire action potentials at high frequency. Interestingly, at these frequencies, the NMJ undergoes various forms of short-term plasticity (lasting up to a few minutes) both during and immediately following high-frequency stimulation. There are two main categories of plasticity events: potentiating events (when EPP amplitude is increased) and depressing events (when EPP amplitude is decreased). First, the increase of synaptic transmission is named facilitation (when lasting hundreds of milliseconds [ms]), augmentation (when lasting a few seconds) or potentiation (when lasting up to several minutes). Facilitation can be observed when the time between two stimulations is less than some tens of microseconds. Augmentation and post-tetanic potentiation can be obtained for stimulation lasting tens of seconds with frequencies usually from tens to hundreds of Hertz. Second, the decrease in synaptic transmission is named synaptic depression. Interestingly, and most importantly, the same level of synaptic activity induces potentiating events (augmentation and potentiation) as well as synaptic depression (for review, see Zucker and Regher, 2002). As a consequence, the net plasticity phenomenon that one observes is often the result of a summation of different plastic events. Or, plastic events (e.g., depression and post-tetanic potentiation) can also be temporally spaced and result in a succession of different plastic events. Potentiation and depression are better differentiated when the level of transmitter release is changed by modifying the external Ca^{2+} concentration: potentiation is more pronounced when basal synaptic transmission is decreased while depression is more pronounced when basal synaptic transmission is increased. Thus, the plasticity event that prevails in normal physiological conditions is synaptic depression.

In addition to ACh, there are other neurotransmitters and modulators that influence synaptic activity. For instance, synaptic vesicles also contain a significant amount of ATP (ACh/ATP = 5 to 10) (Dowdall et al., 1974; Wagner et al., 1978) that, once released, is quickly degraded to adenosine by ectonucleotidases (Keller and Zimmermann, 1983). However, only one-half of adenosine acting at the NMJ comes from hydrolysis of released ATP; the other half comes by direct adenosine release from the activated muscle fiber (Smith, 1991). Adenosine contributes to synaptic depression expressed during 1–2-Hz nerve stimulations (Redman and Silinsky, 1993). ATP, however, has a potentiating effect during embryonic development (Fu and Huang, 1994), but appears to have a depressing effect on ACh release at the adult NMJ (Giniatullin and Sokolova, 1998). Dense-core vesicles that contain calcitonin-gene-related-peptide (CGRP) and substance P (Matteoli et al., 1988, 1990) are also present in nerve terminals of frog NMJs. They are located on the margin of active zones, adjacent to perisynaptic Schwann cells, and are released after a prolonged nerve stimulation (Pécot-Dechavassine and Brouard, 1997). The effects of substance P and CGRP on synaptic transmission have not been fully elucidated. Although ACh and ATP are released together in any condition of synaptic activity, additional release of neuromodulators such as substance P or CGRP occurs during high-frequency, long-lasting stimulation.

PERISYNAPTIC SCHWANN CELLS ARE SENSITIVE TO NEURONAL ACTIVITY

Perisynaptic Schwann cells surround the nerve terminal at the NMJ. In the frog, they send finger-like processes every 1–3 μm around nerve terminal. These processes are at the proximity of presynaptic active zones and thus are ideally situated to detect synaptic activity (Couteaux and Pécot-Dechavassine, 1974).

Perisynaptic Schwann cells at amphibian as well as mammalian NMJs are sensitive to synaptic activity. Indeed, high-frequency nerve stimulation, in ranges that induce the plasticity phenomena discussed above, induce an intracellular calcium increase in perisynaptic Schwann cells (Jahromi et al., 1992; Reist and Smith, 1992; Rochon et al., 2001). This Schwann cell activation occurs in a frequency-dependent manner, since a low-frequency stimulation, such as 0.2 Hz, does not lead to increased levels of intracellular calcium. The amplitude of responses can vary at the same junction from one perisynaptic Schwann cell to the next, suggesting a sensitivity, and possibly even a differential effect, of each perisynaptic Schwann cell on synaptic transmission. Moreover, a rundown in the amplitude of Ca^{2+} responses is observed following repeated glial trains of nerve stimulations (Jahromi et al., 1992; Reist and Smith, 1992; Rousse and Robitaille, 2001). Finally, the glial calcium response has a relatively slow onset (~2 s after the beginning of nerve stimulation). Thus, it is quite unlikely that glial cells will influence the synaptic activity that induced the glial activity itself but, rather, that glial cells will influence the synaptic events that follow the glial activation.

What do Schwann cells detect: presynaptic action potential frequency, neurotransmitter release, or muscle fiber activation? Perisynaptic Schwann cells detect neurotransmitter release, since ω-conotoxin GVIA, through inhibition of presynaptic N-type calcium channels responsible for synaptic vesicle exocytosis, induced a strong inhibition of the glial calcium increase (Jahromi et al., 1992). Moreover, the glial calcium re-

sponse does not depend on muscle fiber activation, as it is unaffected by antagonism of postsynaptic nicotinic receptors (Jahromi et al., 1992) and is even induced despite muscle fiber degeneration (Reist and Smith, 1992).

In frog, perisynaptic Schwann cells express many receptors for neurotransmitters and neuromodulators that are present at the NMJ. They possess muscarinic ACh receptors of as yet unidentified subtype (Robitaille et al., 1997), metabotropic (P2Y) and ionotropic (P2X) ATP receptors, A1 adenosine receptors (Robitaille 1995), and NK1 substance P receptors (Bourque and Robitaille, 1998). All of these receptors, except P2X receptors, are G-protein-coupled receptors whose activation can lead to G-protein-activated pathways and in particular to the release of calcium from intracellular stores. Moreover, the activation of P2X receptors is linked to an L-type calcium channel activation on perisynaptic Schwann cells (Robitaille, 1995).

What neurotransmitter(s) are important for the activation of glial cells during synaptic activity? ACh, ATP, and adenosine are all able to mimic high-frequency synaptic activity associated glial calcium increases (Jahromi et al., 1992; Robitaille, 1995). Among these, ATP at the frog NMJ seems of great importance because suramin, an inhibitor of purinergic receptors, is able to block 87% of the glial calcium response following high-frequency nervous stimulation (Robitaille, 1995).

At the mouse NMJ, perisynaptic Schwann cells also possess muscarinic receptors, P2X and P2Y purinergic receptors as well as A1 adenosine receptors (Rochon et al., 2001; Rousse et al., 2003). However, in this case, ACh and adenosine appear to be responsible for glial activation, as both muscarinic and adenosine receptor antagonists are able to decrease the Schwann cell calcium response strongly in an additive fashion (Rochon et al., 2001).

Other endogenous substances act as modulators of perisynaptic Schwann cell activity. Indeed, at the frog NMJ, application of substance P decreases the amplitude of the glial calcium responses evoked by ACh or ATP (Bourque and Robitaille, 1998). Thus, substance P does not appear to contribute directly to the induction of glial calcium increase that follows high-frequency nervous stimulation; however, it could be responsible for the rundown of glial calcium responses during repeated stimulations. In this way, an NK1 receptor antagonist, that inhibits substance P receptors, prevents the reduction of the glial calcium responses that follow a prolonged nerve stimulation, thus maintaining glial calcium responses at a heightened level (Bourque and Robitaille, 1998).

Thus, the release of neurotransmitters and modulators during high-frequency-induced synaptic transmission elicits perisynaptic Schwann cells activity mainly via G-protein-coupled receptor activation that leads to the release of calcium from internal stores.

PERISYNAPTIC SCHWANN CELLS MODULATE SYNAPTIC TRANSMISSION

Owing to the preponderance of the G-protein-coupled receptors and the involvement of internal Ca^{2+} stores in perisynaptic Schwann cells activation, it is quite likely that these cellular mechanisms are involved in any potential modulation of synaptic functions by perisynaptic Schwann cells. We will now present evidence indicating that perisynaptic Schwann cell G-protein activity and calcium internal stores are implicated in the modulation of synaptic activity and plasticity at the NMJ. To obtain clear results regarding the involvement of perisynaptic Schwann cells on synaptic transmission, it is necessary to use an approach that will warrant the specificity of the action on these glial cells, leaving the presynaptic terminal and the muscle fiber untouched. An experimental protocol was designed to allow specific iontophoretic injection of pharmacological compounds in the cell body of a perisynaptic Schwann cell in conjunction with a focal recording of synaptic activity (focally recorded EPPs are termed end-plate current [EPC]). This enabled the detection of the modulation that a single Schwann cell exerts on the activity of the nerve terminal covered by this Schwann cell. Using this protocol, one would predict that the injection of antagonists in the perisynaptic Schwann cells would inhibit the plasticity event studied during high-frequency nerve stimulation (e.g., inhibiting the decrease of EPPs during depression). However, the use of agonists should reproduce the plastic event (e.g., decrease of the amplitude of EPPs) without high-frequency stimulation, i.e., during basal low-frequency stimulation (0.2 Hz).

GTP-γS is a nonhydrolyzable analogue of GTP that maintains G-proteins in an active state and should promote perisynaptic Schwann cell activity. Injection of GTP-γS in the cell body of a perisynaptic Schwann cell induced a decrease in the basal amplitude of EPPs, suggesting the ability of perisynaptic Schwann cells to depress synaptic transmission. However, injection of GTP-γS did not modify the amplitude of miniature EPP (mEPP) indicating that the sensitivity of postsynaptic receptors to spontaneous neurotransmitter release was not affected by glial G-protein activation. This suggests that the effects of glial G-protein activation are likely presynaptic, modulating transmitter release. In addition, frequency of mEPP was not affected by GTP-γS injection, suggesting that it did not affect the presynaptic calcium homeostasis controlling neurotransmitter release. Thus, the presynaptic modulation of the glial G proteins may be downstream from the calcium entry in the nerve terminal (Robitaille, 1998).

The evidence that a massive activation of G-proteins in perisynaptic Schwann cells induces a reduction in transmitter release only indicates that the glial cells at the NMJ have the capacity to modulate synaptic transmission, likely to depress transmitter release. However, it does not provide any insight as to whether these cells do modulate endogenous synaptic activity.

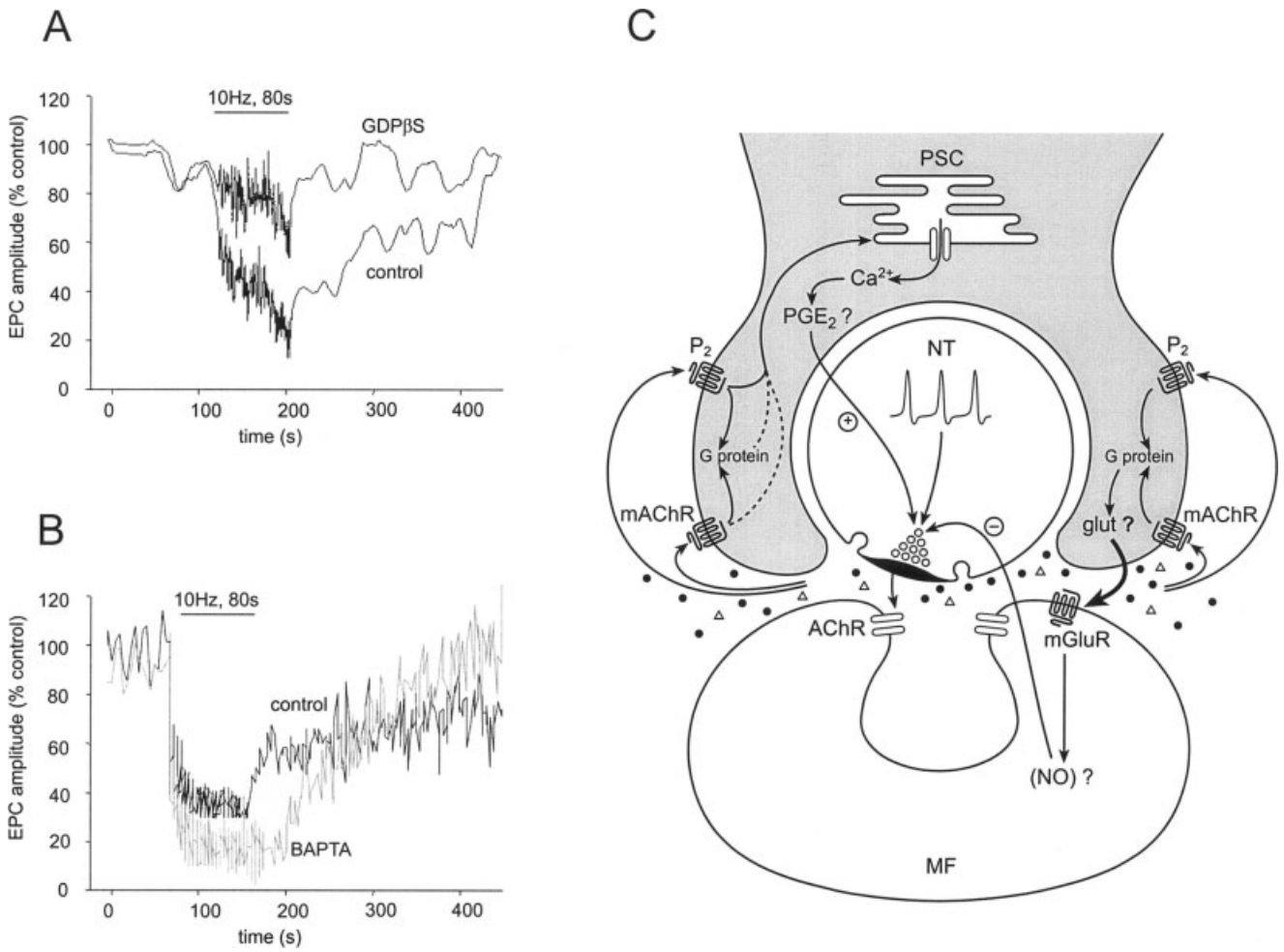

Fig. 1. Perisynaptic Schwann cell modulation of synaptic activity at the neuromuscular junction (NMJ). **A:** The inhibition of glial G-protein pathways by injection of GDPβS in a perisynaptic Schwann cell reduced synaptic depression induced by high-frequency stimulation (10 Hz, 80 s). Focal electrode recording of end-plate currents (EPC) were performed to assess synaptic activity adjacent to the injected Schwann cell. (Adapted from Robitaille, 1998.) **B:** Focal electrode recording of end-plate currents (EPC). The inhibition of intracellular calcium elevation by injection of BAPTA in the cell body of a perisynaptic Schwann cell increased synaptic depression induced by 10-Hz, 80-s nerve stimulation. (Adapted from Castonguay and Robitaille, 2001.) **C:** Model of perisynaptic Schwann cell modulation of synaptic activity. On the left part of the scheme, increase of glial calcium leads to potentiation of transmitter release. We hypothesize that it could be mediated by a release of prostaglandins that increase transmitter release at the NMJ. On the right part of the scheme, glial G-protein activation leads to depression of transmitter release. We propose that activated perisynaptic Schwann cells could release glutamate that activate postsynaptic metabotropic glutamate receptors. Muscle fiber activation by glutamate would induce synthesis and release of nitric oxide (NO) that could lead to a subsequent decrease of transmitter release.

Hence, knowing that the activation of perisynaptic Schwann cells occur at higher frequency of nerve activity and, as indicated above, that the main synaptic plasticity event observed in these conditions is synaptic depression, the natural consequence of these observations was to propose that perisynaptic Schwann cells may be involved in synaptic depression at the frog NMJ. To test this possibility, we used GDP-βS, a nonhydrolyzable analogue of GDP, that maintains G-proteins in an inactivated state, to block perisynaptic Schwann cell activation. The specific injection of GDP-βS in perisynaptic Schwann cells, reduced by half the synaptic depression induced by a stimulation of 10 Hz during 80 s (Robitaille, 1998) (Fig. 1). Glial cells, via G proteins, are thus involved in short-term plasticity at the NMJ.

Since the Ca^{2+} rise in PSCs is one of the main events occurring upon G-protein stimulation and to obtain a more precise picture of the role of the Ca^{2+}-dependent pathways, experiments were performed to test specifically the role of perisynaptic Schwann cells internal stores of Ca^{2+} in the regulation of transmitter release.

We tested the effects of injection of IP_3 in perisynaptic Schwann cells, an inductor of the calcium release from intracytoplasmic stores, since the internal stores of Ca^{2+} in perisynaptic Schwann cells are controlled by IP_3 receptors (Castonguay and Robitaille, 2002). Injection of IP_3 in perisynaptic Schwann cells induced an increase in the amplitude of EPC adjacent to the Schwann cell. This is likely due to presynaptic effects, since mEPP amplitude was not affected (Castonguay and Robitaille, 2001). These results suggest that the

glial calcium increase that follows the release of neurotransmitters leads to a potentiation of synaptic activity at a presynaptic level.

To test this possibility, a chelator of Ca^{2+} (BAPTA) was injected in perisynaptic Schwann cells. What could be expected from such an experiment? As indicated above, several events of short-term plasticity (depression and potentiation) occur simultaneously during high-frequency train of stimuli. Knowing that a direct release of Ca^{2+} from internal stores results in a potentiation of transmitter release, one would predict that preventing the Ca^{2+}-dependent activation of perisynaptic Schwann cells should limit the expression of the potentiating events and, thus, result in an increase in synaptic depression. Indeed, injection of BAPTA induced an increase in synaptic depression elicited by a stimulation of 10 Hz during 80 s (Fig. 1) (Castonguay and Robitaille, 2001).

How to reconcile the observation that the G-protein pathways manipulation, that includes Ca^{2+} internal stores, resulted in a depression of transmitter release whereas the specific manipulation of Ca^{2+} stores resulted in a potentiation of transmitter release? A likely explanation is that the G-protein manipulation leads to multiple pathways that sum up to produce a final effect of depression. In this sum of events, the Ca^{2+} increase is only one of the many events that occur. Based on these observations, it appears that perisynaptic Schwann cells could modulate neurotransmission during depression and potentiation.

The requirement of G-protein activity, as well as the relatively slow onset of the glial modulation, may suggest that second messengers producing modulatory substances may be involved in the glial modulation of synaptic transmission and plasticity.

What could these glial-derived neuromodulators be? At the frog NMJ, the amplitude of synaptic depression induced by a high-frequency stimulation is decreased by metabotropic glutamate receptor inhibitors (Pinard et al., 2003). We can hypothesize that glutamate released by glial cells could mediate synaptic depression. But it may not be a simple interaction between perisynaptic Schwann cells and nerve terminal. Indeed, metabotropic glutamate receptors are expressed by muscle fiber at the end-plate, and glutamate depression depends on nitric oxide (NO) (Pinard et al., 2002). A possible hypothesis is that activated perisynaptic Schwann cells release glutamate acting on postsynaptic muscle fiber and leading to indirect depressing effect on neurotransmitter release via NO produced by the muscle fiber (Fig. 1).

Synaptic potentiation by glial cells could be due to prostaglandin release. Prostaglandins are very diffusible lipidic mediators synthesized by cyclooxygenases from arachidonic acid. At the frog NMJ, prostaglandin E_2 (PGE_2) has a potentiating effect where it induces an increase in basal EPP amplitude. Conversely, application of indomethacin, a blocker of prostaglandin synthesis, decreased the EPP amplitude (Madden and Van der Kloot, 1982; Pappas et al., 1999). Moreover, enzymes in the prostaglandins synthesis pathway, notably phospolipase A_2 and cyclooxygenases, are present in perisynaptic Schwann cells (Pappas et al., 1999). However, the direct implication of prostaglandins in the potentiation of glial origin remains to be demonstrated.

In summary, we propose that the release of neurotransmitters or modulators induces the activation of glial receptors mainly coupled to G proteins that generates a large number of second messengers and, in particular, an intracellular calcium increase. Our results indicate that the Ca^{2+}-dependent pathway leads to a potentiation of transmitter release while other unknown G-protein-coupled cascades lead to depression. It is quite likely that any activation of perisynaptic Schwann cells by high-frequency stimulation will result in a combined potentiation and a depression modulation, the sum of which accounts for the final glial modulating effect.

CONCLUSION

Perisynaptic Schwann cells contribute to short-term plasticity at the NMJ during high-frequency stimulations. The glial modulation of synaptic activity influences a significant amount of the short-term plasticity at the NMJ, considering that 20–50% of synaptic depression and approximately 10% of synaptic potentiation are related to glial cells activity. However, when taking into account the safety factor of NMJs, the glial modulation of synaptic activity may not make a large impact on muscle contraction. Under physiological conditions, release of neurotransmitters, even when modulated by glial cells, leads to a muscular action potential.

In spite of this, the glial contribution to modulation may be most important for improving the reliability of synaptic transmission. For instance, the synaptic depression that is influenced by glial neuromodulation can lead to a reduction in the level of neurotransmitters released for each stimulation and thus prevent an exhaustion of synaptic vesicles. What is the interest of glial modulation compared to already existing neuronal modulation? It is likely that glial modulation brings an additional level of safety to the NMJ. Moreover, because Schwann cells are sensitive to other stimuli such as inflammation, or a lesion, they could act also as integrators of other information, notably with regards to the physiological status of the NMJ or the muscle, and adjust synaptic activity accordingly. Under pathological conditions, one can consider that Schwann cells could change their modulation of synaptic activity, in response to structural, functional or environmental modifications at the NMJ. These possibilities are currently a research focus of our laboratory.

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