Neuronal Glutamate and GABA<sub>A</sub> Receptor Function in Health and Disease


Neuronal Glutamate and GABA<sub>A</sub> Receptor Function in Health and Disease

Peter R. Moult<sup>1</sup>
Neurosciences Institute, Division of Medical Sciences, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, U.K.

**Abstract**
Glutamate and GABA (γ-aminobutyric acid) are the predominant excitatory and inhibitory neurotransmitters in the mammalian CNS (central nervous system) respectively, and as such have undergone intense investigation. Given their predominance, it is no wonder that the reciprocal receptors for these neurotransmitters have attracted so much attention as potential targets for the promotion of health and the treatment of disease. Indeed, dysfunction of these receptors underlies a number of well-characterized neuropathological conditions such as anxiety, epilepsy and neurodegenerative diseases. Although intrinsically linked, the glutamatergic and GABAergic systems have, by and large, been investigated independently, with researchers falling into the ‘excitatory’ or ‘inhibitory’ camps. Around 70 delegates gathered at the University of St Andrews for this Biochemical Society Focused Meeting aimed at bringing excitation and inhibition together. With sessions on behaviour, receptor structure and function, receptor trafficking, activity-dependent changes in gene expression and excitation/inhibition in disease, the meeting was the ideal occasion for delegates from both backgrounds to interact. This issue of *Biochemical Society Transactions* contains papers written by those who gave oral presentations at the meeting. In this brief introductory review, I put into context and give a brief overview of these contributions.

GABA<sub>A</sub>Rs [GABA (γ-aminobutyric acid) type A receptors]
GABA<sub>A</sub>Rs are heteropentameric Cl<sup>−</sup>-selective ligand-gated ion channels that mediate fast inhibition within the mammalian CNS (central nervous system). Deficits in GABA<sub>A</sub>R function occur in a range of neuropsychiatric disorders, including autism, anxiety, schizophrenia and epilepsy. They are also key therapeutic targets for benzodiazepines, barbiturates, neurosteroids and general anaesthetics. The ultimate message highlighted at this meeting was that the diversity of receptors subtypes generated from different subunit combinations and the RNA editing of specific subunits imparts the diversity of function upon the GABA<sub>A</sub>R. This understanding is fuelling the development of more specific, and clinically effective, ligands for the treatment of diseases associated with GABA<sub>A</sub>R dysfunction by limiting some of the undesirable side effects derived from the activation of unwanted receptor subtypes.

Given their therapeutic importance, there is significant interest in how neurons regulate the number of functional GABA<sub>A</sub>Rs. Like many glutamatergic receptors, GABA<sub>A</sub>Rs also undergo fast constitutive recycling; however, the role that membrane trafficking of these receptors plays in neuronal network activity is poorly understood. Recent work on a β
receptor subunit identified the mechanism of AP2 (clathrin adaptor protein 2) binding and GABA<sub>A</sub>R endocytosis ([11] and Vithlani and Moss [2], pp. 1355–1358). Neurons expressing β<sub>3</sub>S408A/S409A subunits show not only increases in GABA<sub>A</sub>R surface expression, but also increases in the number and size of inhibitory synapses and consequential increased inhibitory synaptic transmission. Interestingly, neurons expressing β<sub>3</sub>S408A/S409A subunits showed deficits in the number of mature spines (excitatory synapses), an effect that is reversed by the pharmacological blockade of GABA<sub>A</sub>Rs. Thus the regulation of GABA<sub>A</sub>R membrane trafficking may play an important role in regulating spine maturity, which would have significant implications for synaptic plasticity and behaviour [1]. Although much current research has focused on the plasticity of glutamatergic synapses and its receptors, it is now very much established that GABAergic synapses and receptors can also undergo dynamic, constitutive and activity-driven long-term modifications. This is exemplified further during development when the α<sub>1</sub>β<sub>2</sub>δ-containing GABA<sub>A</sub>Rs undergoes down-regulation during puberty. Here THP (3α-hydroxy-5[alpha]-pregnan-20-one), a steroid released during stress actually increases anxiety in female prepubescent mice, whereas it reduces anxiety in adults ([3] and Shen and Smith [4], pp. 1378–1384). This apparent contradiction is explained by the inhibition of α<sub>1</sub>β<sub>2</sub>δ containing GABA<sub>A</sub>Rs, which are only highly expressed during puberty in the hippocampal CA1 area, where they generate outward currents.

GABA<sub>A</sub>Rs containing the α<sub>5</sub> subunit have proved to be promising candidate targets for memory-enhancing drugs. There is nothing new in the concept that GABA<sub>A</sub>R activity modulates learning and memory. However, non-selective inverse agonists that have been shown to improve memory performance also have anxiogenic, convulsant and proconvulsant properties that limit their clinical use. However, if the α<sub>5</sub>-containing receptors are selectively targeted with inverse agonists (e.g. α5I4A or α5IAll), in some instances, they have been shown to enhance certain forms of memory [5,6] without the unwanted side effects associated with the non-specific activation of other GABA<sub>A</sub>Rs. Interestingly, these compounds may also be useful for the reversal of amnesic effects caused by some anaesthetics such as etomidate or isoflurane [7,8]. Although discrepancies exist, α<sub>5</sub> GABA<sub>A</sub>Rs offer a realistic target for the development of memory-modifying therapies (Martin et al. [9], pp. 1334–1337). This highlights the specificity of function attributed to subtypes of GABA<sub>A</sub>Rs delineated by some fascinating work using knockin point mutations of GABA<sub>A</sub>R α subunits [10,11]. Here it is revealed that it is the α<sub>2</sub>, and not the α<sub>3</sub>, α<sub>5</sub>, or α<sub>6</sub> GABA<sub>A</sub>R subunit which accounts for the anxiolytic effects of benzodiazepines. Similarly, these same knockin approaches have been used to show that spinal α<sub>2</sub> and α<sub>3</sub> subunits are powerful modulators of pain. Critically, the α<sub>2</sub>/α<sub>3</sub> ligand L-838417 has been shown to be effective at suppressing inflammatory as well as neuropathic pain, while being devoid of any sedative effects [12]. Finally, the influence of GABAergic drugs on EEG (electroencephalogram) demonstrates the strong influence that the GABAergic system has on the control of oscillatory neuronal activity. Thus inhibition is also about network timing, not network shutdown. Changes in sleep pattern and EEG frequency associated with classical benzodiazepines are attributable to most GABA<sub>A</sub>Rs (other than α<sub>1</sub>-containing receptors), with α<sub>2</sub> receptors having the most profound influence. A point mutation of α<sub>1</sub> was used to make it diazepam-insensitive, and this strongly attenuated the usual suppression of δ waves and increases in both β and θ waves [13]. In contrast, the sedative effect of benzodiazepines was seen to be modulated almost exclusively by α<sub>1</sub>-containing receptors ([14] and Möhler [15], pp. 1328–1333). This small snapshot of subtype-selective GABA<sub>A</sub>R function underlines the importance for the development of more subunit-specific ligands. Such compounds should show stronger clinical efficacy and lessen undesired side effects sometimes displayed by currently available therapies.

As well as specific subunits conferring certain properties upon GABA<sub>A</sub>Rs, RNA editing also plays an important role in GABA<sub>A</sub>R function. To this end, an important RNA-editing site on the α<sub>1</sub> subunit was recently discovered [16]. Here, editing of the Gabra3 (GABA<sub>A</sub>R α<sub>1</sub>) transcript causes an isoleucine-to-methionine amino acid change in the TM3 (third transmembrane domain) of the α<sub>1</sub> subunit. Receptors containing this edited form of the subunit have smaller amplitude responses, slower activation and faster deactivation kinetics [17]. Preliminary data suggest that this editing may affect assembly and delivery of receptors to the plasma membrane (Daniel and Ohman [18], pp. 1399–1403).

As well as rapid trafficking of glutamate and GABA<sub>A</sub>Rs from readily available pools, many forms of synaptic plasticity are dependent upon a precise balance between the protein turnover (synthesis against degradation) of others. The zinc-finger protein Zif268 has emerged as an important transcription factor involved in the late phase of some forms of plasticity and is up-regulated during hippocampal LTP (long-term potentiation) [19]. Two crucial proteins for the synaptic localization of GABA<sub>A</sub>Rs, gephyrin and ubiquilin, have been identified as possible transcriptional targets of Zif268 [20]. In these studies, Morris and colleagues show that both the mRNA and protein levels of gephyrin and ubiquilin are down-regulated by increased Zif268 levels (McDade et al. [21], pp. 1375–1377). Hippocampal LTP is associated with a down-regulation of inhibitory events. Thus one of the functional consequences of increased Zif268 could be a reduction in synaptic GABA<sub>A</sub>Rs and therefore facilitation of LTP.

While discussing the modulation of neuronal function via glutamate and GABA<sub>A</sub>Rs, one must not forget the role of glial cells. Although glial cells make up the majority of cells within the CNS, they have largely been ignored until recent years. Of emerging importance is the communication between glial cells and neurons. Astrocytes ensheath neurons and as such are exposed to the neurotransmitters released by these cells. Indeed, they possess a number of neurotransmitter receptors which, when activated, can trigger downstream Ca<sup>2+</sup> signalling cascades culminating in the vesicular release of ‘gliotransmitters’ such as δ-serine, ATP and glutamate. Recent work demonstrates that glial cells can release ATP in response to receptor
stimulation in sufficient quantities to activate neighbouring P2X purinoreceptors [22,23]. The activation of P2X receptors inhibits NMDAR [NMDA (N-methyl-D-aspartate) receptor] function [24] and thereby profoundly affects synaptic plasticity [25,26]. More recent evidence also suggests a Ca\(^{2+}\)-dependent link between P2X receptors and the inhibition of GABA\(_\text{A}\)Rs ([27] and Lalo et al. [28], pp. 1407–1411).

With respect to subunit composition, it is not just what you have got that is important. It also matters where you put it. The use of subunit concatenation (physically tethering subunits and forcing their positioning within the pentameric receptor) followed by functional characterization is a powerful technique which allows an investigation into the relative contribution of the positioning of subunits (Sigel et al. [29], pp. 1338–1342). Here, it has been used to study the localization of novel binding sites for the benzodiazepine flurazepam. The classical benzodiazepine-binding site is located on the \(\alpha/\gamma\) interface. This modelling has predicted the existence of a second site at the \(\alpha/\beta\) interface that regulates the allosteric signalling seen at higher concentrations.

A note of caution, however, has to be employed when designing and interpreting ion channel studies. A recent study highlighted this issue while investigating the decay kinetics of GABAergic inhibitory synaptic currents. The decay time was considerably shorter when recorded with physiological internal Cl\(^-\) concentrations than with symmetrical Cl\(^-\) solutions used normally [30]. The effect of intracellular Cl\(^-\) is independent of the net direction of current flow through the ion channel and is a direct modulation of the GABA\(_\text{A}\)R. Importantly, this means that the time window during which GABAergic inhibition can influence excitatory inputs is much shorter under physiological conditions than previously thought using other Cl\(^-\) concentrations in the recording medium. This is expected to have implications for neuronal network excitability and neurodevelopment, and for our understanding of pathological conditions, such as epilepsy and chronic pain, where intracellular Cl\(^-\) concentrations can be altered.

**Glutamate receptors**

Glutamate is the most prominent neurotransmitter in the mammalian CNS and exerts its actions via ionotropic [NMDA, AMPA (\(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and kainite] receptors and mGluRs [metabotropic (G-protein-coupled) glutamate receptors]. Although many CNS disorders have a complex aetiology, neuronal dysfunction is normally attributable to a combination of defects, but almost always involve excitatory (or inhibitory) neurotransmission. As glutamate receptors mediate most of the excitatory synaptic transmission in the mammalian CNS, they pose seductive targets for therapeutic intervention in a number of CNS disorders. As such, much effort has been directed at elucidating the structure and mechanisms of function of these types of receptor.

A crucial feature of neuronal glutamate receptors is, as discussed for the GABA\(_\text{A}\)R, their ability to traffic rapidly in and out of synapses. Many synaptic plasticity events occur as a result of the rapid removal or insertion of receptors from the synaptic membrane. Ca\(^{2+}\) influx plays an important role in many different types of synaptic plasticity and in triggering such trafficking events. Emerging evidence is now highlighting the importance of members of the NCS (neuronal calcium sensor) protein family in plasticity, in particular NCS-1 and hippocalcin. Hippocalcin is highly expressed in the hippocampus and is involved in both spatial and associative memory [31,32]. Of particular interest, in terms of synaptic plasticity and AMPAR (AMPA receptor) trafficking, is recent evidence that hippocalcin acts as a molecular link between Ca\(^{2+}\) entry (through the NMDAR) and subsequent AMPAR internalization during LTD (long-term depression) [33]. In contrast, NCS-1 appears to have no involvement in NMDAR-dependent LTD, but has recently been shown to be critical for the induction of another form of LTD that is dependent upon the activation of mGluRs [34]. Both forms of plasticity involve postsynaptic Ca\(^{2+}\) entry and Ca\(^{2+}\) release from internal stores and both forms of LTD are expressed via the internalization of synaptic AMPARs, but depend on different Ca\(^{2+}\) sensors (Amici et al. [35], pp. 1359–1363). The subtle differences and complexity of the many mechanisms of AMPAR trafficking are highlighted by this work on Ca\(^{2+}\) sensors, and it seems likely that many more mechanisms are yet to be elucidated. Indeed, exciting new work is emerging demonstrating the ability of the anti-obesity hormone, leptin, to act as a powerful stimulant of AMPAR trafficking and modulator of synaptic function (Moulton and Harvey [36], pp. 1364–1368). Leptin converts STP (short-term potentiation) into LTP [37], promotes rapid increases in dendritic filopodial outgrowth and subsequently increases the density of hippocampal synapses [38]. Leptin is also capable of depotentiating CA1 synapses by selectively removing GluA2-lacking receptors from potentiated sites [39]. Leptin’s modulation of glutamatergic transmission is not limited to AMPARs; indeed, NMDAR function can also be potentiated [37]. This work has significant implications, not only for synaptic plasticity, as it also highlights the wider importance of hormones such as leptin in basic synaptic function.

Benzodiazepines modulate their anxyolitic, hypnotic and anti-convulsant effects through the allosteric enhancement of GABA\(_\text{A}\)R function, but long-term use can lead to tolerance and dependence which can manifest itself as anxiety-like behaviour. Evidence now suggests that this anxiety-like behaviour is also due to the modulation of hippocampal glutamatergic transmission via a synaptic insertion of GluA1 homomers (i.e. GluA2 lacking AMPARs) ([40,41] and Shen et al. [42], pp. 1394–1398). This increase in synaptic function is CaMKII (Ca\(^{2+}\)/calmodulin-dependent protein kinase II)-dependent and, as such, appears analogous to activity-dependent models of LTP. Taken together with the hormonal modulation of hippocampal synaptic strength and recent work on LTP, this highlights the importance of not only trafficking of receptors, but also the selective trafficking of a specific subunit composition of receptor, thus changing the dominant receptor identity at the synapse.
Such trafficking of glutamate receptors and subsequent long-term modification of synaptic efficacy is thought to underlie learning and memory. Development of various glutamate receptor subunit-knockout mice has enabled the dissociation of very specific types of memory and the elucidation of how these types of memory depend on different glutamate receptor subtypes (Bannerman [43], pp. 1323–1327). For example, using the GluA1 AMPAR subunit-knockout mouse, researchers have been able to differentiate a GluA1-dependent associative short-term memory mechanism, important for spatial working memory tasks, and a GluA1-independent long-term associative memory mechanism, important for spatial reference memory tasks [44,45]. Similar techniques have now been employed to reveal differences in the contribution of NMDAR subtypes to different forms of memory. GluN2A (NR2A) knockout impairs rapidly acquired spatial working memory, but causes no deficits in spatial reference memory [46]. GluN2B (NR2B) deletion results in an apparent global memory impairment, but this subunit may be important for distinguishing between spatial locations based on familiarity [47]. Such studies highlight the subtleties in the function and make up of glutamate receptors, and no doubt similar techniques will be used to tease out specificities of GABA_A R function.

There are many similarities between these activity-dependent forms of learning and memory and cognitive enhancement and stroke recovery [48,49]. Indeed, recent work from Carmichael and colleagues have shown that increases in cortical excitation via similar processes can improve post-stroke functional performance and that manipulating classical learning and memory pathways can offer a novel means for promoting post-stroke recovery (Clarkson and Carmichael [50], pp. 1412–1414).

Although AMPARs mediate much of the last excitatory transmission in the mammalian CNS, regulation and trafficking of NMDARs also plays an important role in many forms of plasticity. Wyllie and colleagues have generated various chimaeric subunit combinations of the NMDAR subunits in order to study ligand binding at these sites. Unlike the ubiquitously expressed GluN1 (NR1) subunit, the expression of the GluN2 (NR2) subunit is regulated both temporally and spatially throughout the mammalian brain. The GluN1 subunit contains the binding site for the co-agonist glycine, whereas the GluN2 subunit contains the binding site for glutamate. The specific subtype of the GluN2 subunit (GluN2A–GluN2D) within a given NMDAR confers important properties on the receptor, including glycine/glutamate potencies, single-channel conductance, duration of activation and sensitivity to voltage-dependent Mg^{2+} block. The largest of these differences is seen between NMDARs containing either GluN2A or GluN2D. Wyllie and colleagues have developed a series of chimaeric GluN2A/2D subunits in order to investigate the factors which determine such differences between the subunits [51–53]. These chimaeric subunits have been employed successfully to gain important pharmacological and biophysical information about the different regions of the subunits responsible for subunit differences. These properties are highlighted by O’Leary and Wyllie ([54], pp. 1347–1354). For example, the GluN2 S1 and S2 regions not only determine glutamate potency, but also have an important influence over glycine potency. The M1–M3 regions determine the voltage-dependent Mg^{2+} block, but the LBD (ligand-binding domain) also plays a role. M1–M3 regions also determine unitary conductances, whereas the LBD determines the burst lengths.

NMDARs play an important role in the physiology and pathophysiology of the CNS. Too much Ca^{2+} entry through the receptor can result in neuronal loss as occurs during acute brain trauma, ischaemia and in neurodegenerative diseases such as Huntington’s disease. However, the normal physiological activity of NMDARs can promote neuroprotection against both apoptotic and excitotoxic insults. There are numerous scenarios in which NMDAR activation can either be neuroprotective or neurotoxic (Hardingham [55], pp. 1147–1150; Mizielinska et al. [56], pp. 1389–1393; Nicholls [57], pp. 1385–1388) and so an important question remains: what determines whether an episode of NMDAR activity is protective or toxic? Stimulus intensity is clearly important: with modest (physiological) activation being pro-survival, and too little or too much activation being pro-death. This implies that Ca^{2+} concentrations for activating pro-survival pathways [i.e. PI3K (phosphoinositide 3-kinase), ERK1/2 (extracellular-signal-regulated kinase 1/2), CaMKIV (Ca^{2+}/calmodulin-dependent protein kinase IV)] have to be lower than those required for activating toxic events such as calpain activation, mitochondrial Ca^{2+} uptake or NO production [58–60]. The location of the NMDAR also plays an important role. Ca^{2+} entering cells via activation of synaptic NMDARs is well tolerated by cells, whereas Ca^{2+} entering the cell via activation of extrasynaptic NMDARs, either on their own or in combination with synaptic activation, leads to toxicity [61,62]. There is evidence to suggest that, in mature neurons, extrasynaptic NMDARs are more likely to include GluN2B, whereas synaptic receptors are more likely to include GluN2A, suggesting that the subunit composition of the NMDAR confers pro-survival compared with pro-death characteristics? However, there is little evidence to support this notion entirely. Nevertheless, a recent study does suggest that activation of GluN2A-containing receptors are more likely to promote survival, whereas activation of GluN2B-containing receptors are more likely to promote cell death [63].

Although NMDAR location and perhaps subunit composition are important factors in determining the fate of the cell during NMDAR activation, the time and concentration of exposure to glutamate are by far the overriding factor. Chronic exposure to glutamate exacerbates neuronal damage, and mitochondria play a central role in the fate of the exposed neuron. The mechanisms of excitotoxicity and how this has been studied extensively in primary hippocampal cultures is reviewed by Nicholls [57] on pp. 1385–1388).

An interesting element of excitotoxicity is the particular vulnerability of the dendrites due to the high concentration of excitatory glutamate receptors present. Excitotoxic dendritic injury is characterized by the formation of dendritic beads along the length of the dendrite, separated by...
thin dendritic segments [64,65]. Structural studies show disrupted microtubules and microtubule-associated proteins within beads, suggesting that microtubule collapse may be responsible for the formation of these beads. However, in primary hippocampal cultures, stabilization of microtubules before a bead-inducing insult (100 μM glutamate/10 μM glycine, 20 min, 37°C) does not prevent dendritic bead formation [56]. Dendritic beads also contain a large number of dysfunctional mitochondria; however, depleted ATP levels (due to mitochondrial dysfunction) themselves are not sufficient to cause beading [66]. Thus microtubule and mitochondrial collapse appear to be secondary events, following beading. Regardless, it is abundantly clear that bead formation does not necessarily cause, nor predict, neuronal death. It remains to be established whether indeed these beads play any toxic or protective roles in neuronal survival (see Mizielińska et al. [56], on pp. 1389–1393, for more detail).

Concluding remarks

Although much is understood about both glutamatergic and GABAergic transmission in health and disease and many therapeutic treatments have been developed on the basis of this understanding, one overriding theme kept arising throughout the meeting in St Andrews: the need for more specificity in these treatments. With new developments throughout the meeting in St Andrews: the need for more therapeutic treatments have been developed on the basis of molecular and neuronal substrate for the selective attenuation of anxiety. Science 290, 131–134


References


Received 10 September 2009
doi:10.1042/BST0371317