The Glutamatergic Synapse – a key hub in neuronal metabolism, signalling and plasticity.

Bruno G. Frenguelli*

School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK

*Author for correspondence

Professor Bruno G. Frenguelli

School of Life Sciences

University of Warwick

Coventry

CV4 7AL

UK

b.g.frenguelli@warwick.ac.uk

Abstract

The Special Issue of *Neuropharmacology* on the glutamatergic synapse is one of a series of Special Issues celebrating the 40th anniversary of Dick Evans and Jeff Watkins's seminal review on excitatory amino acids (Watkins and Evans, 1981). Through a careful appraisal of the literature extending several decades prior to the 1980s, and their own development and use of ligands for excitatory amino acid receptors, Dick and Jeff provided incontrovertible proof for the veracity and importance of glutamate as a neurotransmitter in the central nervous system. While other Special Issues in this series examine the receptors activated by glutamate (AMPA, NMDA, Kainate, mGluR and Delta/Orphan glutamate receptors) this Special Issue examines the glutamatergic synapse itself, and considers its evolution, metabolism, structure, properties and plasticity that have placed it so firmly at the centre of neuronal signalling in the central nervous system.

The Special Issue on the Glutamatergic Synapse is prefaced by a Question and Answer session with Jeff Watkins and Dick Evans (Watkins et al., 2021). Jeff and Dick, while having retired from active research, have no less enquiring minds and posed a series of questions to their "fellow glutamateers", the contributors to this series of Special Issues on Glutamate Receptors. The questions ranged from whether L- or D-aspartate was a neurotransmitter at the "glutamatergic" synapse, to the therapeutic potential of agents based on GluRs. The glutamateers duly responded and provided a useful contemporary account of the state of play of our understanding of glutamate receptors and glutamatergic signalling, that nicely sets the scene for this series of Special Issues.

This topical overview and stage-setting is followed by a long look back across time by Moroz et al.(Moroz et al., 2021) and Ramos-Vicente et al. (Ramos-Vicente et al., 2021) who provide fascinating accounts of the evolution of glutamatergic signalling and glutamate receptors (GluRs) dating back to the origins of life on Earth. Moroz et al. describe L-glutamate's peculiar physico-chemical properties that made it a central hub in primordial metabolic and stress signalling processes, a position that L-glutamate retains to this day, some 3.5 to 4 billion years later. They also provide a detailed account of the massive diversity of ionotropic glutamate receptors across over 20 different families and in excess of 15 subfamilies, only four of which persist in vertebrates – AMPA, Kainate, NMDA and Delta/Orphan receptors, with each covered elsewhere in this series of Special Issues. They close by considering the bioenergetic cost of glutamatergic signalling and conclude that through the utilisation of glutamate as an energy source, glutamatergic signalling may, at least in part, pay for itself. The contribution by Ramos-Vicente and colleagues (Ramos-Vicente et al., 2021) complements this account to expand into G protein-coupled metabotropic glutamate receptors (mGluRs), auxiliary proteins associated with AMPA GluRs, and the evolutionary and structural basis of both ligand selectivity and GluR kinetics. They synthesise this data into an account of the functional implications of GluR-mediated signalling across vertebrates and invertebrates. Both contributions testify to the evolutionary importance of glutamatergic signalling across species as diverse as bacteria, fungi, sponges, plants and primates.

The theme of metabolism is continued in the contribution by Andersen et al. (Andersen et al., 2021), while the energetic cost of the glutamatergic synapse is revisited by Lezmy and co-workers (Lezmy et al., 2021). Anderson et al. introduce both the importance of astrocytes at the glutamatergic synapse (considered further by Lalo et al. (Lalo et al., 2021)), and the implications of dysregulated glutamate metabolism in neurodegenerative disorders. In the former, the glutamate-glutamine cycle sees the conversion of glutamate to glutamine by astrocytes and the delivery of this glutamine to neurones, where it is converted back to glutamate to enter synaptic vesicles, along with the glutamate taken up by neurones per se. As alluded to by Moroz et al. (Moroz et al., 2021), this glutamate can be used as an energy substrate by both neurons and astrocytes, fuelling their own contributions to glutamatergic synaptic transmission. These authors also describe how dysregulated glutamate metabolism occurs in neurodegenerative diseases and focus on two in particular – Alzheimer's disease and Huntington's disease – and in which disruption of glutamate uptake and the enzymes involved in glutamate synthesis and metabolism may be key drivers of pathology (Andersen et al., 2021).

How the need for metabolically- and energetically-expensive glutamatergic synaptic transmission is balanced by the available energy supply is addressed by Lezmy and colleagues (Lezmy et al., 2021). They explain that optimizations have occurred at both the presynaptic terminal and postsynaptic dendritic spine in terms of the probability of glutamate release and glutamate receptor number, respectively. These optimizations have allowed maximal information transfer (ie the generation of action potentials) with the minimal amount of energy (ie ATP) expenditure. This optimization is maintained on the fly, as it were, by the metabolism of ATP to adenosine, which, through its activation of the inhibitory adenosine A_1 receptor, can suppress glutamate release and hyperpolarise membranes, and hence reduce energy demands. Such actions would especially be necessary during conditions of energy crisis such as during epileptic seizures or cerebral ischemia, which provoke both ATP depletion and extracellular adenosine accumulation (Dale and Frenguelli, 2009), and opportunities for the rapid diagnosis of acute brain injury, and therapeutic restoration of cerebral ATP (Frenguelli and Dale, 2020).

The utilization of glutamate as a metabolic substrate and neurotransmitter could not occur without an efficient system of glutamate removal from the synaptic cleft, which serves valuable additional functions of maintaining phasic glutamatergic signalling and preventing glutamate-mediated excitotoxicity. Initially overviewed by Andersen and colleagues in this Special Issue (Andersen et al., 2021), a fuller treatment is provided by Rodríguez-Campuzano and Ortega (Rodríguez-Campuzano and Ortega, 2021), who describe the five plasma membrane glutamate transporters (Excitatory Amino Acid transporters 1- 5; EAAT1 – 5), the vesicular glutamate transporters (VGLUT1-3), and the cystine/glutamate transporter (System Xc^-), which is responsible for the *release* of glutamate into the extracellular space upon cystine import. Detailed descriptions of the transcriptional, epigenetic and post-translational regulation of the transporters are provided, as are their roles in regulating the synaptic vesicle and intra-and extracellular concentrations and kinetics of glutamate, and their part in modulating synaptic transmission and plasticity. With such fundamental roles in the regulation of glutamatergic synaptic concentrations and transmission, Rodríguez-Campuzano and Ortega end with an account of the neurodegenerative, neurological and psychiatric disorders in which glutamate transporters are implicated, including amyotrophic lateral sclerosis, schizophrenia and depression.

Glutamate transporters clearly influence synaptic and extrasynaptic glutamate signals. Indeed this is the very topic of the contribution by Rusakov and Stewart (Rusakov and Stewart, 2021), who use electron microscopic ultrastructural analysis of glutamatergic synapses to give a detailed account of the diversity of synaptic elements, including those associated with perisynaptic astroglial processes. They complement this direct observational approach with functional and computational studies of receptor activation to demonstrate the dynamic nature of synaptic structures – especially of perisynaptic astroglial processes - and their importance in determining phasic, point-to-point synaptic transmission, versus more diffuse or distributed volume transmission across synapses. The importance of astrocytes in glutamatergic transmission is emphasised by Lalo and colleagues (Lalo et al., 2021) in their description of the tripartite synapse, a concept that extends the synapse from pre- and postsynaptic neuronal elements to include the astrocyte as an active and intimately-associated partner (Araque et al., 1999). Lalo et al. succinctly describe the expanding plethora of receptors and (glio)transmitters possessed and released by astrocytes, and the

very many physiological and pathological roles in which they play a key part. Coupled to this is the motility of astrocytic elements, which, as we saw in Rusakov and Stewart's contribution (Rusakov and Stewart, 2021), has dramatic influences on the spread of glutamate across the neuronal parenchyma.

The structural organisation of the glutamatergic synapse is explored by Feng and colleagues (Feng et al., 2021) who describe how key features of the glutamatergic synapse – glutamate-containing synaptic vesicle pools, the presynaptic active zone, the postsynaptic density, and perhaps the synaptic cleft itself - represent biomolecular condensates of proteins that come together through liquid-liquid phase separation, and which, remarkably, can be recapitulated in cell-free in vitro assays. These physical/chemical features of the protein constituents of these features explain such fundamentals of the glutamatergic synapse as the coupling of voltage-gated calcium channels to vesicle fusion and glutamate release, the interactions of AMPARs with auxiliary proteins, the wide-ranging interactions of CaMKII with many proteins of the postsynaptic density, and the nanocolumns that concentrate proteins across the synapse. These proteins of course have a finite lifetime. Angela Mabb provides an informative account of protein ubiquitination at the glutamatergic synapse, a primary mechanism by which proteins are targeted for degradation (Mabb, 2021). Mabb describes how the post-mitotic nature of neurons especially necessitates an efficient protein clearance mechanism, which likely explains the 20-fold higher levels of ubiquitin in the nervous system compared to muscle. Importantly, Mabb explains that ubiquitin-based protein degradation may not just be a cellular house-keeping function, but potentially a means of allowing protein turnover in an activity-dependent manner, and which may explain the specialised nature of protein ubiquitination in the nervous system. Such examples include synaptogenesis, axonal outgrowth, and the remodelling of mature structural and functional pre and post-synaptic elements, including glutamate receptors themselves.

The theme of activity-dependent modifications at the glutamatergic synapse is considered by Collingridge and Abraham (Collingridge and Abraham, 2021), through an account of how pharmacological and technical innovations provided by Jeff Watkins, Dick Evans and their colleagues led to many seminal discoveries of the functional properties of the glutamatergic synapse. Largely based on their own personal experiences, but with reference to work conducted by others, Collingridge and Abraham describe the role of glutamate

receptors in synaptic transmission, plasticity and cognition, the activity-dependence of NMDAR activation in the induction of long-term potentiation (LTP), and the role of mGluRs in metaplasticity, the process by which the induction of synaptic plasticity is influenced by prior synaptic experience.

The final contribution to the Special Issue by Cooper and Frenguelli (Cooper and Frenguelli, 2021) further explores the influence of experience on the glutamatergic synapse. Ever since the work of legendary Canadian psychologist Donald Hebb (whose name is invoked to describe synapses demonstrating the activity-dependent changes he postulated should occur - "Hebbian"), we have known that sensory experience has a profound influence on neuronal structure, synaptic function and cognitive processes. At the glutamatergic synapse this is evidenced by changes in the density of dendritic spines, the induction of synaptic plasticity, and in performance on behavioural tasks. Positive experience (environmental enrichment) of the type first described by Donald Hebb when he took lab rats home to be raised as pets and found they were subsequently smarter than the rats that remained in the lab (Hebb, 1947, 1949), increases spine density, enhances synaptic plasticity, and improves cognition. Cooper and Frenguelli summarise enrichment studies in the hippocampus and propose that an enzyme, mitogen- and stress-activated protein kinase 1 (MSK1), activated by brain-derived neurotrophic factor (BDNF) mediates several aspects of enrichment, including the regulation of dendritic spines, the increase in the dynamic range of synapses, enhancements in some aspects of cognition, and a genomic homeostasis characterised by the *downregulation* of key plasticity-related proteins and genes, including Arc and Egr1. Such a downregulation may be required to avoid runaway protein synthesis or plasticity associated with continued experience- and activity-dependent neuronal stimulation and may be a metaplastic adaptation to improve the genomic and synaptic signal-to-noise ratio when a new experiential challenge is encountered.

The Special Issue also, and sadly, includes a tribute to Professor Mike Stewart who tragically and unexpectedly died during the final stages of the Special Issue. The tribute contains contributions from his close friends and colleagues and attests to Mike's importance and legacy as a neuroscientist. The Special Issue of *Neuropharmacology* on the glutamatergic synapse is dedicated to Mike's memory.

Conflicts of Interest:

BGF is the Editor-in-Chief of *Neuropharmacology.*

References

Andersen JV, Markussen KH, Jakobsen E, Schousboe A, Waagepetersen HS, Rosenberg PA, Aldana BI (2021) Glutamate metabolism and recycling at the excitatory synapse in health and neurodegeneration. Neuropharmacology 196:108719.

Araque A, Parpura V, Sanzgiri RP, Haydon PG (1999) Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 22:208-215.

Collingridge GL, Abraham WC (2021) Glutamate receptors and synaptic plasticity: The impact of Evans and Watkins. Neuropharmacology:108922.

Cooper DD, Frenguelli BG (2021) The influence of sensory experience on the glutamatergic synapse. Neuropharmacology 193:108620.

Dale N, Frenguelli BG (2009) Release of adenosine and ATP during ischemia and epilepsy. Curr Neuropharmacol 7:160-179.

Feng Z, Wu X, Zhang M (2021) Presynaptic bouton compartmentalization and postsynaptic density-mediated glutamate receptor clustering via phase separation. Neuropharmacology 193:108622.

Frenguelli BG, Dale N (2020) Purines: From Diagnostic Biomarkers to Therapeutic Agents in Brain Injury. Neurosci Bull 36:1315-1326.

Hebb DO (1947) The effects of early experience on problem-solving at maturity. American Psychologist 2:306-307.

Hebb DO (1949) The Organization of Behaviour. New York: John Wiley.

Lalo U, Koh W, Lee CJ, Pankratov Y (2021) The tripartite glutamatergic synapse. Neuropharmacology 199:108758.

Lezmy J, Harris JJ, Attwell D (2021) Optimising the energetic cost of the glutamatergic synapse. Neuropharmacology 197:108727.

Mabb AM (2021) Historical perspective and progress on protein ubiquitination at glutamatergic synapses. Neuropharmacology 196:108690.

Moroz LL, Nikitin MA, Poličar PG, Kohn AB, Romanova DY (2021) Evolution of glutamatergic signaling and synapses. Neuropharmacology 199:108740.

Ramos-Vicente D, Grant SGN, Bayés À (2021) Metazoan evolution and diversity of glutamate receptors and their auxiliary subunits. Neuropharmacology 195:108640.

Rodríguez-Campuzano AG, Ortega A (2021) Glutamate transporters: Critical components of glutamatergic transmission. Neuropharmacology 192:108602.

Rusakov DA, Stewart MG (2021) Synaptic environment and extrasynaptic glutamate signals: The quest continues. Neuropharmacology 195:108688.

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 21:165-204.

Watkins JC et al. (2021) 21st century excitatory amino acid research: A Q & A with Jeff Watkins and Dick Evans. Neuropharmacology 198:108743.