



Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Smillie, KJ;Cousin, MA;Gordon, SL

Title:

Preface to the Special Issue “Presynaptic Dysfunction and Disease”

Date:

2021-04-01

Citation:

Smillie, K. J., Cousin, M. A. & Gordon, S. L. (2021). Preface to the Special Issue “Presynaptic Dysfunction and Disease”. *Journal of Neurochemistry*, 157 (2), pp.102-106. <https://doi.org/10.1111/jnc.15319>.

Persistent Link:

<https://hdl.handle.net/11343/298352>

DR. KAREN JANET SMILLIE (Orcid ID : 0000-0003-4369-0470)

DR. MICHAEL ALAN COUSIN (Orcid ID : 0000-0002-1762-160X)

DR. SARAH L GORDON (Orcid ID : 0000-0003-2281-3160)

Article type : Invited Editorial Highlight

m.cousin@ed.ac.uk

Presynaptic Dysfunction and Disease

Karen J. Smillie^{1,2}, Michael A. Cousin^{1,2,3} and Sarah L. Gordon⁴

1 - Centre for Discovery Brain Sciences, Hugh Robson Building, George Square, University of Edinburgh, Edinburgh, Scotland, EH8 9XD

2 - Muir Maxwell Epilepsy Centre, Hugh Robson Building, George Square, University of Edinburgh, Edinburgh, Scotland, EH8 9XD

3 - Simons Initiative for the Developing Brain, Hugh Robson Building, George Square, University of Edinburgh, Edinburgh, Scotland, EH8 9XD

4 - The Florey Institute of Neuroscience and Mental Health, Melbourne Dementia Research Centre, The University of Melbourne, Melbourne, Victoria, 3011, Australia.

Keywords

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/JNC.15319](https://doi.org/10.1111/JNC.15319)

This article is protected by copyright. All rights reserved

JNeurochem Special issue “Presynaptic Dysfunction and Disease”; calcium; neurotransmitter; presynapse; synaptic vesicle; neurodevelopmental disorder; neurodegeneration

Abstract

The synapse is formed between a presynapse (which releases neurotransmitter) and the postsynapse (which transduces this chemical signal). Over the past decade, presynaptic dysfunction has emerged as a key mediator of a series of neurodevelopmental and neurodegenerative disorders. This special issue will highlight some of the important presynaptic molecules and mechanisms that are disrupted in these conditions and reveal potential routes for therapy.

Abbreviations

SV - Synaptic vesicle; LRRK2 - leucine rich repeat kinase 2.

[Main text, no separate header]

The fundamental principle underlying brain communication is the tightly controlled release of chemical neurotransmitter in response to action potential stimulation. Neurotransmitter release occurs at presynaptic terminals. These highly specialized subcellular structures, enriched in neurotransmitter-containing synaptic vesicles (SVs), lie directly opposed to postsynaptic compartments adorned with receptors, which ensures timely and efficient neurotransmission. Nerve terminals are usually located at the extreme termini of the neuronal axon, sometimes over a meter from the cell body, and due to their isolation from the neuronal soma, they have evolved a series of adaptations that allow them to function as an almost autonomous entity. These include: 1) a modifiable energy supply in the form of mobile presynaptic mitochondria (Devine & Kittler 2018), 2) a highly organized microanatomy to ensure clustering of key molecules for neurotransmitter release and facilitate instantaneous and highly concentrated increases in intracellular free calcium (Emperador-Melero & Kaeser 2020) and, 3) a series of recycling modes to regenerate SVs with a high degree of speed and fidelity (Chanaday *et al.* 2019). The efficient recycling of SVs at the presynapse is essential to sustain neurotransmitter release and thus complex patterns of brain activity. During this life cycle, SVs are first filled with neurotransmitter, before being organized into specific functional pools that are mobilized by particular patterns of neuronal activity (Rizzoli & Betz 2005). Neurotransmitter release occurs at the active zone, a presynaptic region with strikingly complex microarchitecture. Evoked release is initiated by the invasion of action

potentials, which cause the synchronous activation of highly clustered voltage-dependent calcium channels, thereby creating a microdomain of exceptionally high intracellular calcium (Dolphin & Lee 2020). This triggers the sub-millisecond fusion of SVs that are physically tethered to the active zone plasma membrane. After this point, both SV proteins and lipids are retrieved from the plasma membrane via a variety of endocytosis modes that are recruited with different timescales in response to different patterns of neuronal activity (Chanaday et al. 2019).

It has been acknowledged for decades that acute presynaptic dysfunction can precipitate serious human disease. However, its role in more prolonged congenital or degenerative disorders remained contentious, since defects in this process would seemingly be incompatible with life. However, in the past 10 years a leap in our understanding of the relationship between presynaptic dysfunction and neurological disorders has occurred. This revolution has been driven by advances in genomic sequencing and has revealed that presynaptic dysfunction can either contribute to, or be directly causal for, a range of neurodevelopmental and neurodegenerative disorders. These interrelated topics will be explored in this special issue of *The Journal of Neurochemistry*.

The recent expansion in data, particularly from –omics based techniques, has directly or indirectly implicated many presynaptic genes and proteins as having a potential role in the expression of pathogenic phenotypes. This has highlighted a requirement to be able to interrogate the function and dysfunction of the presynapse in different models of human disease. Harper et al (2020), discuss both established and emerging techniques to monitor presynaptic function with a particular emphasis on SV recycling. The range of independent approaches through which SV recycling can be investigated is relatively limited and most notably includes amphiphilic dyes, fluid phase markers, electrophysiology and genetically-encoded reporters. However, each technique can be adapted in a variety of ways to address a surprising breadth of parameters, allowing distinct aspects of the SV cycle to be teased apart and specific aberrant processes to be exposed. Identification of presynaptic dysfunction is of limited value however, unless the molecular defect can also be revealed. Therefore, techniques that define protein targets, identify alterations to protein-protein interactions and pinpoint where these occur in the cell, are central to establishing both mechanism and phenotype. The integrated application of these approaches, as described by Harper et al (2020), is key to resolving how presynaptic dysfunction may culminate in human disease.

One of the first demonstrations that disturbance of presynaptic function can cause neurological impairment came from pioneering studies into the mechanisms underlying neurotoxin action. These studies revealed that neurotoxins, such as tetanus and botulinum toxin (from clostridial bacteria) and α -latrotoxin (from the widow family of spiders), cause a cessation or dysregulation of SV fusion (Sudhof 2001, Dong *et al.* 2019). Several decades after these studies, the genetic revolution is now revealing that the core exocytic machinery is perturbed in a wide variety of neurological disorders, including neurodevelopmental, neuromuscular and neurodegenerative disorders. (Melland *et al.* 2020) overview how disturbance of the SV fusion machinery via either toxin action or genetic mutation leads to altered neurotransmitter release and neurological dysfunction. They audit the specific mutations across each of the SV fusion proteins and explore the biochemical and physiological mechanisms likely underlying disease pathogenesis. In cataloguing these various neurological disorders associated with dysregulation of the exocytic machinery, Melland *et al.* (2020) reveal common overlapping clinical features that link these seemingly divergent disorders of SV fusion, as well as highlighting some surprising points of heterogeneity.

STXBP1/Munc18-1 is one of the most commonly mutated SV fusion proteins associated with neurological dysfunction, and is therefore arguably one of the most extensively investigated in terms of pathogenicity (O'Brien *et al.* 2019). Mutations in the *STXBP1* gene have long been associated with various forms of epileptic encephalopathies, however, as discussed by (Abramov *et al.* 2020), we now understand that intellectual disability or developmental delay is a universal feature of *STXBP1* encephalopathies, with epilepsy being a highly prevalent but not requisite concomitant feature. There is remarkable heterogeneity in the types of disease-causing mutations and their location within the *STXBP1* gene, with little genotype-phenotype correlation. Many of these mutations (including missense variants) cause instability or reduced expression of the STXBP1/Munc18-1 protein, and given this context, Abramov *et al.* (2020) compare evidence for haploinsufficiency versus dominant-negative effects as causative of underlying pathogenesis of the disorder. Given the prominence of *STXBP1* mutations as a source of human disease, *STXBP1* encephalopathies are also acting as a forerunner in terms of investigations into potential avenues of treatment – including disease-modifying therapies - of synaptopathies.

A presynaptic contribution to neurodevelopmental disorders goes much wider, however, than just the disorders of SV fusion outlined above. As highlighted by (Bonnycastle *et al.* 2020), mutations in genes

responsible for almost every aspect of the SV life cycle are implicated in neurodevelopmental disorders of genetic origin. These aspects include SV filling with neurotransmitter, organization of functional SV pools, SV cargo clustering and retrieval post-fusion, as well as the various SV endocytosis modes. These mutations in genes encoding the SV recycling machinery can result in either a loss or gain of function of the respective gene products, making interpretation of the molecular, cellular and circuit consequences of these insults difficult to decode without detailed systematic analysis. The impact of these variants on brain function can be potentially diverse, with the outcome dependent on a series of factors, including the activity patterns of specific neuronal circuits, expression in different neuronal subtypes, or the developmental trajectory of specific parameters within the SV life cycle. However, a rapidly increasing knowledge base on the effect of these mutations has started to suggest potential avenues of treatment, not just at the level of gene correction, but also by overriding the defective mutation by either boosting intracellular signaling or parallel pathways.

The discovery of individuals harboring disease-causing mutations allows the stratification of patients that exhibit common phenotypes, to reveal common pathways and networks. This topic is explored by (John *et al.* 2020). In this review, they applied a functional network framework of published studies of neurodevelopmental disorders with identified mutations in genes encoding the SV recycling machinery and systematically collated phenotypes across both genes and publications. In addition they performed parallel quantitative analysis of the DECIPHER database, to determine the most commonly presenting clinical phenotypes characteristic of these disorders. By stratiating these across presynaptic functions, John *et al.* (2020) were able to collate the most common neurological features present when distinct aspects of the SV cycle are perturbed. While this approach has limitations due to the rarity of some of the mutations it nevertheless illustrates that for most, if not all, disorders of SV recycling, there is unlikely to be one unique phenotype, but instead a range of clinical outcomes.

The work within the studies discussed above is essential for enhancing the clinical understanding and diagnosis of these neurological disorders. However, the broader goal is the identification of potential treatment strategies. The most direct method to combat monogenic disorders is to correct the genetic insult at source. (Turner *et al.* 2020) discuss progress in this sphere in the context of neurodevelopmental disorders with epilepsy. Neurodevelopmental disorders with epilepsy as a comorbidity can arise from mutations in specific genes that cause changes in gene expression, ion channel function and/or synaptic transmission. To design effective therapies, preclinical models which

accurately reflect both the genetic insult (construct validity) and the clinical condition (face validity) are essential. Advances in gene editing technologies have resulted in a large expansion of rodent models, which has vastly improved construct validity to a degree previously impossible. However, the face validity of these models in many of these instances can be complex when relating to the human condition and is dependent on the mutation, making translational studies more challenging. Such obstacles are being overcome through the concomitant development of human *in vitro* models, which have the advantage of possessing the same genetic information of the affected individual, and can be corrected using gene editing to obtain isogenic controls. A powerful refinement of standard two-dimensional cultures are organoids, which have the potential to more accurately model the early stages of brain development. The most potent strategy is to apply both approaches in parallel, to balance both species and systems complexity limitations. A critical consideration in the application of disease modifying therapies is whether these genetic insults can be corrected early enough in neuronal development for clinical phenotypes to be reversed. Exploratory studies, while still in their infancy, are now beginning to be performed and will reveal the potential therapeutic window for gene therapy. The incredible pace of development of gene and base editing technologies have the potential to radically transform this research field; a key determinant in the clinical translation of these strategies will be whether the viral delivery systems can match the progress of these genetic editing tools.

At the opposite end of the developmental spectrum are neurodegenerative diseases. These disorders have also been linked to presynaptic dysfunction, in particular perturbation of SV endocytosis. A series of independent endocytosis modes are present at the presynapse, to ensure that SVs are rapidly regenerated from the presynaptic plasma membrane with the correct cargo molecules (Chanaday et al. 2019). However, these pathways are not limited to SV cargo retrieval and SV regeneration. Instead, they are intricately linked to a series of intracellular trafficking pathways and cellular degradation processes that converge on the lysosome, such as the endolysosomal system and autophagy (Birgisdottir & Johansen 2020, Hu et al. 2015). Recognition of the importance of autophagy in maintaining presynaptic physiology has emerged over the past decade, however its interdependence with endocytosis and how this impacts presynaptic function requires further examination. (Overhoff et al. 2020) examine the common molecular players that are utilized in both the neuronal endocytic and autophagy pathways, and highlight that these have overlapping mechanisms and interlinked components. They discuss how these two connected pathways sculpt both SV regeneration and axonal trafficking of presynaptic cargo

molecules and explore how mutations in specific components can both impact on, and culminate in, neurodegeneration.

One such neurodegenerative disorder is Parkinson's Disease, which - while likely having a complex etiology - can be caused by rare mutations in a series of genes which encode proteins with presynaptic roles, including α -synuclein, Parkin, DJ-1 and leucine rich repeat kinase 2 (LRRK2) (Klein & Westenberger 2012). Mutations in the large multidomain kinase and GTPase LRRK2 are one of the most common causes of familial Parkinson's Disease, with most variants likely causing dysfunction via increasing kinase activity. Notably, LRRK2 phosphorylates several presynaptic proteins and thereby regulates diverse aspects of presynaptic function, including SV endocytosis, exocytosis, and the mobilization of distinct SV pools. (Pischedda & Piccoli 2020) provide a comprehensive overview of the current understanding of LRRK2 function in the synapse-specific modulation of SV dynamics. One tantalizing discovery is that LRRK2 may act differentially in glutamatergic and dopaminergic synapses, a finding with potentially important implications for understanding the pathogenesis underlying Parkinson's Disease.

Perspectives

The past decade has been a golden age for identifying new pathogenic mutations in presynaptic genes across a diverse range of neurological disorders. But how can this information be best harnessed and leveraged to 1) reveal mechanisms underlying disease pathogenesis, 2) identify new treatment strategies, and 3) improve clinical care outcomes?

It is becoming apparent that to determine the pathogenesis underlying these presynaptic synaptopathies, sophisticated studies are required that investigate the specific impacts of alterations to the presynaptic machinery in an appropriate disease context. Whilst studies using knockout / knock-in systems have proved informative for investigating protein function, they provide limited clinically relevant information, especially for heterozygous disorders (which represent the vast majority of presynaptic neurological disorders). Therefore there is an urgent need for an expansion of studies that investigate the impact of specific mutations in appropriate disease model contexts. This improved construct validity should uncover whether disease variants induce pathogenesis by loss of function or dominant-negative effects. However, a note of caution must be applied at this stage, since model systems with accurate construct validity can display extremely mild phenotypes. Therefore, robust outcome measures will be critical when investigating these more disease-relevant model systems.

Furthermore, in terms of mutations to presynaptic genes, not all mutations can be considered equal. In multiple cases there is a notable heterogeneity in the impact on presynaptic activity from different mutations to the same gene. While molecular modelling may also prove useful in informing predictions of the impacts of specific mutations, there remains a need for unbiased screening of the effect of distinct mutations, using multiple complementary assays that can dissect specific pleiotropic effects within the nerve terminal. To this end, employing the most powerful and informative assays in the most appropriate model systems is of paramount importance. This may finally reveal new insights into genotype-phenotype relationships, which will have important implications in diagnosis of presynaptic disorders and has the potential to enable individualized clinical management in the future.

Finally, interrogation of genetic neurological disorders is powering an explosion into our understanding of the precise molecular mechanisms underlying presynaptic function. Studies utilizing these genetic variants have not only uncovered the multifaceted roles played by a range of proteins, but are also revealing the importance of specific residues within those proteins and their intramolecular interactions. Application of this reverse-translational approach – using mutations that cause human disease to inform our investigation into protein function – provides researchers with unprecedented insight, down to an atomic scale, of how the presynaptic activity is coordinated.

Acknowledgements

This work was supported by an Ideas Grant from The National Health and Medical Research Council (NHMRC) of Australia (SLG, grant reference – GNT2003710), an Investigator Award from The Wellcome Trust (MAC, grant reference -204954) the Simons Foundation Autism Research Initiative (SFARI) to the Simons Initiative for the Developing Brain (MAC, grant reference - 529805) and Cure Huntington’s Disease Initiative (KJS and MAC, grant reference – A-14021). The Florey Institute of Neuroscience and Mental Health acknowledges the strong support from the Victorian Government and in particular the funding from the Operational Infrastructure Support Grant.

Conflict of interest

The authors declare no conflict of interest beyond having edited the current special issue “Presynaptic Dysfunction and Disease”.

Reference list

- Abramov, D., Guiberson, N. G. L. and Burre, J. (2020) STXBP1 encephalopathies: Clinical spectrum, disease mechanisms, and therapeutic strategies. *Journal of neurochemistry*.
- Birgisdottir, A. B. and Johansen, T. (2020) Autophagy and endocytosis - interconnections and interdependencies. *Journal of cell science*, **133**, jcs228114.
- Bonnycastle, K., Davenport, E. C. and Cousin, M. A. (2020) Presynaptic dysfunction in neurodevelopmental disorders: Insights from the synaptic vesicle life cycle. *Journal of neurochemistry*.
- Chanaday, N. L., Cousin, M. A., Milosevic, I., Watanabe, S. and Morgan, J. R. (2019) The Synaptic Vesicle Cycle Revisited: New Insights into the Modes and Mechanisms. *J Neurosci*, **39**, 8209-8216.
- Devine, M. J. and Kittler, J. T. (2018) Mitochondria at the neuronal presynapse in health and disease. *Nature reviews. Neuroscience*, **19**, 63-80.
- Dolphin, A. C. and Lee, A. (2020) Presynaptic calcium channels: specialized control of synaptic neurotransmitter release. *Nature reviews. Neuroscience*, **21**, 213-229.
- Dong, M., Masuyer, G. and Stenmark, P. (2019) Botulinum and Tetanus Neurotoxins. *Annual review of biochemistry*, **88**, 811-837.
- Emperador-Melero, J. and Kaeser, P. S. (2020) Assembly of the presynaptic active zone. *Current opinion in neurobiology*, **63**, 95-103.
- Harper, C. B. and Smillie K. J. (2021) Current molecular approaches to investigate presynaptic dysfunction *Journal of neurochemistry*
- Hu, Y. B., Dammer, E. B., Ren, R. J. and Wang, G. (2015) The endosomal-lysosomal system: from acidification and cargo sorting to neurodegeneration. *Transl Neurodegener*, **4**, 18.
- John, A., Ng-Cordell, E., Hanna, N., Brkic, D. and Baker, K. (2020) The neurodevelopmental spectrum of synaptic vesicle cycling disorders. *Journal of neurochemistry*.
- Klein, C. and Westenberger, A. (2012) Genetics of Parkinson's disease. *Cold Spring Harb Perspect Med*, **2**, a008888.
- Melland, H., Carr, E. M. and Gordon, S. L. (2020) Disorders of synaptic vesicle fusion machinery. *Journal of neurochemistry*.
- O'Brien, S., Ng-Cordell, E., Study, D. D. D., Astle, D. E., Scerif, G. and Baker, K. (2019) STXBP1-associated neurodevelopmental disorder: a comparative study of behavioural characteristics. *J Neurodev Disord*, **11**, 17.

- Overhoff, M., De Bruyckere, E. and Kononenko, N. L. (2020) Mechanisms of neuronal survival safeguarded by endocytosis and autophagy. *Journal of neurochemistry*.
- Pischedda, F. and Piccoli, G. (2020) LRRK2 at the presynaptic site: a 16-years perspective. *Journal of neurochemistry*.
- Rizzoli, S. O. and Betz, W. J. (2005) Synaptic vesicle pools. *Nature reviews. Neuroscience*, **6**, 57-69.
- Sudhof, T. C. (2001) alpha-Latrotoxin and its receptors: neurexins and CIRL/latrophilins. *Annu Rev Neurosci*, **24**, 933-962.
- Turner, T. J., Zourray, C., Schorge, S. and Lignani, G. (2020) Recent advances in gene therapy for neurodevelopmental disorders with epilepsy. *Journal of neurochemistry*.

Author Manuscript