SYNAPTIC PROJECTIONS OF MOTONEURONS WITHIN THE SPINAL CORD

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Abstract (up to 150 words)

Motoneurons have long been considered as the final common pathway of the nervous system, transmitting the neural impulses that are transduced into action.

While many studies have focussed on the inputs that motoneurons receive from local circuits within the spinal cord and from other parts of the CNS, relatively few have investigated the targets of local axonal projections from motoneurons themselves, with the notable exception of those contacting Renshaw cells or other motoneurons.

Recent research has not only characterised the detailed features of the excitatory connections between motoneurons and Renshaw cells but has also established that Renshaw cells are not the only target of motoneurons axons within the spinal cord. Motoneurons also form synaptic contacts with other motoneurons as well as with a subset of ventrally located V3 interneurons. These findings indicate that motoneurons cannot be simply viewed as the last relay station delivering the command drive to muscles, but perform an active role in the generation and modulation of motor patterns.

There are notable exceptions to Sherrington's statement of motoneurons as the final common pathway (Sherrington 1906). For instance, the autonomic, enteric, and neuroendocrine systems each constitute components of the nervous system whose output pathways evade somatic motoneurons. The magnocellular neurosecretory cells of the ventral hypothalamus for example project infundibular axons to the posterior pituitary from where they secrete peptide hormones directly into the bloodstream. While the associated neuronal circuitry is not as well characterised as that of the somatic motor system where the first recurrent circuit was discovered (Renshaw 1946), it has long been known that magnocellular neurosecretory cells also send recurrent fibres, which project back to the ventral hypothalamus (Dreifuss and Kelly 1972).

Motifs of recurrent connectivity may thus be a feature common to the different efferent components of the nervous system. It has been proposed that the intercommunication between magnocellular neurosecretory cells performs an important role in modulating hypothalamic outputs for physiological neurohormone release (Leng and Dyball 1983). By analogy, it is highly likely that modulation of motoneuron activity by recurrent circuitry is essential for shaping their outputs for normal motor function (Hultborn et al. 1979).

Projections of motoneurons to Renshaw cells

Renshaw cells were first identified in the cat and distinctively respond with prolonged bursts of spikes in response to antidromic ventral root or nerve stimulation (Eccles et al. 1954). While there have been numerous experimental recordings from motoneurons, intracellular access to the Renshaw cells have proved more difficult mostly because of their smaller size. However, field recordings have confirmed the presence of prolonged bursts of activation following motoneuron firing (Ryall and Piercey 1971). Extracellular recordings in cat preparations have provided evidence of strong excitatory motoneuron synapses onto Renshaw cells. Continuous firing in a motoneuron can increase spiking in Renshaw cells (Ross et al. 1975) and drive discharge rates up to ≥40–60 Hz (Ross et al. 1976). Ventral root stimulation in decerebrated cats and rabbit preparations (Renshaw 1946) can elicit bursts of discharges from putative Renshaw cells and stimulation of a single motoneuron alone can be sufficient to evoke multiple spikes (Van Keulen 1981). On the

basis of these observations, the motoneuron to Renshaw cell synapse has always been postulated to be a strong synapse with Renshaw cells reliably propagating the firing in motoneurons. The motoneuron to Renshaw cell synapse might thus be considered as a ‘relay synapse’, giving a faithful image of motoneuron firing.

The strength of this synapse has been confirmed using paired recordings (Moore et al. 2015 Fig. 1a) performed in neonatal (P8-14) mice spinal cord slices. Quantal analysis of these recordings revealed that each motoneuron forms 5-10 contacts with its post-synaptic Renshaw cell, with high vesicular release probabilities (>~0.5) following a single nerve impulse. In many of such recordings, firing in a single motoneuron could evoke a spike in the Renshaw cell.

In addition to the unitary conductance, the degree of convergence from within a motoneuron pool onto a single Renshaw cell will determine the size of excitation.

Immunohistochemical studies have have showed that in rats each Renshaw cell receives 20-140 synaptic contacts from VAChT immunoreactive terminals originating from motoneurons (Alvarez et al. 1999). This would correspond to tens of motoneurons converging onto a single Renshaw cell. A functional gauge of convergence was estimated using electrophysiological recordings and quantal analysis of ventral root stimulation induced responses in Renshaw cells (Moore et al. 2015), that indicated that 5-10 motoneurons contact a single Renshaw cell. While the results may appear somewhat discrepant, the electrophysiological studies were performed in a different species (mouse) and, more importantly, in a reduced ‘oblique slice’ spinal cord preparation with the ventral root attached, but with a thickness less than that of a single segment. Since motoneurons axon collaterals can extend for more than one segment, contacting more distant Renshaw cells, estimates obtained from electrophysiological recordings from slices should be considered as a lower bound for the degree of convergence. A similar caveat is applicable to the estimate of the number of reciprocal connections between single motoneurons and Renshaw cells (Fig. 1b): Moore et al. (2015) found that within the spinal slice, ~1/3 of motoneuron to Renshaw cell synapses were reciprocal. Given the inevitable severance of connections in the slice preparation as highlighted above, this proportion is likely to be much larger in an intact system.

In addition to the synaptic strength of individual connections between motoneuron and Renshaw cells, the degree of convergence and divergence between each cell type are a key determinants of

their function. While we have estimates of the convergence, with respect to the number of Renshaw cells contacting each motoneuron and the number of motoneurons contacting each Renshaw cell (Moore et al. 2015), we do not know the level of divergence. Given the strong effect of a single Renshaw cell input on a motoneuron (Bhumbra et al. 2014), if the same Renshaw cell were to contact several motoneurons, it is likely that this configuration would increase synchronicity of motoneuron firing.

A consequence of the combination of a considerable degree of convergence and high reliability of the motoneuron to Renshaw cell synapse is that the Renshaw cell can follow the firing of motoneurons with high fidelity. Indeed, even with ventral root stimulation at 100 Hz, near the upper range of physiological firing frequencies in motoneurons, the Renshaw cells show just ~50% of synaptic depression and can follow reliably the firing in the motoneuron pool (Moore et al. 2015).

The precise role of Renshaw cells in the control of motor outputs is unknown. While they could just prevent excessive motoneuron firing, several other hypotheses were raised about their function (Windhorst 1996), including a role as a variable gain regulator (Hultborn and Pierrot-Deseilligny 1979) or in correlating or decorrelating (Maltenfort et al. 1998) motoneuron firing. In a recent report (Enjin et al. 2017), selective ablation of Renshaw cells input to motoneurons was achieved using a knockout strategy, taking advantage of the selective expression of the neuronal nicotinic receptor subunit α2 in Renshaw cells (Perry et al. 2015), Constitutive ablation of the vesicular inhibitory amino acid transporter (VIAAT) from Renshaw cells terminals however did not result in any apparent motor phenotype, but these results are potentially confounded by compensatory changes occurring within the motor circuits.

A more recent model (Brownstone et al. 2015) has suggested that the combination of Renshaw cell connectivity and components of spinal circuitry projecting to motoneurons themselves may contribute to sensorimotor learning. It is proposed that an ‘internal model’ of muscle action can learn as a result of differences between feed-forward predictive (recurrent) information and feedback reactive (proprioceptive) information. The advantage of tuning the internal model to feedforward information is that by nature it is more immediately available compared to the feedback information that inherently can only be transmitted later in time (Brownstone et al. 2015).

Co-transmission to Renshaw cells

When Dale's principle was initially proposed, it stated only that the chemical function of a neuron was the same at all its terminals. It is important to note that only two putative transmitters were known at the time: acetylcholine and noradrenaline (at the time thought to be adrenaline, Dale 1935). Consequently, Dale could not have envisaged co-release of both transmitters. This statement was subsequently re-appropriated and misinterpreted in a form that states that all synaptic terminals of the same neuron release the same transmitter onto all its different targets, perhaps in part due to Eccles' early formulation (Eccles et al. 1954) that used the word 'transmitter' in the singular. The large number of cases in which this statement is clearly inaccurate led many to describe the discovery of co-release (of different inhibitory amino-acids, or neurotransmitter and peptides or gases) as a violation of Dale's principle. However, we owe it to Eccles to clarify his more faithful interpretation of Dale's principle in which he reformulated the originally loose statement by Dale in more precise terms: 'Dale's Principle be defined as stating that at all the axonal branches of a neuron, there was liberation of the same transmitter substance or substances' (Eccles et al. 1976). With this definition, it is clear that the many instances in which co-release has been demonstrated do not constitute any violation of Dale's principle.

It is however important to emphasise that where there is co-release (meaning the release of vesicles containing two or more transmitters), it does not necessarily follow that transmission at that synapse is mediated by both transmitters. While both transmitters could be released, their corresponding detections depend on the repertoire of post-synaptic receptors (Chery and De Koninck 1999; Lu et al. 2008). Eccles' observation that ACh blockers do not completely abolish the Renshaw cell discharge elicited by antidromic nerve stimulation is consistent with a possible second transmitter released from motoneurons. At the neuromuscular junction, some studies reported the presence of glutamate *(W*ærhaug and Ottersen 1993; Meister et al. 1993) while others failed to detect it (Herzog et al. 2004; Kraus et al. 2004). Similarly conflicting results were reported from studies of immunoreactivity for vesicular glutamate transporters in the motoneurons terminals opposed to Renshaw cells, with some authors detecting the expression of Vglut2

(Oliveira et al. 2003; Herzog et al. 2004; Nishimaru et al. 2005) or Vglut RNA (Schäfer et al. 2002) and others failing to detect its presence (Mentis et al. 2005).

The issue of a second transmitter was finally resolved using direct electrophysiological recordings of the excitatory response evoked by ventral root stimulations in the neonatal spinal cord. Two groups independently observed a non cholinergic component in the ventral root evoked EPSC recorded in Renshaw cells (Mentis et al. 2005; Nishimaru et al. 2005) that could be blocked by glutamatergic antagonists. These recordings confirmed that motoneurons could release an excitatory amino acid from their central synapses. Without unequivocal identification of mechanisms for excitatory amino acid loading at the motoneuron terminals, glutamate remains only a putative transmitter at this synapse. Since motoneuron terminals opposed to Renshaw cells are enriched with aspartate (Richards et al. 2014), glutamate might not be the only feasible candidate.

The situation is potentially more complex on the post-synaptic side, where at least 4 different subtypes of receptors contribute to the post-synaptic response (Lamotte d'Incamps and Ascher 2008). A fast α7 mediated response is followed by a slower nicotinic response mediated by heteromeric receptors of unknown subunit composition, although it has been suggested that two classes of receptors with different αβ stoichiometry might be involved (Lamotte d'Incamps and Ascher 2014). Furthermore, both AMPA and NMDA receptors are activated post-synaptically. The combined cholinergic and glutamatergic contributions to the connections to Renshaw cells results in a synapse with two transmitters and at least four different types of post-synaptic receptors. Bursts of spikes observed in response to ventral root stimulation may result from a 'priming' depolarisation, mediated by AMPA and nicotinic receptors, relieving magnesium blockade and prolonging activation of NMDA receptors (Lamotte d'Incamps and Ascher, 2008).

Definitive confirmation of the synaptic activation of AMPA receptors is somewhat inconsistent with aspartate as the second transmitter, since aspartate, while being an agonist at NMDA receptors, does not activate AMPA receptors (Patneau and Mayer 1990), even though some reports suggest that AMPA receptors can be weekly activated by direct application of aspartate, at least in a subset of dopaminergic neurons (Krashia et al. 2016). Motoneurons thus appear to communicate using

only ACh at the neuromuscular junction, and using both ACh and glutamate (or similar excitatory amino-acid) at the motoneuron to Renshaw cell synapse.

Contrary to GABA and glycine co-transmission in the spinal cord (Jonas et al. 1998; Singer et al. 1998), that is largely confined to the early developmental stage (Bhumbra et al. 2012; Jiang and Alstermark 2015) co-transmission of ACh and glutamate is preserved at a mature age (Lamotte d'Incamps et al. 2017). Most of the electrophysiological evidence for co-transmission was obtained from recordings of Renshaw cell responses to ventral root stimulation, that activates a large number of motoneurons. Compound responses in single Renshaw cells could thus result from mixed transmission whereby some motoneurons released ACh only and others released a glutamate like substance. Paired recordings from connected motoneurons and Renshaw cells (Lamotte d'Incamps et al. 2017) have excluded this possibility by unmasking of a non-nicotinic component following blockade of ACh receptors. Since responses to single motoneurons were recorded, the residual non-cholinergic component confirms the occurrence of both modes of transmission at the level of single motoneurons.

Kinetic analysis of spontaneously occurring miniature synaptic currents and of asynchronous mEPSC originating from the motoneurons revealed a considerable degree of segregation between the two transmitters systems: namely, miniature events were either mediated by the two nicotinic receptors or by the two glutamate receptors, but no mixed cholinergic and glutamatergic mEPSC were observed (Lamotte d'Incamps et al. 2017), suggesting that transmission occurs with only one transmitter at each individual terminal.

It is possible that all four receptors are present at the post-synaptic Renshaw cell membrane, but each terminal of any given motoneuron can release vesicles containing either ACh or glutamate, but not both (Fig. 2c). Alternatively, single synaptic vesicles can contain both transmitters, but the post-synaptic receptors expressed Renshaw cells are either GluRs or AChRs, thus precluding mixed transmission at individual contacts (Fig. 2d). The function of any such arrangements is unclear, since at the level of individual synapses between motoneurons and Renshaw cells, that are made up of several functional contacts (Moore et al. 2015), transmission is always mixed. In order to distinguish between the pre-synaptic and post-synaptic configurations for segregation, it will be necessary to perform experiments aimed at evoking release from single motoneuron

terminals onto Renshaw cells. Such experiments might employ spatially restricted light activation of excitatory opsins or of caged calcium compounds loaded into the pre-synaptic cell.

Recurrent excitation between motoneurons

Inter-communication between motoneurons in vertebrates has often been attributed to gap-junctions (Fulton et al. 1980). While gap junctions are expressed during early mammalian development (Fulton et al. 1980; Hinckley and Ziskind-Conhaim 2006), their density tend to decrease in more mature animals (Walton and Navarrete 1991; Chang et al. 1999; Personius et al. 2007), even though the presence of mixed electrical and chemical synapses in the spinal cord has been detected also in adult rats motoneurons (Rash et al. 1996).

Most motor pools, with the exception of those innervating the more distal paw muscles (Cullheim and Kellerth 1978; McCurdy and Hamm 1992) show extensive axonal branching. Anatomical studies in the cat lumbar spinal cord have revealed that motoneurons axon collaterals also invade the motor nuclei, potentially forming synapses onto other motoneurons (Cullheim et al. 1977; Cullheim et al. 1987). The first functional synapses between motoneurons were observed in frog embryos (Perrins and Roberts 1995). Such synapses are often mixed exhibiting electrical and chemical components, with the latter mediated by ACh and transmitted across spinal segments. However, there are no reports of similar connectivity in adult frogs. In mammals, reports of synaptic connectivity between motoneurons were scarce and somewhat contradictory. In neonatal rats, ventral root simulation not only evoked the expected di-synaptic inhibition of motoneurons mediated via Renshaw cells, but also a smaller response, sensitive to glutamate receptors antagonists (Schneider and Fyffe 1992) and whose reversal potential was compatible with a mixed cation mediated current (Jiang et al. 1991). The presence of this excitatory input was attributed to the invasion of primary afferent fibers through the ventral roots. This view was supported by some anatomical evidence (Coggeshall 1980) and by the capacity of glutamate receptors antagonists to block the excitation (Jiang et al. 1991). More recent studies (Mentis et al. 2005) however have excluded the presence of primary afferent in ventral roots, indicating that the source of glutamatergic excitation could be from the motoneurons themselves. This suggestion is supported by the observed close juxtaposition between motoneurons terminals loaded with different dyes

through adjacent ventral roots (Mentis et al. 2005) and evidence of glutamate enrichment in a number of these terminals.

Ventral root evoked excitatory responses in motoneurons were also described in neonatal mice, P0-4 (Nishimaru et al. 2005). Such responses were mixed glutamatergic-cholinergic, even though in 7/9 recorded cells, the excitatory response was dominated (>80%) by the glutamatergic component. On the contrary, recurrent excitation measured in neonatal rats (Ichinose and Miyata 1998) was abolished in 3/5 cases by cholinergic antagonist, but glutamate antagonists were not tested in the remaining cases.

A more recent systematic study of recurrent excitation between motoneurons (Bhumbra and Beato 2018) confirmed the presence of recurrent excitatory connectivity between motoneurons. This was shown not only by ventral root stimulation, but also from paired recording between synaptically coupled motoneurons. Both paired recordings and ventral root stimulation established that excitation between motoneurons is entirely mediated by glutamate and that excitatory inputs from motoneurons can propagate across neighbouring segments. Furthermore, while previous studies were limited to neonatal mouse preparations (up to 10 days old), (Bhumbra and Beato 2018) showed that recurrent excitation is not a transient phenomenon in early development, but it is present also in older animals up to P20, when motor systems are considered mature and the animal is capable of executing most motor tasks normally. In summary, the presence of recurrent excitation between motoneurons has been consistently observed in different species and ages, but while one recent study suggested that transmission is purely glutamatergic (Bhumbra and Beato 2018), previous works (Ichinose and Miyata 1998; Nishimaru et al. 2005) point at mixed glutamatergic-cholinergic trnasmission, a view that is also supported by the presence of cholinergic boutons originating from motoneurons onto other motoneurons (Mentis et al. 2005).

One remarkable feature of recurrent excitation is that putative fast motoneurons, identified by their firing properties (Leroy et al. 2014), receive almost 10 times more excitation than putative slow motoneurons, characterized by their early firing behaviour (Figure 3a-d). However, it is not known whether the pre-synaptic origins of excitation of fast motoneurons are predominantly slow or fast units (Fig. 3e,f). Hennemann’s size principle (Henneman 1957) states that motor units are recruited

in a specific order, with slow units being recruited first, and progressively more involvement of fast units as the required force increases. If the preferential pattern of connectivity favoured connections between slow to fast units (Fig. 3e), recurrent excitation could be a further mechanism for the implementation of the size principle and could mediate the progressive recruitment of faster fibers in synergy with. progressively increasing input strength received by different motor units. Preferential connectivity from pre-synaptic slow motoneurons to post-synaptic fast motoneurons would favour the recruitment of fast units in a graded way, facilitating a coordinated increase in the strength of muscle contractions.

If on the other hand, the preferential pattern of connectivity only involves fast units (Fig. 3f), recurrent excitation would lead to a closed loop amplification that could increase firing rates, and potentially coherence between fast units after a sufficient proportion are activated. In order to distinguish between these two possibilities, a complete electrophysiological characterization of pre and post-synaptic partners is necessary, and the interpretation of results should be supported by detection of genetic (Muller et al. 2014) or immunohistochemical (Enjin et al. 2010; Leroy et al. 2014) markers of fast and slow motoneurons.

While synaptic connectivity between individual motoneurons has been demonstrated through paired recordings between motoneurons belonging to the same motor nucleus (lateral gastrocnemius), it remains to be ascertained whether this connectivity extends also across different nuclei. Experiments using Ca^{2+} imaging across two segments showed that the wave of excitation evoked by ventral root stimulation could be observed throughout the scanned segments in virtually all motoneurons, that presumably belonged to different motor nuclei. However, motoneurons also make synaptic contact with V3 interneurons, and the poor time resolution of Ca^{2+} imaging does not allow to distinguish between direct motoneuron to motoneuron excitation and excitation mediated by a potential excitatory disynaptic loop through V3 interneurons (Chopek et al. 2018). Therefore, to date, there is no conclusive evidence that motoneuron connectivity extends across different nuclei.

While the presence of connectivity among synergist muscles would again be consistent with a role of recurrent excitation in amplifying the muscle contractions by providing positive interactions

between muscles that are normally co-activated during movements, the presence of connectivity extending across antagonist units is more difficult to interpret. Since recurrent excitation might be effective only when a critical number of motor units are activated, it is possible that excitation between antagonist motor units might play a role in the preparation and execution of ballistic movements, such as throwing, jumping, or lifting heavy weights, wherein an explosive movement is characteristically preceded by isometric co-contraction of antagonist muscles.

Investigation of these possibilities will require exploration in the future, possibly using electrophysiological recordings from labelled motor units from different muscles or using trans-synaptic tracing methods (Stepien et al. 2010; Tripodi et al. 2011) or selective expression of excitatory opsins in different motor nuclei.

Even once the connectivity pattern is unravelled, establishing the exact role of recurrent excitation will not be straightforward.

Other synaptic targets of motoneurons

It is generally accepted that in many invertebrate species motoneurons actively contribute to the generation and execution of motor patterns through either gap junctions or chemical synaptic connections, in vertebrate species the only identified post-synaptic targets were Renshaw cells and other motoneurons. The recurrent inhibitory and excitatory loops, could down- or up-scale firing in motoneurons, thus altering the strength of muscle contraction. A seminal paper however showed that antidromic activation of motoneurons through ventral root stimulation could elicit episodes of fictive locomotion in an intact neonatal spinal cord in vitro (Mentis et al. 2005). The consequence of this observation is that motoneurons terminal must be capable of activating some elements of the central pattern generator, resulting in locomotor like pattern throughout the spinal cord at least at a developmental stage. Further confirmation came from studies in neonatal rats (Machacek and Hochman 2006, Fig. 4b), showing that ventral root stimulation can entrain rhythmic bursting activity following blockade of synaptic inhibition. These experiments also show that noradrenaline unmasks a connectivity pattern between motoneurons and an unidentified class of interneurons. Similarly, during drug-activated fictive locomotion, light induced activation of motoneurons,

following selective expression of the excitatory opsin channelrhodopsin, increases the bursting frequency of the locomotor pattern (Falgairolle et al. 2017).

The initiation of locomotion, entrainment of disinhibited bursting activity, and up-regulation of the frequency of the locomotor patterns are incompatible with an effect mediated by recurrent excitation alone or by activation of Renshaw cells, due to their inhibitory nature, even accounting for projections from Renshaw cells to Ia inhibitory interneurons and to ventral spinocerebellar neurons (Jankowska and Hammar 2013). Furthermore, even if Renshaw cells had synaptic projections to some elements of the CPG, their effect would most likely be inhibitory, especially since, already at the neonatal stage, the chloride reversal potential is more negative than the membrane potential (Delpy et al. 2008).

Evidence of motoneuron-induced effects on the CPG suggests that motoneuron axon collaterals directly contact an unspecified group of interneurons associated with the CPG. It is possible that modulation of motoneuron activity on fictive locomotion result from excitation of large motor pools, through antidromic or light stimulation, activating the CPG not through direct synaptic contacts. Instead, the modulatory effects may be mediated through either ephaptic transmission (Jefferys 1995), or through local increases in extracellular potassium, that is known to contribute to the initiation of locomotor bursts following dorsal root stimulation (Marchetti et al. 2001a; Marchetti et al. 2001b). However, this explanation is inconsistent with results from optogenetic experiments (Falgairolle et al. 2017) that show that during drug-activated fictive locomotion, light-induced inhibition of motoneurons, selectively expressing the inhibitory opsin halorhodopsin, results in a remarkable slowing down of the rhythmic pattern. Furthermore, this effect is strongly attenuated following blockade of AMPA receptors, but unchanged by partial block of gap junctions. This elegant set of experiments indicates not only that motoneurons communicate with interneurons other than Renshaw cells and that such interneurons are associated with the CPG, but also that such communication is not mediated through gap junctions but through an excitatory amino acid that activates AMPA receptors, such as glutamate.

Direct evidence of communication between motoneurons and other CPG related interneurons came initially from zebrafish preparations (Song et al. 2016), where it was shown that activation of

motoneurons perturbs the frequency of the swimming pattern. The study identified a novel motoneuron synaptic target in the population of V2a interneurons, with connections that are either electrical, chemical, or a combination.

Mammalian V2a interneurons however do not receive any input from motoneurons, whether chemical or electrical (Bhumbra and Beato 2018), raising the possibility that other classes of interneuron might be responsible for the motoneuron induced modulation of locomotor activity. Potential alternative candidates have been identified among V3 interneurons, that are known to send commissural axons and contact contralateral motoneurons (Zhang et al. 2008). It has been recently shown that within a spinal cord slice, V3 interneurons also send direct ipsilateral projection to motor pools (Chopek et al. 2018). Remarkably in the subset of the most ventrally located V3 interneurons, this pattern of connectivity appears to be reciprocal, with at least a proportion of interneurons receiving excitatory glutamatergic inputs from motoneurons (Chopek et al. 2018). This was the first direct evidence of connectivity between motoneurons and an identified class of excitatory interneurons within the mammalian spinal cord.

While identification of these excitatory projections does not fully explain the modulatory effects of motoneuron activity on the frequency of the locomotor rhythm, it at least introduces a candidate class of interneurons that could feasibly mediate this effect.

V3 interneurons contribute to the CPG for locomotion through contralateral connections (Danner et al. 2017) and their acute or chronic ablation (Zhang et al. 2008) causes changes the regularity, though do not abolish, the motor pattern.

New methods establishing the connectivity patterns of cells in the CNS may reveal further classes of neurons post-synaptic to motoneurons that could modulate the central pattern generators.

Conclusions

It seems that the era of perceiving motoneurons confined to a purely passive role integrating inputs from the brain and local spinal cord circuits is coming to an end as evidence emerges of motoneurons as active protagonists in the generation of motor patterns.

Recent evidence concerning the nature of the different post-synaptic targets of motoneurons have unveiled a peculiar diversity of neurotransmitter phenotypes. Activation at the neuromuscular junction is purely mediated by ACh, motoneurons excite Renshaw cells via a combination of ACh and glutamate, while transmission from motoneurons to themselves and to V3 interneurons is purely glutamatergic.

motoneurons thus communicate in different languages according to their specific post-synaptic target (Fig. 5). This arrangement is not unique in the central nervous system, but as far as we know it is certainly rare. To the best of our knowledge, the only other case of such neurotransmitter dissociation is at the synapse between Golgi cells and unipolar brush cells and granule cells in the cerebellum, where the first transmitting with glycine and the latter with GABA (Dugue et al. 2005). In both this case and for motoneurons, it is difficult to envisage a rationale or a function for such differentiation in the transmitter used, especially since there seems to be no relation between the size of the post-synaptic target and the kinetics of the post-synaptic receptors involved. It is yet to be determined whether segregation of the different neurotransmitter systems occurs at the pre or post-synaptic sites. Attempts of answering this question are challenged by the disparate immunohistochemistry evidence at motoneurons terminals and by our ignorance of the exact mechanism of glutamate loading in the vesicles.

It is humbling to admit that we do not know the exact role of the recurrent inhibitory loop in the control and execution of motor tasks, despite the fact that it is one of the first characterized closed loop circuits in the CNS and 70 years of subsequent research. The recent discovery of two further recurrent loops, both excitatory, one between motoneurons themselves and one between motoneurons and V3 interneurons, raises the question of which one of these feedback loops is dominant during the execution of motor tasks. Addressing this question requires knowledge of the specific connectivity patterns between each element of the feedback loops.

While it is accepted that recurrent inhibition is largely confined to homonymous or synergist motor nuclei (McCurdy and Hamm 1994), it is known that there is differential degree of convergence between fast and slow motoneurons: slow motoneurons receive more inhibition than fast ones (Hultborn et al. 1988a), and contact fewer Renshaw cells (Hultborn et al. 1988b). Similarly, within

the recurrent excitatory loop, fast motoneurons receive 10 times greater recurrent excitation than slow ones, while the relative strengths of synaptic connections in the loop between motoneurons and V3 interneurons is unknown. It is tempting to speculate that the differential pattern of connectivity within each loop might relate to the differential order of recruitment of slow and fast motor units, conferring dominance to one circuit or the other depending on the task being performed.

Whatever the relationship between the recurrent loops, it is clear that motoneurons must assume a central active role in the control of motor tasks, well beyond their postulated role within the final common pathway as simple passive units relaying information from the CNS to the muscles.

Figure legends

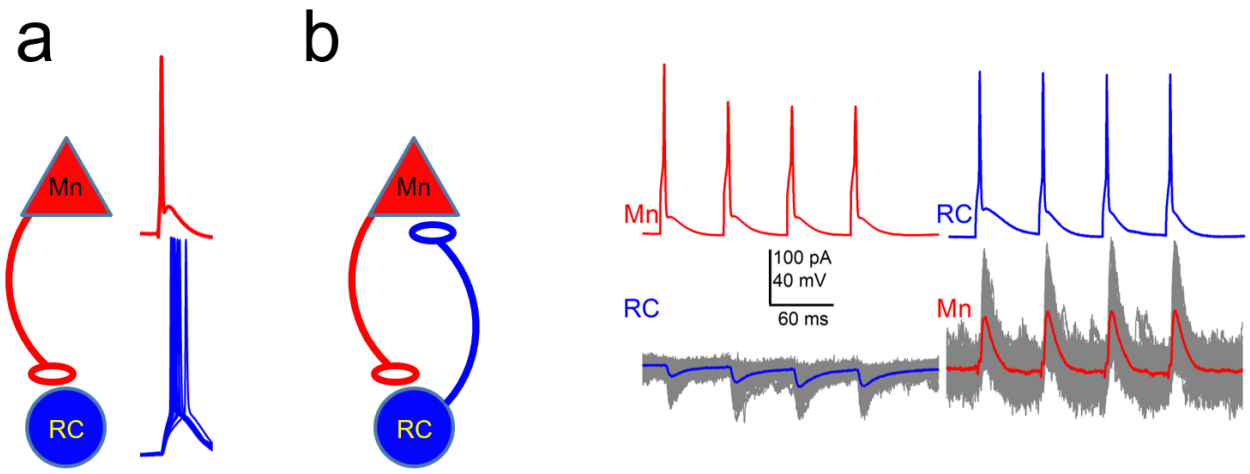

Fig. 1 Synapses between motoneurons and Renshaw cells: In a paired Motoneuron-Renshaw cell recording, a spike evoked in the motoneuron always induces firing in the connected Renshaw cell (panel a). Reciprocal connections between individual motoneurons and Renshaw cells occurred in ~30% of paired recordings (adapted from (Moore et al. 2015). Excitatory and inhibitory currents are consistently evoked by a train of spikes induced in the motoneuron or in the Renshaw cell.

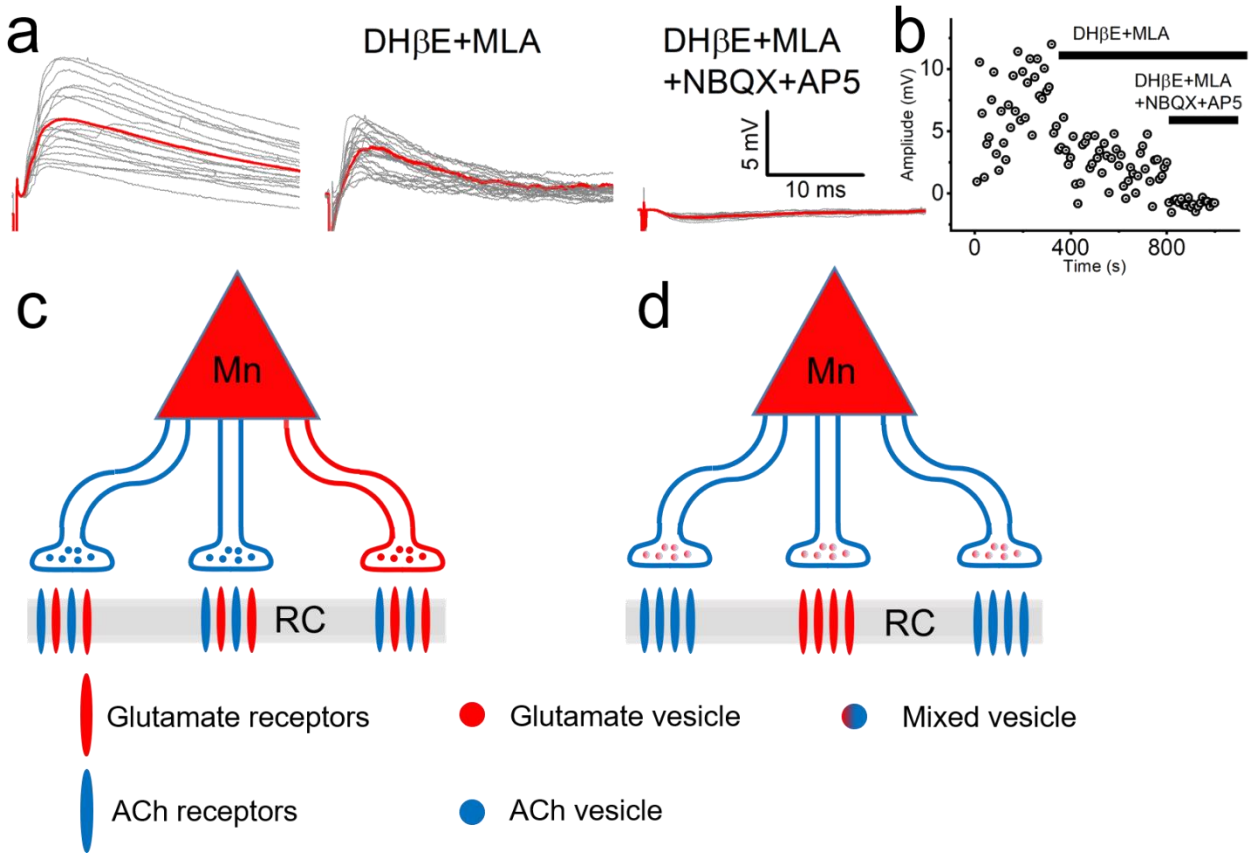

Fig. 2 Modes of transmissions between motoneurons and Renshaw cells. An example of a ventral root evoked response in a Renshaw cell from a mature (P18) spinal cord slice, showing the progressive decrease in the evoked potential following application of cholinergic (middle panel) and glutamatergic (right panel) antagonists. The full time course of the experiment is shown in panel b. Segregation of glutamate and acetylcholine neurotransmission at the motoneuron to Renshaw cell synapse can occur either at the pre-synaptic level (panel c), with individual terminals from a single motoneuron containing only one of the two transmitters, or at the post-synaptic level (panel d), if all pre-synaptic terminals contain mixed content vesicles, but the post-synaptic membranes opposed to each terminal contain either glutamate or nicotinic receptors.

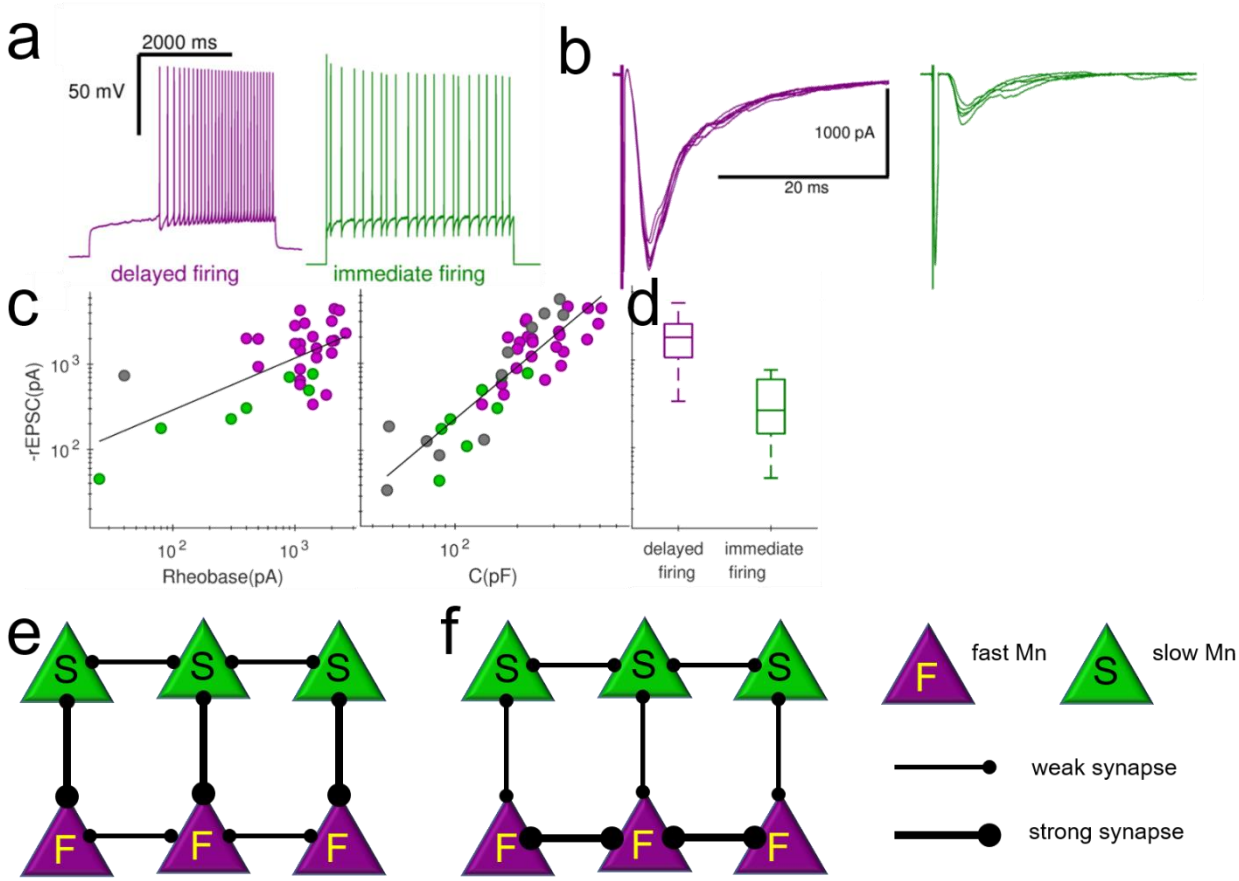

Fig. 3 Motoneurons can be distinguished based on their firing properties, with delayed firing and immediate firing motoneurons associated with fast and slow units respectively (panel a). Recurrent excitation is larger in delayed firing motoneurons (panel b,d) and its size positively correlates with rheobase and cell capacitance (panel c, adapted from (Bhumbra and Beato 2018). Larger recurrent excitation in fast units could be due to stronger connectivity of slow to fast motoneurons (panel e) or to stronger connectivity between fast motoneurons themselves (panel f).

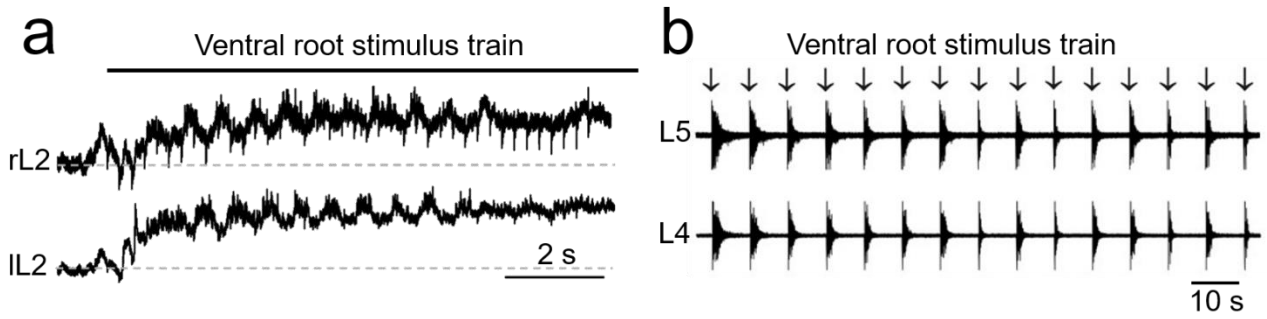

Fig. 4 Motoneurons influence the activity of central pattern generators: a train of ventral root stimulations can induce a locomotor like pattern recorded in the lumbar ventral roots (panel a, adapted from (Bonnot et al. 2009). Similarly, single ventral root stimulations can entrain the spontaneous bursting pattern induced by block of inhibition (panel b, adapted from (Machacek and Hochman 2006) Copyright 2006, Society for Neuroscience).

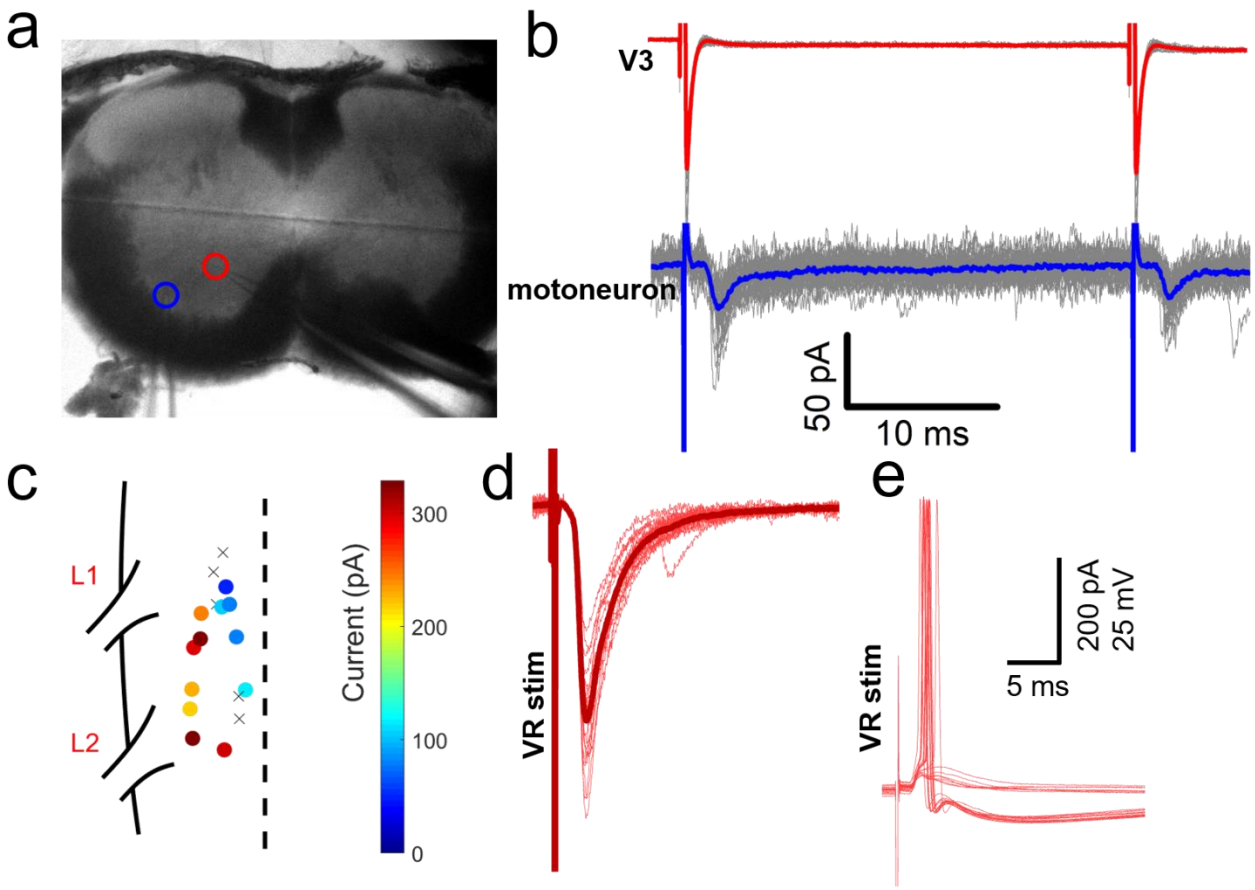

Fig. 5 Reciprocal connectivity between motoneurons and V3 interneurons. V3 interneurons are monosynaptically connected to ipsilateral motoneurons. An example of a paired recording is shown in panels a,b, with the location of the recorded cells indicated by an open circle. A spike evoked in the V3 interneuron in the loose cell-attached configuration (panel b, upper trace) evokes a post-synaptic response in the recorded motoneuron (panel b, lower trace). In a longitudinal spinal cord preparation with dorsal horn ablated (panel c), some V3 interneurons respond to ventral root stimulation (size of the postsynaptic response is colour coded in panel c, crosses correspond to not responding cells) with a large postsynaptic current (panel d) that can bring the V3 interneurons to threshold for the generation of an action potential (panel e) (adapted from Chopek et al. 2018).

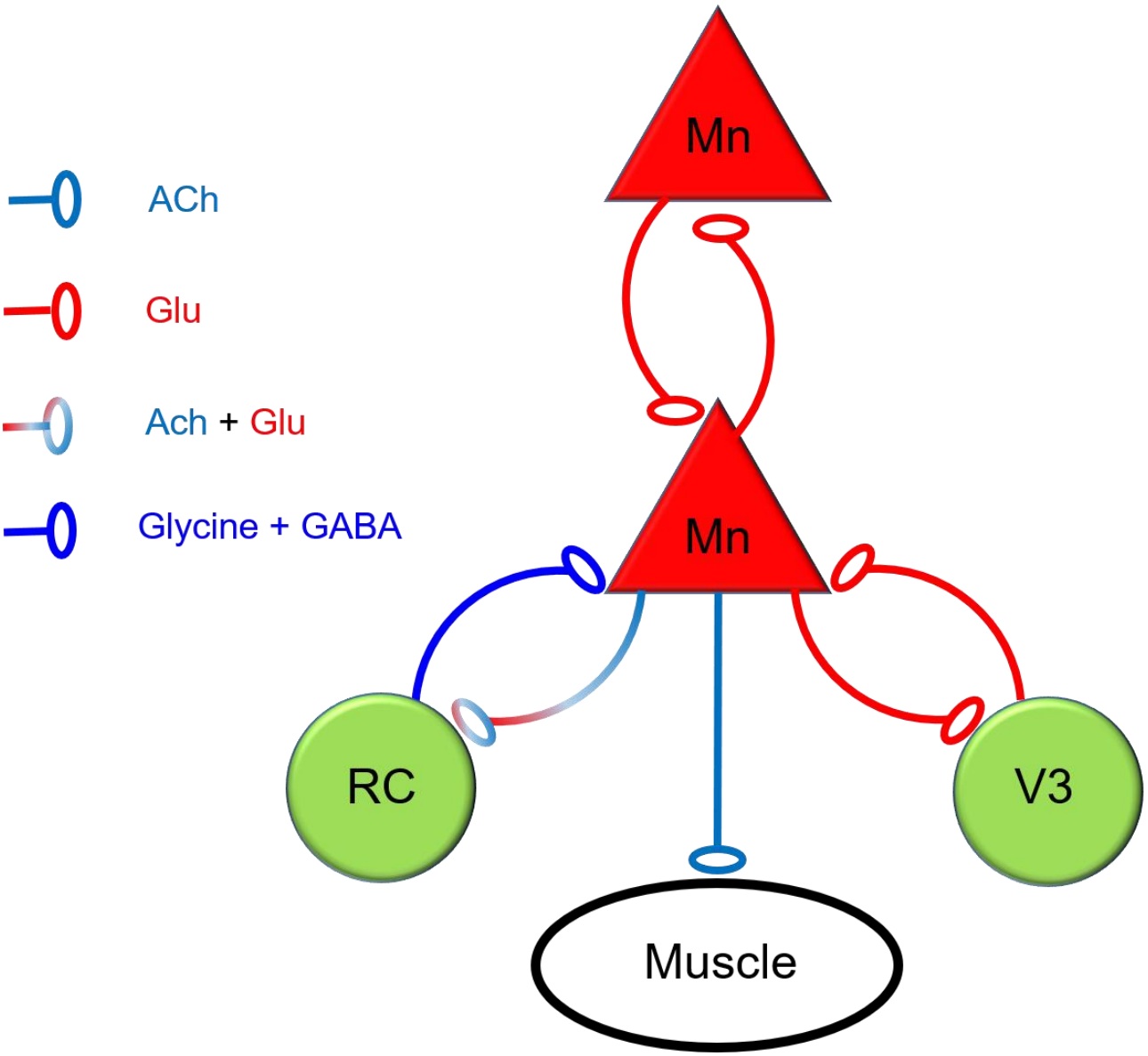

Fig. 6 Modes of synaptic communication from motoneurons: synaptic transmission is entirely cholinergic at the neuromuscular junction, glutamatergic at the motoneuron to motoneuron and motoneuron to V3 interneurons synapses, but mixed at the motoneuron to Renshaw cell synapse.

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