Seminars in Cell & Developmental Biology 60 (2016) 146–154

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Seminars in Cell & Developmental Biology

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Cell-cell fusion in the nervous system: Alternative mechanisms of development, injury, and repair

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ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 14 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
Cell-cell fusion
Axonal regeneration
Dendrite remodelling
Heterokaryon
Neuron theory
Fusogen

ABSTRACT

Over a century ago, the seminal work of Ramón y Cajal revealed that the nervous system is made of individual units, the neurons, which are related to each other by contiguity rather than continuity. This view overturned the idea that the nervous system was a reticulum of fibers, a *rete diffusa nervosa*, as proposed and defined by Camillo Golgi. Although the neuron theory has been widely confirmed in every model system studied and constitutes the basis of modern neuroscience, evidence accumulated over the years suggests that neurons, similar to other types of cells, have the potential to fuse their membranes and undergo cell-cell fusion under certain conditions. This concept adds a substantial layer to our view of the nervous system and how it functions. Here, we bring together past and more recent discoveries on multiple aspects of neuronal fusion, discussing how this cellular event is generated, and what consequences it has for our understanding of nervous system development, disease, injury, and repair.

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Contents

1. Introduction 147
2. Neuronal self-fusion 147
   2.1. Dendrite fusion to remodel a developing dendritic arbor 147
   2.2. Axonal fusion to repair an injured axon 147
3. Neuronal cell-cell fusion and its implications 149
   3.1. Viral-induced neuronal cell-cell fusion 149
   3.2. Ectopic neuronal cell-cell fusion during axonal regeneration 150
   3.3. Cell-cell fusion with glia and between glial cells 151
   3.4. Cell-cell fusion between neurons and stem cells 151
4. Membrane nanotubes between neurons and between neurons and glia 152
5. Expression of fusogens in the nervous system in health and disease 152
6. Conclusion 153
   Acknowledgements 153
   References 153

Abbreviations: AFF-1, anchor cell fusion failure-1; AMsh, amphid sheath; AMso, socket cells; DRG, dorsal root ganglia; EFF-1, epithelial fusion failure-1; gB, glycoprotein B; HSV1, herpes simplex virus type 1; PrV, pseudorabies virus; PS, phosphatidylserine; TNTs, tunnelling nanotubes; VZV, varicella-zoster virus.

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http://dx.doi.org/10.1016/j.semcdb.2016.06.019
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1. Introduction

The neuron theory proposed by Ramón y Cajal, according to which neurons exist as individual cells, has been the foundation of modern neuroscience and has paved the way for our current understanding of how the nervous system develops and functions. Neurons are highly polarized cells that extend two functionally and morphologically different compartments from the soma: dendrites and an axon. Dendrites are specialized to receive and process electrochemical inputs, whereas the axon transmits the electrochemical messages to other neurons or a target tissue via chemical or electrical synapses. Although a number of other tissues normally develop through cell-cell fusion, thereby generating multinucleate syncytia (placenta, muscle, osteoclasts, macrophages), this cellular mechanism was thought to be absent from the nervous system. However, several reports suggest that neurons, similar to other cells, have the capacity to fuse their plasma membranes, and that these events occur under both physiological and pathological conditions. This raises a number of intriguing questions. Is it possible that the true extent of neuronal fusion across species has been overlooked due to the complexity of the nervous system? What consequences do these cellular events have for the identity and function of the neurons involved, and for the circuits they form? Could neuronal fusion be part of the etiology underlying certain neurological diseases? What is the molecular machinery that governs neuronal fusion? This review aims to present the current evidence in the field, and to highlight the potential impact of recent advances on our understanding of the nervous system.

2. Neuronal self-fusion

One of the simplest examples of the capacity of neurons to fuse their membranes is observed during neuronal self-fusion. This term defines the ability of a neuron to fuse sections of its own neuronal processes (dendrites or axon), in order to remodel them during development or repair them following injury (Fig. 1 and Table 1).

2.1. Dendrite fusion to remodel a developing dendritic arbor

The PVDs (left and right) are a bilateral pair of mechansensory neurons in *Caenorhabditis elegans*, each of which extends anteriorly and posteriorly directed dendrites, and a ventrally directed axon. Each dendrite extends several multibranched units (named menorahs based on their shape) both ventrally and dorsally, covering almost the full body of the animal (Fig. 1A). This highly branched and stereotypical dendritic arbor has made PVD one of the best-characterized neurons in terms of dendrite development and repair [1–7]. Using this cell as a model system, Oren-Suissa and colleagues have described a new mechanism for dendritic arbor development involving membrane fusion [7]. They have elegantly shown that, during development, the PVD dendritic arbor is pruned and shaped through branch retraction and, most interestingly, through loop formation by neurite self-fusion. Both these processes were shown to be mediated by the nematode-specific fusogen Epithelial Fusion Failure-1 (EFF-1), a *bona fide* fusogen previously shown to mediate cell-cell fusion during development in other *C. elegans* tissues [8]. The authors proposed that assembly of EFF-1 complexes in *cis* causes membrane curvature and retraction, whereas interactions between EFF-1 molecules in *trans* across closely apposed membranes causes dendrite fusion. EFF-1 sculpts these neurons in a dose-dependent manner to maintain the angle of neurites at branching points and avoid overlapping branches. This process could be compared to the self-contact elimination process described decades ago for the development of neuronal growth cones *in vitro* [9]. A similar self-contact elimination by membrane self-fusion has recently been characterized in epithelial cells [10] and in the vascular endothelial cells of zebrafish embryos [11], raising the possibility that it might be a common mechanism to shape cellular processes (Fig. 1B). These findings show that some neurons express a functional fusogen, and that dendrites are capable of membrane fusion.

2.2. Axonal fusion to repair an injured axon

Another example of neuronal self-fusion is the process of axonal fusion observed during axonal regeneration. In this case, following transection of the axon, the proximal axonal fragment that is still attached to the cell body regrows toward and fuses with its own separated axonal fragment in an end-to-end or end-to-side configuration (Fig. 1C), re-establishing membrane and cytoplasmic continuity and therefore the original axonal tract. This process has been recognized for more than 50 years, and has been described in the motor neurons of crayfish [12], sensory neurons of the leech [13,14], giant axons of the earthworm [15], dissociated *Aplysia* sensory neurons *in vitro* [16] and, more recently, in the mechanosensory neurons of the nematode *C. elegans* [17,18]. In these studies, cytoplasmic continuity after rejoining of the two separated fragments was confirmed by electron microcopy [13,15,17,18], by injection of high molecular weight dyes (such as horseradish peroxidase) into the soma [14], or by expressing genetically encoded photoconvertible fluorophores such as Kaede [18], which were able to diffuse through the fusion site, from the soma to the distal axonal fragment. In some models, neuronal function has also been shown to recover fully at the electrophysiological [12,13,15,16] and behavioral levels [12,15].

Although the process of neuronal self-fusion during axonal regeneration has been well characterized at the morphological level, it was not until recent studies in *C. elegans* that the molecular mediators of this fusion process were identified [17,19]. In the *C. elegans* mechanosensory neurons, membrane fusion of the rejoining axonal fragments is mediated by the nematode fusogen EFF-1 [17,19]. In this process, EFF-1 is the final effector of a pathway involving changes in membrane lipid composition, which mediates the recognition of the separated distal fragment by the regrowing proximal fragment. In particular, these studies revealed that, following axonal transection, the lipid phosphatidylserine (PS) becomes exposed on the outer leaflet of the plasma membrane of the distal axonal fragment. Exposed PS itself, or PS bound by specific secreted ligands (such as the transthyretin TTR-52 or the lipid-binding protein NRF-5), is detected by transmembrane receptors present on the regrowing fragment (such as the PS receptor PSR-1, and possibly the TTR-52-binding receptor CED-1), thereby mediating recognition between the two separated axonal fragments prior to specific membrane fusion [19].

It is not known whether the role of these molecules in mediating axonal self-fusion during regeneration is conserved among species, but it is likely that similar molecular pathways are involved in other organisms, given that membrane fusion is an active process that requires specialized molecular players. PS exposure and recognition by cell surface receptors is a common mechanism for many cell-cell fusion events, and has been implicated in the fusion of myoblasts [20,21], syncytiotrophoblast cells in the placenta [22], and macrophages [23,24], as well as in the fusion that mediates the entry of some viruses into host cells [25,26]. Finally, it is likely that species-specific fusogens act as the last effectors in the mediation of membrane fusion. Taken together, these findings demonstrate that neurons of different classes and from different invertebrate species likely express functional fusogens and can fuse their membranes as a mechanism of repair.

Table 1
Self-fusion and cell-cell fusion of neurons and glia.

Biological process	Result of fusion	Neuronal or glial class	Site of fusion	Organism	Fusogen
Self-fusion					
Dendrite development	Self-contact elimination	Mec	Dendrites	*C. elegans*	EFF-1 [7]
Axonal development	Self-contact elimination	DRG *in vitro*	Axon	Chicken [9]	
Axonal regeneration	Rejoined axonal fragments	Motor	Axon	Crayfish [12]	
		Interneurons/Mec	Axon	Leech [13,14]	
		Giant axon	Axon	Earthworm [15]	
		Sensory *in vitro*	Axon	*Aplysia* [16]	
		Mec	Axon	*C. elegans*	EFF-1 [17–19]
Cell-cell fusion					
Axonal development	Neuronal syncytium (giant axon)	Third order giant fibers	Axon	Squid [27]	
Axonal regeneration	Neuronal syncytium	Mec	Axon	Leech [14]	
		Sensory	Axon	*C. elegans*	EFF-1 [18,19]
Sensory organ remodeling	Glial syncytium	Amphid sheath glia	Glia processes	*C. elegans*	AFF-1 [48]
Aging	Heterokaryon	BMDC/Purkinje	Soma?	Mouse/rat [60,65]	
Axosomatic synapses	Neuronal syncytium	PAG neurons	Axon/soma	Cat [93]	
In vitro culture	Neuronal syncytium	Neurons *in vitro*	Dendrites/soma	Snail [94]	
Contacting neuronal cell bodies and neurites	Neuronal syncytium	Hippocampal and cerebellar neurons	Soma	Rabbit [95]	
		Sympathetic neurons	Axon/dendrites	Cat [96]	
Viral infection	Neuronal syncytium (altered activity)	Superior cervical ganglia sympathetic neurons	Axon/dendrites [36] Soma [37]	Rat	PrV gB
	Neuronal syncytium (altered activity)	Submandibular ganglia peripheral neurons [38]	Axon	Mouse	PrV gB
	Heterokaryon	DRG and satellite cells [39]	Soma	Humans (xenographs in mice)	(VZV)
	Neuronal syncytium or heterokaryon	Sympathetic neurons and MeWo cells [40]	Axon/soma	Humans (*in vitro*)	(VZV or HSV1)
	Heterokaryon	Pyramidal and microglia [42]	Dendrites/ microglia	Mouse	(MRC)
SPC transplantation	Heterokaryon	Cortical/microglia/NSC [47]	Dendrites/soma	Mouse/rat	
		BMDC/Purkinje [51–53]	Soma?	Humans/ mouse	
		BMDC/cortical [55]	Soma?	Mouse	
		BMDC/spinal motor [71]	Soma?	Mouse	
BMDC transplantation in a model of diabetes	Apoptotic heterokaryon	BMDC/DRG [74]	Soma?	Mouse	
Hematopoietic SPC transplantation in retinal damage	Apoptotic or reprogrammed heterokaryon	Hematopoietic SPC/retinal neurons [72]	Soma?	Mouse	

BMDC: bone marrow derived cells; Cortical: cortical neurons; DRG: dorsal root ganglia neurons; gB: glycoprotein B; HSV1: herpes simplex virus type 1; Mec: mechanosensory neurons; Motor: motorneurons; MRC: murine retrovirus type-C; NSC: neural stem cells; PAG: periaqueductal gray substance; PrV: pseudorabies virus; Purkinje: Purkinje neurons; Pyramidal: pyramidal neurons; Sensory: senroy neurons; SPC: stem and progenitor cells; VZV: varicella-zoster virus.

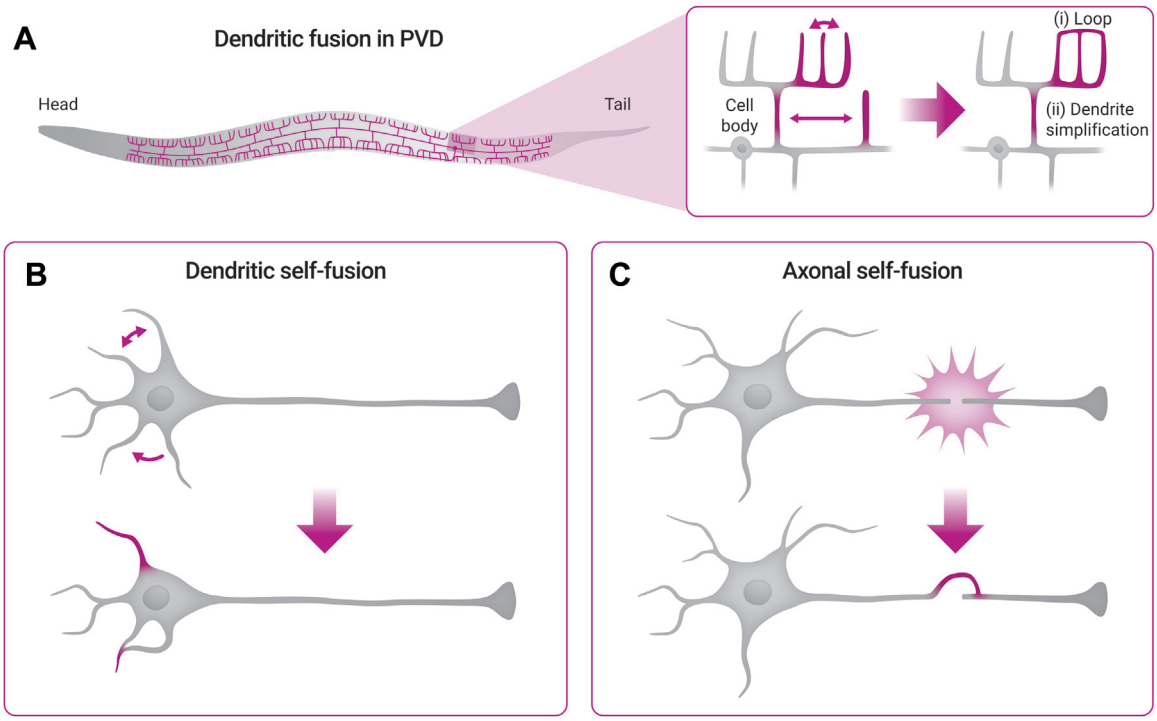

Fig. 1. Different forms of neuronal self-fusion. (A) *C. elegans* mechanosensory PVD neurons and their branched, stereotypical dendritic arbors. PVD dendrites can undergo fusion leading to the formation of loops (i), or to the simplification of the dendritic arbor (ii). (B) Self-contact elimination as a form of shaping dendrites. (C) Axonal self-fusion after injury. The separated proximal and distal axonal fragments fuse in an end-to-side configuration during axonal regeneration.

3. Neuronal cell-cell fusion and its implications

It is also possible for neurons to undergo fusion with other neurons or with other cell types, a phenomenon that we will refer to as neuronal cell-cell fusion. A select number of studies have shown that neuronal cell-cell fusion may occur spontaneously. For example, the giant axons of the squid are generated by complete fusion of multiple neurons (third order giant fibers) and the syncytial axon is enclosed in a single sheath cell [27]. In vertebrates, some studies using electron microscopy and brightfield microscopy have reported the formation of neuronal syncytia *in vitro* and *in vivo* [28–32]; unfortunately, however, these results were not quantified, making it difficult to determine their general relevance. Overall, the vast majority of neuronal cell-cell fusion events in different species have been described in the context of specific conditions or insults, which include viral infection, axonal injury, or the presence of stem or precursor cells (Fig. 2 and Table 1).

3.1. Viral-induced neuronal cell-cell fusion

It is well established that some viruses cause the formation of syncytia in infected tissues as a means of propagation and spreading. This ability to mediate cell-cell fusion of infected cells was used in early experiments as a technique to study gene expression and nuclear reprogramming in different cell types, including neurons. Using inactivated Sendai virus (one of the Para-influenza I group of Myxoviruses) [33], neurons were fused for the first time with undifferentiated green monkey kidney fibroblasts *in vitro* to form heterokarya [34,35]. Recent evidence shows that viral-mediated cell-cell fusion also occurs *in vivo* in the nervous system, with infected neurons forming viable syncytia (with either other neurons or different cell types). Currently, the mechanisms of viral-mediated neuronal cell-cell fusion, and the effects of these fusion events on the function of the nervous system, are just beginning to be elucidated.

A number of neuroinvasive viruses from the Herpesviridae family have been shown to induce neuronal cell-cell fusion *in vitro* and *in vivo*. Pseudorabies virus (PrV) was first shown to induce fusion between pre-and post-synaptic membranes of neurons in infected rat sympathetic and sensory ganglia, as evidenced by the observation of fusion pores at the electron microscopy level [36]. Later studies confirmed that PrV infection was able to induce fusion between infected sympathetic neurons *in vitro*, based on the diffusion of high molecular weight dyes from the soma of an injected neuron to neighboring neurons [37]. More recently, neuronal cell-cell fusion caused by PrV infection has been observed *in vivo* in the autonomic ganglia of infected mice [38]. Similarly, varicella-zoster virus (VZV) has been shown to induce fusion between neurons and satellite cells in human dorsal root ganglia (DRG) xenographs in mice. Half of the neuron-satellite cell complexes analyzed showed signs of cell-cell fusion, which is consistent with the ability of VZV to induce cell-cell fusion in skin lesions [39]. VZV can also induce fusion of DRG neurons with other non-neuronal cells that are in contact with the DRG axons, and potentially generates syncytia containing neurons and keratinocytes [40]. Finally, herpes simplex virus type 1 (HSV1) is capable of fusing DRG neurons with non-neuronal cells [40], although unlike VZV, it has not been found to mediate fusion with satellite cells [41]. Another example of viral-mediated neuronal cell-cell fusion comes from an experimental setting designed to label dividing cells in the mouse brain. Replication-incompetent murine retrovirus type-C, from the Retroviridae family, was found to induce fusion between neurons and microglia in the mouse neocortex [42]. Taken together, these lines of evidence show that some viral infections can generate viable neuronal syncytia or heterokarya, presenting a paradigm to study neuronal cell-cell fusion and, most importantly, the consequences of this fusion in terms of nervous system function.

In order to understand the mechanisms of cell-cell fusion between infected neurons, McCarthy and colleagues investigated the temporal and spatial formation of the fusion pores between

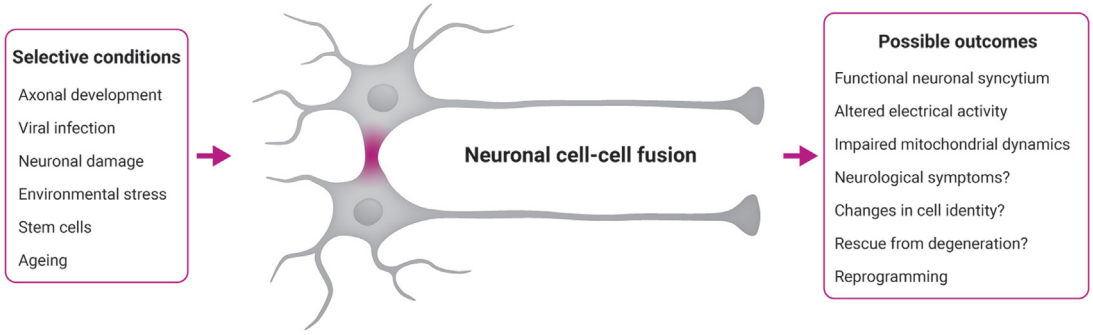

Fig. 2. Factors leading to neuronal cell-cell fusion and its consequences.

neurons *in vitro* [37]. Using a combination of dyes with different molecular weights, they found that PrV infection first induced the formation of small pores, which allowed the diffusion of only low molecular weight dyes between neurons, followed by the formation of larger fusion pores later in the course of infection, as evidenced by the diffusion of high molecular weight dyes. As infection progressed, signs of membrane fusion became apparent at the level of the soma, with multi-nucleated syncytia clearly visible.

Interestingly, viral-induced cell-cell fusion might occur in a specific compartment of the neuron. McCarthy and colleagues observed that *in vitro* PrV-infected neurons underwent fusion at the level of the soma, whereas PrV infection *in vivo* led to neuronal fusion at the axonal level (unmyelinated axons). Indeed, a PrV strain defective for the anterograde transport of virions and viral proteins into the axon was not able to induce the formation of neuronal syncytia [38]. VZV-induced fusion observed between DRG neurons and infecting cells occurred at either the axon or the soma [40]. In contrast, HSV1 was able to induce the fusion of DRG neurons with cells contacting the axon, but not with satellite cells, which are in contact with the soma. It has been proposed that the HSV1 virion, similar to PrV, may require transport into the axon to be functional, and therefore might only mediate fusion in this compartment [40]. Lastly, during fusion of neurons and microglia in murine C-type retrovirus-infected mouse brains, Ackman and colleagues observed a consistent location of fusion between the apical dendrites of neurons and the processes of the associated microglia [42]. This, together with the absence of other cell-cell fusion partners, pointed to a unique interaction between microglia and neurons that is favorable to membrane fusion. Taken together, these results indicate that viruses are able to hijack a neuron and make it transition into a fusion-competent cell, possibly in a spatially controlled manner to target particular neuronal compartments (Fig. 3). Although the molecular mechanisms of this transition are still to be identified, a likely prediction is that infected neurons are forced to express fusogens on their membranes. Some evidence does suggest that fusion is mediated by the same viral membrane fusion machinery that mediates the entry of the virus into the host cell. A mutated version of PrV lacking a functional viral fusion glycoprotein B (gB) (necessary for cell infection by the virus and its propagation to other cells), is unable to mediate the formation of neuronal syncytia following infection [37,38,43].

An important question that arises from these discoveries is what are the physiological and behavioral consequences of the formation of neuronal syncytia? Remarkably, it has been shown that PrV-induced syncytia in cultured neurons have altered electrophysiological activity, as fused neurons display synchronous electrical activity, as well as elevated rates of spontaneous action potential firing [37]. Similar results have been found *in vivo*, with neurons in the ganglia of PrV-infected mice showing a synchronous and cyclical calcium pattern [38]. This spontaneous and synchronous cyclic activity had already been reported decades ago in neuronal tissues infected by PrV virus [36,44,45]. Abnormal electrical activity is hypothesized to be the cause of the characteristic symptoms caused by alpha-herpesviruses, *i.e.* numbness and tingling, and the sensations of itching and pain. At the cellular level, the formation of neuronal syncytia was also shown to affect mitochondrial dynamics *in vitro* [43]. After PrV infection, the formation of syncytia was followed by an increase in the intracellular calcium level, which in turn disrupted the association of the mitochondrion membrane anchored-Miro1 with Kinesin-1 through its calcium binding sites; this resulted in disruption of recruitment of mitochondria to Kinesin-1 and, as a consequence, altered mitochondrial transport. It is important to note that such impairment of mitochondrial dynamics could lead to neuronal degeneration.

Overall, viral-induced neuronal syncytia are viable, but their formation alters the normal electrophysiological properties of the neurons, which in turn could result in an altered "behavioral" output. Moreover, cell-cell fusion also alters cellular processes required for maintaining neuronal homeostasis.

3.2. Ectopic neuronal cell-cell fusion during axonal regeneration

Fusion between individual neurons has also been reported to occur during axonal repair after injury, first in the leech several decades ago [14] and more recently in *C. elegans* [18,19]. Although the fusion between the regrowing proximal fragment and its own distal fragment is generally specific, it has been reported that when two or more fasciculating axons are simultaneously transected, the regrowing proximal axonal fragment of one neuron can fuse with the proximal or distal axonal fragment of the nearby injured neuron [14,18]. In *C. elegans*, this event has been well characterized in two pairs of tightly associated neurons: PLM-PLN and ALM-ALN [18]. The axons of PLM and PLN extend from their respective cell bodies on the posterolateral side of the animal, and run in close association under the epidermis toward the midbody. Similarly, the axons of ALM and ALN run in close association on the anterolateral side under the epidermis, toward the head of the animal. When both axons in each pair were transected, the transfer of fluorophore from one cell to the other revealed fusion between PLM and PLN in ~10% of cases, and between ALM and ALN in 13% of cases [18]. Remarkably, PLM-PLN neuronal fusion has also been observed in a different model of axonal injury, induced by the lack of the cytoskeletal component UNC-70/β-Spectrin that causes axonal fragility [19,46]. Importantly, it has been demonstrated that PLM-PLN fusion depends on the same molecular machinery that mediates self-fusion between the PLM proximal and distal axonal fragments, requiring the fusogen EFF-1 [19]. It remains unclear whether this type of cell-cell fusion occurs by accident, or if it plays a physiological role. It is also unknown whether it has consequences in terms of the identity and function of the neurons involved. However, the very low frequency at which such fusion is observed makes

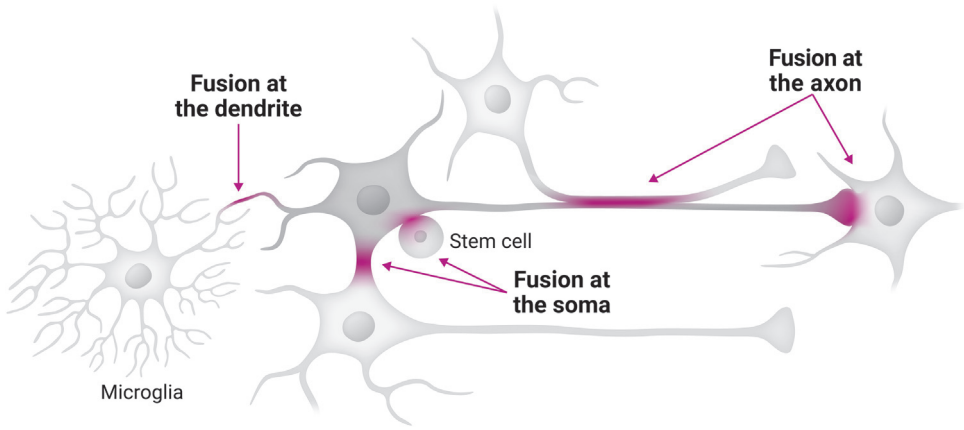

Fig. 3. Different neuronal compartments can engage in cell-cell fusion.

it tempting to speculate that a specific molecular mechanism favors specific self-fusion repair and prevents cell-cell fusion.

3.3. Cell-cell fusion with glia and between glial cells

As briefly mentioned above, cell-cell fusion events involving neurons may occur with a glial partner. VZV infection causes fusion between neurons and the support cells of the DRG [39], and replication-incompetent C-type retrovirus injected in the mouse neocortex causes very specific fusion between neurons and microglia [42]. Fusion has also been shown to occur between microglia and neuronal stem cells [47]. However, one of the clearest examples of developmentally regulated glial fusion occurs in the major sensory organs of *C. elegans*, the amphids, which are located on the left and right sides of the nematode's head. Two support cells, the amphid sheath (AMsh) and socket (AMso) cells, provide the structural support and a channel for the sensory cilia of 12 neurons that are exposed to the environment and detect external stimuli. In harsh environmental conditions, such as the absence of food, high temperature, or high population density, *C. elegans* enter a diapause stage (*Dauer* stage), which is characterized by a dramatic remodeling of body structures, including the amphids [48,49]. Cell-cell fusion occurs between the left and right AMsh glial cells, and this event is required for the correct morphological and functional changes to the ciliated receptive endings of the chemosensory neurons. This AMsh glial fusion event is mediated by the second known *bona fide C. elegans* fusogen, Anchor cell Fusion Failure-1 (AFF-1) [48]. These examples reveal that not only neurons but also glial cells can engage in cell-cell fusion events.

3.4. Cell-cell fusion between neurons and stem cells

In the past decade, it has become clear that cell-cell fusion is a key mechanism through which stem or progenitor cells of one tissue give rise to differentiated cells of another tissue. In 2002, Ying and colleagues demonstrated that progenitor cells of the central nervous system were able to fuse with embryonic stem cells, giving rise to pluripotent hybrid cells [50]. Other milestone discoveries revealed that bone marrow cells were able to fuse spontaneously with neuronal progenitors *in vitro* [51], and that following bone marrow transplantation, bone marrow-derived cells fused with Purkinje neurons *in vivo*, forming binucleated heterokarya [51–53]. This phenomenon has been demonstrated in both humans and mice, and can also occur between cells of different species [54]. Interestingly, these binucleated heterokarya retain the Purkinje neuron cell fate, as the nucleus of the stem cell (usually round with condensed chromatin) acquires a Purkinje neuron-like nucleus shape (with dispersed chromatin), and also expresses some Purkinje neuron-specific genes [51,55,56]. Most importantly, these heterokarya have been shown to act as electrically active Purkinje neurons [57], suggesting that in this case fusion is not detrimental to the neuron.

A series of important subsequent studies further characterized the formation of these heterokarya and their possible physiological role (reviewed in [58]). The frequency of heterokaryon formation in both humans and rodents is very low, and ranges between ~0.1% and 0.4% of the total Purkinje neuron population [51–54,59]. However, several studies indicate that this rate of fusion increases (10–100 fold) with damage [60–62], inflammation [56,61,63], neurodegenerative disease [54,56,59,64] or chemically induced degeneration [65]. Although some authors defend the hypothesis that fusion is almost exclusively a consequence of damage and irradiation [61], others report that fusion is not caused by the irradiation and transplantation procedure itself, as blood chimerism obtained by surgically joining two mice (parabionts) also leads to the formation of heterokarya between the hematopoietic cells of one mouse and the Purkinje neurons of the other mouse [56]. Most importantly, other studies have revealed that neuronal fusion might also occur during normal development and during aging in non-manipulated mice [60,65]. An increase in fusion rate with aging has also been found in irradiated models [53,60], supporting the idea that aging somehow favors the acquisition of fusion competence by neurons. The contribution of hematopoietic cells to Purkinje neurons can occur in physiological conditions; however, these cells were found to be mononucleated, suggesting that fusion might be a transient event or that another mechanism is in place [63].

Despite considerable evidence of heterokaryon formation accumulated during the past decade, it is still unclear if this process plays a specific physiological role. As noted above, the heterokarya remain functional Purkinje neurons, and a study by Bae and colleagues found that the degeneration of Purkinje neurons in a mouse model of Niemann-Pick disease type C1 (a disease that affects the function of the cerebellum leading to impaired motor function) was alleviated after transplantation of bone marrow-derived mesenchymal stem cells [64]. Similar results were obtained in a mouse model of cerebellar ataxia, although the exact role of cell fusion in this functional recovery was not clear [66]. Overall, it has been hypothesized that the fusion of progenitor or stem cells with damaged neurons provides a healthy nucleus that is able to rescue these highly complex neurons that would otherwise be impossible to replace. This is similar to the regenerative role of cell-cell fusion

and heterokaryon formation first described in skeletal muscle [67] and liver [68,69] (for a review see [70]).

Fusion in the nervous system has also been observed outside the cerebellum, with reports that bone marrow-derived stem cells can fuse with cortical neurons [55] and spinal motor neurons [71] following transplantation. Fusion has also been described between embryonic stem cell-derived neuronal stem cells and microglia *in vitro*, and between neuronal stem cells and mature pyramidal neurons in the neocortex of mouse and rats *in vivo* [47].

Another important study has revealed that injured retinal neurons can undergo fusion with transplanted hematopoietic stem and progenitor cells [72]. Remarkably, following activation of the Wnt/beta-catenin signaling pathway, these hybrids are programmed to a precursor stage, proliferate and develop into differentiated neurons, providing partial regeneration of the damaged retina and functional rescue. However, lack of activation of the Wnt/beta-catenin signaling pathway leads to apoptosis of the newly formed heterokarya [72].

It is important to note that some neuronal types seem to be refractory to fusion, even under the same conditions in which Purkinje neurons form heterokarya [61,73]. This raises the possibility that some neurons may be more prone to fusion than others. It also suggests that there might be an inherent resistance to cell-cell fusion, and that neuronal fusion is potentially harmful in some contexts. Consistent with this, fusion of bone marrow-derived cells with DRG neurons has been implicated in the pathogenesis of diabetic neuropathy. In a mouse model of diabetes, cells thought to arise from these fusion events following bone marrow transplantation displayed abnormal calcium homeostasis and accelerated apoptosis [74].

In summary, the presence of progenitor cells or stem cells is able to facilitate neuronal fusion under certain circumstances, an effect that can be enhanced by different types of neuronal insults as well as aging. However, the cellular and molecular mechanisms underpinning these fusion events are still unknown. For example, it is not known whether a specific fusogen becomes expressed on the surface of progenitor cells or neurons, making them more prone to fusion, or if specific neuronal types have a particular proteolipidic membrane composition that makes them fusion competent. Moreover, it is still controversial whether these cell-cell fusion events are beneficial or detrimental to the neurons involved. Answers to these questions might allow us to control this biological process and potentially expand it for beneficial medical purposes, a concept already under consideration [58,75,76].

4. Membrane nanotubes between neurons and between neurons and glia

In 2004, a seminal study revealed the existence of specific nanotubular membrane structures that formed *de novo* between neuroendocrine cells, thereby allowing the transfer of vesicles, molecules, and organelles, and providing a new route for long-distance cell-to cell-communication [77]. These peculiar structures, named tunnelling nanotubes or TNTs, have a diameter of 50–200 μm and a length of several cell diameters [78]. In addition to their role in HIV-1 transmission in T-cells [79], an important study has shown that TNTs represent an efficient route for spreading of prions between neurons in the central nervous system, as well as between immune cells and neurons [80]. Importantly, TNTs formed between neurons and distant astrocytes have also been shown to facilitate electrical coupling and calcium signaling [81]. Thus, TNTs represent a *de facto* neuron-neuron or neuron-glia membrane fusion event, albeit they are mostly temporary, they transport only selected cargo, and they do not form true syncytia. It is still unclear, and of the utmost interest, what molecular elements regulate the formation of TNTs, how the recognition proceeds between the cells involved, and most importantly what fusogenic elements facilitate the actual fusion of the two distinct membranes and what limits it to a transient process.

5. Expression of fusogens in the nervous system in health and disease

Most of the studies on neuronal or glial cell-cell fusion report these events at the cellular level, studying the circumstances and consequences of fusion; however, only a few have explored the underlying molecular mechanisms. Membrane fusion is an active process and therefore requires specific protein and lipid effectors. Viral-induced neuronal cell-cell fusion is likely to be mediated by the viral fusogens expressed by infected neurons [37,38,43]. Studies on neuronal fusion in *C. elegans* have identified the nematode fusogen EFF-1 as the main effector of self-fusion during PVD dendrite development [7] and axonal regeneration of PLM neurons [17,19]. EFF-1 is also responsible for PLM-PLN cell-cell fusion following axonal injury [19], and the second nematode fusogen AFF-1 has been identified as the main effector of AMsh glial cell-cell fusion [48]. Interestingly, when EFF-1 and AFF-1 were first discovered in *C. elegans*, both fusogens appeared to be expressed in neurons and glia [8,82]. However, this was a puzzling discovery, given that at the time, these cells were not known to engage in fusion events. Only recently has it been shown that these fusogens are active in neurons and glia, providing evidence that the *C. elegans* nervous system has the molecular machinery necessary for membrane fusion. With this in mind, could the expression pattern of fusogens in vertebrates be used to predict if these neurons are also fusion competent? In humans, two highly fusogenic proteins of retroviral origin have been identified: Syncytin-1 (encoded by the HERV-W envelope gene located in chromosome 7q21.2) [83,84] and Syncytin-2 (encoded by the HERV-FRD envelope gene located in chromosome 6p24.1) [85]. Both fusogens are almost exclusively expressed in the placenta, and Syncytin-1 and its receptors (the neutral amino acid transporter or type D mammalian retrovirus receptor ASCT 2 and the related protein ASCT 1) are responsible for mediating the fusion events involved in the formation of the syncytiotrophoblast, the syncytial outer layer of the placenta. Interestingly, although expression of these fusogens has not been detected in the nervous system of healthy patients, expression of Syncytin-1 has been found to be upregulated in the brain of patients with multiple sclerosis [86,87], and envelope genes of the HERV-W family have been found in the cerebrospinal fluid of patients with schizophrenia and bipolar disorder [88–90] (for a review on this topic see [91]). How the expression of fusogens might contribute to the etiology of neurological disorders is just beginning to be explored. In multiple sclerosis, the expression of Syncytin-1 in astrocytes and microglia mediates neurotoxicity by triggering a robust inflammatory response that is toxic to oligodendrocytes and leads to demyelination, which explains at least in part the deleterious effect of this fusogen [86]. Whether cell-cell fusion events also contribute to this pathology has not yet been tested, but it is tempting to hypothesize that the expression of Syncytin-1 in the nervous system could lead to cell-cell fusion. Finally, in mice, Syncytin-A and Syncytin-B proteins have been identified as functional fusogens that are also involved in the formation of the syncytiotrophoblast layer of the placenta [92]. These genes are essentially expressed in this tissue; however, Syncytin-A transcript expression is also detected in the brain above background levels [92]. Ultimately, there is evidence that fusogens are expressed in the nervous systems of multiple species; whether this expression occurs in health or disease, it supports the idea that neurons and glia may acquire fusion competence under certain circumstances, and suggests that

unknown self-fusion or cell-cell fusion events remain to be discovered.

6. Conclusion

The concept that neurons can form syncytia or heterokarya is still relatively novel, and the evidence of neuronal fusion discussed here represents the tip of the iceberg. A range of studies addressing different questions in biology (viral infection, tissue regeneration, neuronal development, and more recently the discovery of nanotubes) has converged on the concept of neuronal fusion; however, much remains to be explored in this fascinating field. Are all classes of neurons fusion competent? Under which circumstances do neurons fuse? What are the ultimate consequences of neuronal fusion?

There are a number of factors unique to the nervous system that make *in vivo* studies of cell-cell fusion difficult or impossible to conduct. Firstly, the sheer complexity of the central nervous system, in terms of cellular number, architecture and density, makes observations of neuronal fusion technically challenging. Secondly, neuronal fusion can occur not only at the level of the soma, but also at the level of axons or dendrites, and cellular connections in such a spatially restricted area may be impossible to discern with the resolution of current imaging techniques. Finally, identifying cell-cell fusion in neurons may be further confounded by the fact that such fusion can be transient and result in mono-nucleated diploid cells [63]. There are clearly challenges to address, and progress is most likely to be achieved through a combination of different experimental paradigms in diverse model systems.

We have begun to elucidate some of the molecular mechanisms that mediate neuronal fusion, starting with the EFF-1-containing pathways for neuronal self-fusion and cell-cell fusion in *C. elegans*. The molecules that mediate neuronal fusion in vertebrates, and the role for as-yet-undiscovered fusogens in these systems, are currently unknown. It also remains to be determined whether membrane-exposed PS, an essential requirement for neuronal self-fusion during regeneration, has a common role in neuronal fusion.

Another important step will be to determine how the molecular machinery for neuronal fusion is regulated. The current evidence suggests that the circumstances of neuronal fusion are highly specific. Although very little is known about the physiological advantage or possible detrimental effect of neuronal cell-cell fusion, it is clear that it can have consequences for the neuron at both the subcellular and electrophysiological levels. Exactly how the balance is established between healthy, developmental self-fusion, and protection from ectopic cell-cell fusion, is still to be determined. One hypothesis is that it involves subcellular regulation of cell-specific fusogens, with changes in expression and localization of these molecules occurring in response to specific stimuli or insults.

The finding that neurons can form syncytia or heterokarya might be seen as a challenge to Ramón y Cajal's neuron theory. Conversely, our current understanding of neuronal fusion may instead strengthen this theory, as neurons appear to fuse only under very specific circumstances, and it is likely that mechanisms exist to prevent uncontrolled cell-cell fusion, thereby maintaining neurons (for the most part) as individual units.

Acknowledgements

We thank Rowan Tweedale, Brent Neumann, Fabrice Turpin, Sean Coakley, Alessandra Donato, Eva Kaulich, Xue Yan Ho, and Fiona Ritchie for valuable discussions and comments on the manuscript, and Nick Valmas for help on graphic design. This work was supported by a NHMRC Senior Research Fellowship 1111042, Project Grants 1067461, 1068871, and an ARC Discovery Project 160104359 to M.A.H., a Human Frontier Science Program Long-Term Fellowship LT000762/2012 to R.G.S., and a University of Queensland Research Scholarship to C.L.

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