

## CHAPTER

## 17

## Glutamate and Glutamate Receptors

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## THE AMINO ACID GLUTAMATE IS THE MAJOR EXCITATORY NEUROTRANSMITTER IN THE BRAIN

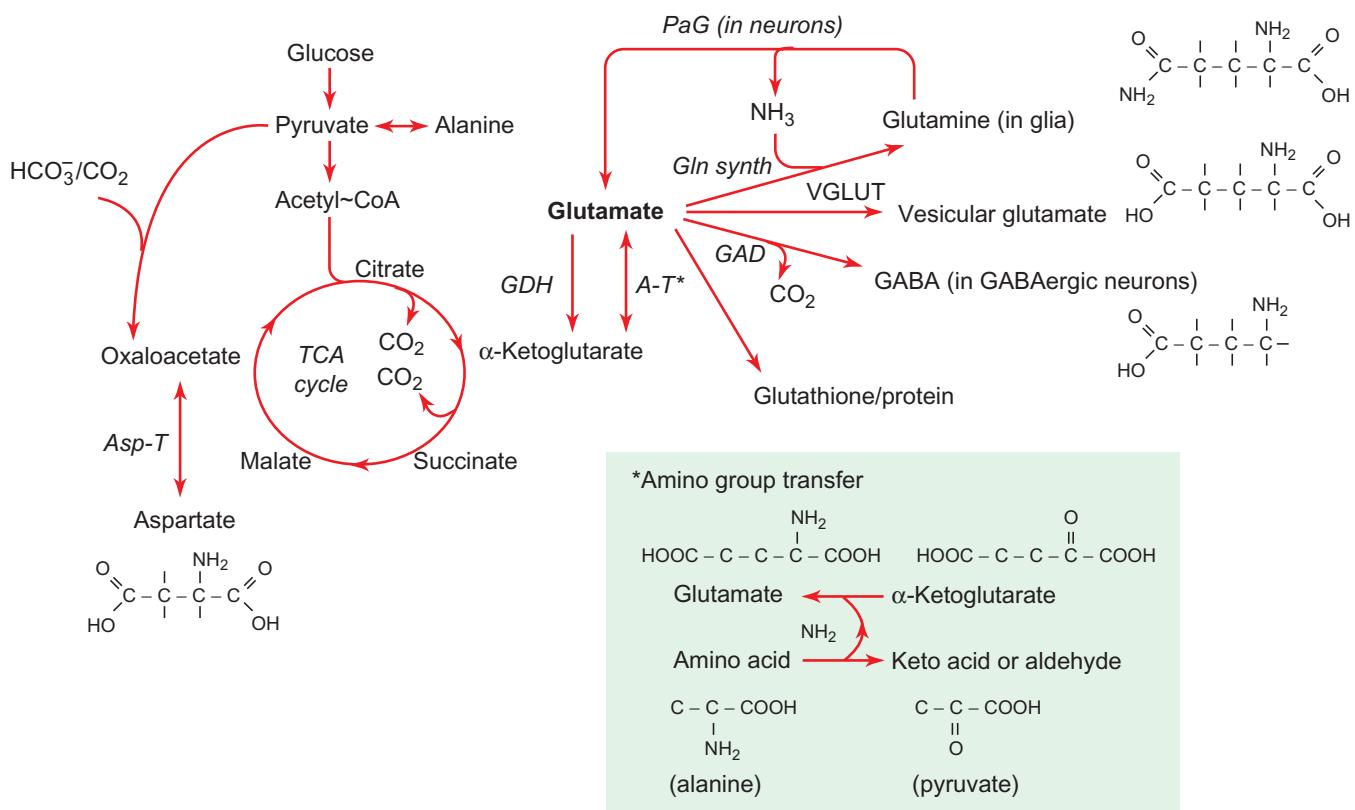
Glutamate mediates most of the fast excitatory neurotransmission in the CNS, and it excites virtually every neuron. Glutamate is the principal mediator of sensory information, motor coordination, emotions and cognition, including memory formation and memory retrieval. As many as 80–90% of the neurons of the brain use glutamate as their neurotransmitter, and approximately 80–90% of the synapses in the brain are glutamatergic (Braitenberg & Schütz, 1998). Repolarization of membranes that are depolarized during glutamatergic activity may account for as much as 80% of the energy expenditure of the brain (Attwell & Laughlin, 2001). The very high consumption of glucose and oxygen by the brain (see Chap. 11) therefore largely fuels glutamatergic activity. The concentration of glutamate in brain gray matter structures varies between 10 and 15 μmol per gram of tissue, higher than in virtually all other tissues of the body. In white matter the glutamate concentration is 4–6 μmol/g. Glutamate takes part in many reactions in the brain (Fig. 17-1). Formation of glutamate is a step in the metabolism of glucose and amino acids; glutamate is a precursor for γ-aminobutyric acid (GABA) in GABAergic neurons and for glutamine in glial cells; it is a constituent of proteins and peptides, e.g., glutathione (γ-glutamyl-cysteinyl-glycine), which is a major defense against oxidative stress in cells. Therefore, all cells of the brain, neuronal and glial, contain glutamate, which is found in both the cytosol and mitochondria of cell bodies and their processes. In glutamatergic neurons, glutamate is concentrated in synaptic vesicles in which compartment it represents ‘transmitter glutamate.’

## BRAIN GLUTAMATE IS DERIVED FROM BLOOD-BORNE GLUCOSE AND AMINO ACIDS THAT CROSS THE BLOOD-BRAIN BARRIER

From a metabolic point of view, glutamate may be thought of as a carbon backbone (with hydrogens and hydroxyl groups attached) on the one hand and an amino group on the other. The carbon backbone derives from serum glucose, which, through glycolysis and the tricarboxylic acid (TCA) cycle (see Chap. 11), is converted to α-ketoglutarate. α-Ketoglutarate then receives an amino group from another amino acid through transamination and becomes converted into glutamate (Fig. 17-1).

Glutamate is in equilibrium with – and continuously reconverted to – α-ketoglutarate and metabolized through the TCA cycle. The cerebral metabolic rate for glucose in human brain is approximately 0.4 μmol/min/g tissue, and the turnover of glutamate is approximately 0.8 μmol/min/g tissue (Shen et al., 1999). This means that virtually all the glucose that enters the brain is metabolized through glutamate, since one molecule of glucose gives rise to two molecules of acetyl-CoA, which enter the TCA cycle to become α-ketoglutarate and hence glutamate (Fig. 17-1). In rat brain the turnover of glutamate is approximately twice that of the human brain. Most of the glutamate in the brain is found in glutamatergic neurons. The high concentration of glutamate in these neurons is due to three factors: (1) the high metabolic rate with rapid metabolism of glucose into α-ketoglutarate; (2) the much higher activity of aspartate aminotransferase (which aminates α-ketoglutarate to glutamate) than of TCA cycle enzyme α-ketoglutarate dehydrogenase, leading to accumulation of glutamate; and (3) the lack of reactions that drain the pool of glutamate much (other than the reversal of the amination and subsequent metabolism of α-ketoglutarate through the TCA cycle). In GABAergic neurons the glutamate pool is drained by the glutamate decarboxylase reaction, which produces GABA, and in astrocytes it is drained by the glutamine synthetase reaction, which produces glutamine. Therefore, in GABAergic neurons and in astrocytes the concentration of glutamate is much lower than in glutamatergic neurons.

The amino group of glutamate derives from serum amino acids that cross the blood-brain barrier. Current evidence points to blood-borne branched-chain amino acids (leucine, isoleucine and valine) as important amino donors for glutamate synthesis. Because the uptake of amino acids into the brain is much less than the uptake of glucose, the amino group of glutamate must be recycled in the brain when the carbon backbone is broken down to CO<sub>2</sub> and water. Aspartate is one important amino group reservoir for glutamate synthesis. The highly active aspartate aminotransferase shuttles the amino group between α-ketoglutarate and another TCA cycle intermediate, oxaloacetate. These two intermediates are converted into glutamate and aspartate respectively when they accept an amino group. Other amino acids, such as alanine and GABA (in GABAergic neurons) are also important amino group reservoirs (Fig. 17-1). Theoretically, glutamate could be formed by reversal of the glutamate dehydrogenase (GDH) reaction: Glutamate + NAD(P) ↔ α-ketoglutarate + NH<sub>3</sub> + NAD(P)H + H<sup>+</sup>. This is unlikely, however, since the K<sub>m</sub> for NH<sub>3</sub> and α-ketoglutarate in the reverse reaction, >12 mmol/l and 1 mmol/l, respectively, are far above normal



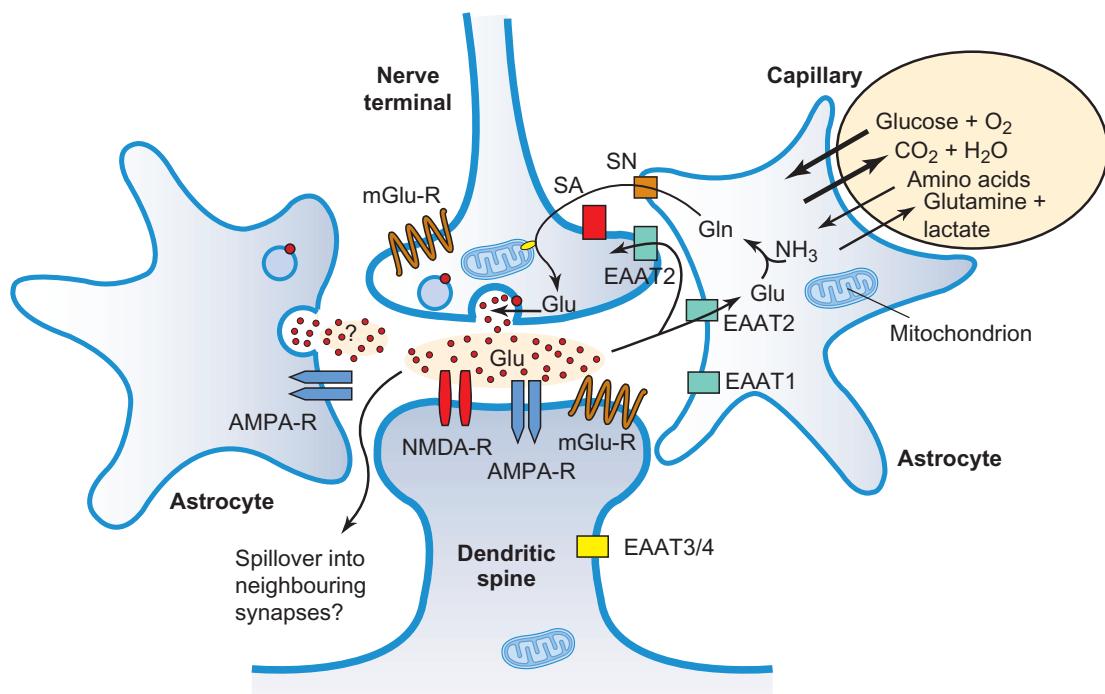
**FIGURE 17-1** The formation of glutamate from  $\alpha$ -ketoglutarate, a TCA cycle intermediate.  $\alpha$ -ketoglutarate, which is formed from glucose, constitutes the carbon backbone of glutamate. The amino group derives from another amino acid, which after donation of its amino group becomes a keto acid or an aldehyde (insert). This amino donor may be aspartate, GABA, alanine (shown in the insert) or some other amino acid. Glutamate takes part in many biochemical reactions: in glial cells it is converted to glutamine, in GABAergic neurons it is converted into GABA. Transmitter glutamate is defined by its accumulation into synaptic vesicles. In the upper right corner the glutamine cycle is shown, with conversion of glutamate into glutamine in glia and conversion of glutamine into glutamate in neurons. To upper left is shown pyruvate carboxylation (anaplerosis), which replenishes the TCA cycle (glial or neuronal) with malate or oxaloacetate to compensate for the loss of glutamate, glutamine, or (in GABAergic neurons) GABA. Asp-T, aspartate aminotransferase; A-T, aminotransferase reaction (Asp-T or other); GABA,  $\gamma$ -aminobutyric acid; GAD, glutamic acid decarboxylase; GDH, glutamate dehydrogenase; Gln synth, glutamine synthetase; PaG, phosphate-activated glutaminase; TCA cycle, tricarboxylic acid cycle; VGLUT, vesicular glutamate transporter. A more comprehensive scheme of glutamate metabolism can be found at <http://www.genome.ad.jp/kegg/pathway/map/map00251.html>.

brain concentrations. The specific role of glutamate dehydrogenase in the brain is not clear but it is an important enzyme in protein degradation. The metabolism of protein-derived amino acids involves transamination of the various amino acids into ketoacids;  $\alpha$ -ketoglutarate accepts the NH<sub>2</sub> group in these transaminations and becomes converted into glutamate. Glutamate dehydrogenase removes the NH<sub>2</sub> group from glutamate as free NH<sub>3</sub> and shunts glutamate back into the TCA cycle as  $\alpha$ -ketoglutarate.

ammonia to form glutamine through the activity of glutamine synthetase, a cytosolic, ATP-dependent enzyme that astrocytes and oligodendrocytes express but neurons do not. This reaction is important even for the detoxification of free ammonia; accumulation of ammonia would interfere with synaptic function. Glutamine, which does not have neurotransmitter properties, is exported to the extracellular fluid and taken up by neurons (see below). In neurons glutamine is converted back to glutamate by phosphate-activated glutaminase, a mitochondrial and possibly neuron-specific enzyme in the brain. The trafficking of glutamate and glutamine between neurons and astrocytes is called the 'the glutamine cycle'. This cycle seems to be very active, its flux has been estimated at approximately 40% of the glutamate turnover rate (Broman et al., 2000), meaning that almost half the glutamate formed in neurons is transferred to glial cells. Whether all the neuronal glutamate taken up by glia is vesicular in origin is not known; reversal of glutamate transporters (see below) or exchange of intracellular glutamate for extracellular cystine may both be important mechanisms by which glutamate can leave neurons (Baker et al., 2003).

## GLUTAMINE IS AN IMPORTANT IMMEDIATE PRECURSOR FOR GLUTAMATE: THE GLUTAMINE CYCLE

Much of the glutamate that is released from nerve terminals is taken up from the extracellular fluid into astrocytic processes that surround synapses (Fig. 17-2). The mechanism of uptake is described later in this chapter. In astrocytes glutamate reacts with



**FIGURE 17-2** A glutamatergic, axodendritic synapse consisting of a presynaptic nerve terminal and a postsynaptic dendritic spine. The nerve terminal contains synaptic vesicles with glutamate transporters (red dot), mitochondria (blue) with glutaminase in nerve terminal (yellow dot), metabotropic glutamate receptors, and transporters for glutamate (EAAT2) and glutamine (SA transporters). The postsynaptic dendritic spine contains glutamate receptors, both ionotropic (AMPA and NMDA type) and metabotropic, and glutamate transporters (EAAT3 and EAAT4, the latter in cerebellar Purkinje cells). Surrounding the synapse are astrocytic processes with glutamate (EAAT1 and EAAT2) and glutamine transporters (SN transporters), glutamate receptors and even glutamate-filled vesicles. Green rectangles in plasma membrane of the axon terminal represent EAAT2 and of the astrocyte represent EAAT1/EAAT2 glu transporters. Glutamate that escapes out of the synapse without being cleared by transporters may spill over into neighboring synapses. AMPA-R, AMPA receptors; EAAT1–4, excitatory amino acid transporters 1–4; Gln, glutamine; Glu, glutamate; mGlu-R, metabotropic glutamate receptors; SA and SN, system A and system N transporters respectively (for glutamine).

Several findings support the concept that glutamine is an important precursor for transmitter glutamate. When brain slices are incubated with radiolabeled glutamine, radiolabeled glutamate is formed that is releasable through a calcium-ion-dependent mechanism. The calcium dependence identifies the released glutamate as vesicular in origin (see Chap. 12). However, neurons may release transmitter glutamate even in the absence of glutamine, as can be seen in brain slices; this finding shows that glutamine is not an obligatory precursor for glutamate, which may also be formed from glucose through α-ketoglutarate, as described, earlier. A way to study the fate of glutamine in the intact brain is to use isotopically labeled acetate to label glutamine. Carbon-13-labeled acetate does not enter neurons but it readily enters astrocytes, where it becomes metabolized to glutamine. After intravenous injection of <sup>13</sup>C-labeled acetate, magnetic resonance spectroscopy of the brain is consistent with the formation of <sup>13</sup>C-glutamine in astrocytes and the formation of <sup>13</sup>C-glutamate from that glutamine in neurons.

### Release of glutamate from nerve endings leads to loss of α-ketoglutarate from the tricarboxylic acid cycle

The glutamine cycle counteracts an important metabolic challenge inherent in glutamatergic neurotransmission: it

helps prevent loss of α-ketoglutarate from the neuronal TCA cycle. When neurons release glutamate and astrocytes take it up, the neuronal TCA cycle loses α-ketoglutarate. If this loss of α-ketoglutarate from the TCA cycle is not countered, the downstream product oxaloacetate will not be available for the formation of citrate (Fig. 17-1; see Chap. 11), and the TCA cycle will eventually grind to a halt, with the consequence that energy metabolism stops. The return of glutamine for transmitter glutamate reduces the loss of neuronal α-ketoglutarate but probably does not prevent it completely, partly because astrocytes metabolize some transmitter glutamate through their own TCA cycle as an energy substrate. Two additional mechanisms exist that maintain the level of glutamate and TCA cycle intermediates in the nerve terminal: one is reuptake of glutamate from the extracellular fluid back into the nerve terminal, and the other is the reaction of pyruvate (derived from glycolysis) with CO<sub>2</sub> to form TCA cycle intermediate malate, the precursor of oxaloacetate. This process is called pyruvate carboxylation. Pyruvate carboxylation in the nerve terminal may be catalyzed by malic enzyme, a reversible enzyme that utilizes CO<sub>2</sub> and NADPH to form malate from pyruvate (Broman et al., 2000). Pyruvate carboxylation also takes place in astrocytes, where it may be catalyzed by pyruvate carboxylase, an ATP-dependent, unidirectional enzyme that utilizes bicarbonate for the carboxylation of pyruvate to oxaloacetate. This reaction may support

a net production and export of glutamine from astrocytes (Broman et al., 2000).

Because the carbon backbone of glutamate derives from serum glucose, the formation and further metabolism of glutamate (e.g., to glutamine or GABA) may be followed in the awake human brain or in animals with magnetic resonance spectroscopy after intravenous injection of  $^{13}\text{C}$ -labeled glucose (Shen et al., 1999). Glutamate and GABA become labeled with  $^{13}\text{C}$  within a few minutes after injection of  $^{13}\text{C}$ -labeled glucose. The labeling of glutamine lags somewhat behind that of glutamate, probably reflecting the time involved in release of glutamate from nerve endings, uptake by astrocytes, and conversion to glutamine (Fig. 17-2).

### SYNAPTIC VESICLES ACCUMULATE TRANSMITTER GLUTAMATE BY VESICULAR GLUTAMATE TRANSPORTERS

One defining characteristic of transmitter glutamate is its accumulation in synaptic vesicles. The concentration of glutamate in synaptic vesicles has been estimated at 60–250 mmol/l. The concentration of glutamate in the cytosol is only a few mmol/l, so glutamate is concentrated inside the vesicles. The inner radius of a glutamate-containing vesicle is on average about 17 nm, which gives a volume of  $2 \times 10^{-20}$  liters; a glutamate concentration of 100 mmol/l (or  $6 \times 10^{22}$  molecules/l) would therefore yield 1200 molecules of glutamate in each vesicle. Specialized proteins in the vesicular membrane, the vesicular glutamate transporters (VGLUTs), cause the accumulation of glutamate in synaptic vesicles. VGLUTs are multimeric protein complexes that are proton/glutamate antiporters (see Chap. 3). Vacuolar (V-type)  $\text{H}^+$ -dependent ATPase establishes a proton-based electric gradient across the vesicular membrane at the expense of ATP: protons accumulate in the lumen of the vesicle, which becomes positive relative to the cytosol. This gradient is the main driving force for the accumulation of glutamate, but the low pH of the vesicular lumen also contributes. However, if we consider the small volume of a vesicle, a vesicular pH in excess of 5 (a measured value) would imply less than 0.1 proton per vesicle. This probably means that a free proton is present in the vesicle less than 10% of the time, and that this is enough to attract glutamate. The VGLUTs have a  $K_m$  for glutamate of 1–2 mmol/l, are stimulated by chloride at a concentration of 4–10 mmol/l (which is the normal cytosolic concentration) and are sodium-independent. Three VGLUTs have been cloned. VGLUT 1 and 2 are localized to different populations of glutamatergic neurons. VGLUT3, on the other hand, is found in GABAergic, cholinergic and monoaminergic neurons, raising the possibility of novel roles for transmitter glutamate (Fremeau et al., 2004). Even some astrocytes have glutamate-containing vesicles with VGLUT 1 and 2, and astrocytes have been shown to release glutamate in a calcium-ion-dependent manner that involves the participation of SNARE proteins (see Ch. 12) (Fig. 17-2).

The fraction of glutamate contained in vesicles in gray matter may be estimated from the density of glutamatergic synapses ( $\sim 1/\text{mm}^3$  or  $1/10^{-15}$  liters), the number of vesicles per terminal

(100–500), the volume of each vesicle ( $\sim 2 \times 10^{-20}$  liters) and the concentration of glutamate in the vesicles (60–250 mmol/l). With a total concentration of glutamate of 12 mmol/kg tissue (or 12 mmol/l), the above values mean that vesicular glutamate may account for 1–20% of the total level of glutamate, depending on the number of vesicles per synapse and the intravesicular concentration of glutamate. This value may seem small considering the high level of glutamate in the brain, but the high level of glutamate is mainly caused by the high metabolic activity of the brain rather than by vesicular glutamate. The higher the metabolic flux, the higher the level of metabolic intermediates, among them glutamate.

### ZINC IS PRESENT TOGETHER WITH GLUTAMATE IN SOME GLUTAMATERGIC VESICLES

Zinc colocalizes with glutamate in subpopulations of synaptic vesicles throughout the brain. Approximately 10% of the synaptic vesicles of glutamatergic terminals in the hippocampus contain a substantial amount of zinc, which is released together with glutamate. Vesicles accumulate zinc through the ZnT3 zinc transporter, which is located in the vesicular membrane. The importance of this colocalization lies in zinc's ability to modulate the activation of glutamate receptors, as described below.

### IS ASPARTATE A NEUROTRANSMITTER?

Aspartate, like glutamate, can be released from brain slices in a calcium-dependent manner by depolarizing the slices with potassium chloride at high concentration, and intriguingly the ratio of released aspartate to glutamate is not fixed, meaning that the two amino acids could be released from different cell types or cellular compartments. Aspartate, which is closely related to glutamate and which has excitatory properties, does not appear to be concentrated in synaptic vesicles and for this reason may not be a transmitter in the classic sense. Aspartate might, however, be released directly from the cytosol of nerve endings. The concentration of aspartate is especially high in the cytosol of GABAergic neurons, but we do not yet know whether aspartate is released from this compartment under physiological conditions. Aspartate exclusively activates NMDA receptors, having no effect (agonist or antagonist) at AMPA receptors (Traynelis et al., 2010). The role of aspartate in excitatory neurotransmission remains to be determined.

### LONG-TERM POTENTIATION OR DEPRESSION OF GLUTAMATERGIC SYNAPSES MAY UNDERLIE LEARNING

Learning is a crucial aspect of our behavior. This is true for the motor, sensory, emotional and cognitive components of behavior (see also Ch. 56). Complex learning processes lie behind our ability to move our body in a purposeful manner, our ability to discern and understand the sensory information that we receive, the perception of stimuli as harmful or good, as

well as memory formation, language acquisition and thought processes. Learning must have an anatomical substrate: the CNS must undergo some form of change that allows the storage of what is to be learnt. Our current understanding is that the changes that underlie such information storage occur at the synaptic level through processes collectively referred to as 'synaptic plasticity'. There is no general agreement as to whether these changes occur predominantly on the presynaptic or on the postsynaptic side of the synaptic cleft, but several molecular mechanisms that could be important for learning have been identified on both sides. Electrophysiologically, two phenomena can be seen that may be components of learning at the synaptic level. These are long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy. A glutamatergic synapse that is briefly but strongly activated by high-frequency stimulation (e.g., 100Hz) may relay impulses more efficiently thereafter and will do so for a long time, for months or longer. This phenomenon, which was first described by Lømo in 1966, is termed LTP; the synapse has been 'potentiated' or strengthened (see also Hebb's postulate of learning, Ch. 56). However, prolonged low-frequency stimulation (1Hz) of a glutamatergic synapse may cause it to relay impulses less efficiently on a long-term basis, a phenomenon termed LTD, reflecting that the synapse has been 'weakened.' Presynaptic mechanisms that may contribute to LTP include a greater success rate for the release of glutamate when an action potential arrives at the terminal and release of greater amounts of glutamate per action potential (Skrede & Malthe-Sørensen, 1981). Postsynaptic mechanisms that mediate LTP and LTD are related to activation and trafficking of glutamate receptors, which are described below.

## THE NEURONAL PATHWAYS OF THE HIPPOCAMPUS ARE ESSENTIAL STRUCTURES FOR MEMORY FORMATION

LTP has been shown in many parts of the brain, but it has been most extensively studied in the hippocampus, a phylogenetically old part of the cerebral cortex that in humans is embedded in the temporal horn and in rats and rabbits lies beneath the parietal and temporal neocortex (Fig. 17-3A). The hippocampus is essential for (declarative) memory formation; in rats the role of hippocampus in acquisition of spatial information has been studied in great detail. In the hippocampus the neuronal connections are highly ordered, so it is easy to identify specific populations of neurons and synapses. One may cut the hippocampus into transverse slices (Fig. 17-3B) in which many of the neuronal connections are maintained. Such slices are excellent for electrophysiological recordings, a technique pioneered by Skrede and colleagues in the early 1970s. The anatomy of the hippocampus was described around 1900 by Ramón y Cajal. Axons from neurons in the entorhinal cortex enter the dentate gyrus as the perforant path (so called because it traverses the hippocampal fissure and 'perforates' the dentate gyrus). These axons form glutamatergic synapses with granule cells of the dentate gyrus. Axons of granule cells, the mossy fibers, form glutamatergic synapses with pyramidal cells of the CA3 region (CA stands for *cornu ammonis*—Ammon's

horn). These neurons send axons to the septum through fimbria as well as collaterals (the Schaffer collaterals) to the CA1 region, where they form glutamatergic synapses with pyramidal cells. In addition to this multisynaptic pathway from the entorhinal cortex to the CA1 region, there is a direct pathway from neurons in layer III of the entorhinal cortex to pyramidal neurons of the CA1 region. The axons of the CA1 pyramidal neurons exit the hippocampus as the alveus and project mainly to the subiculum, but some project to the septum through fimbria (Fig. 17-3B). LTP can be induced at all these glutamatergic synapses.

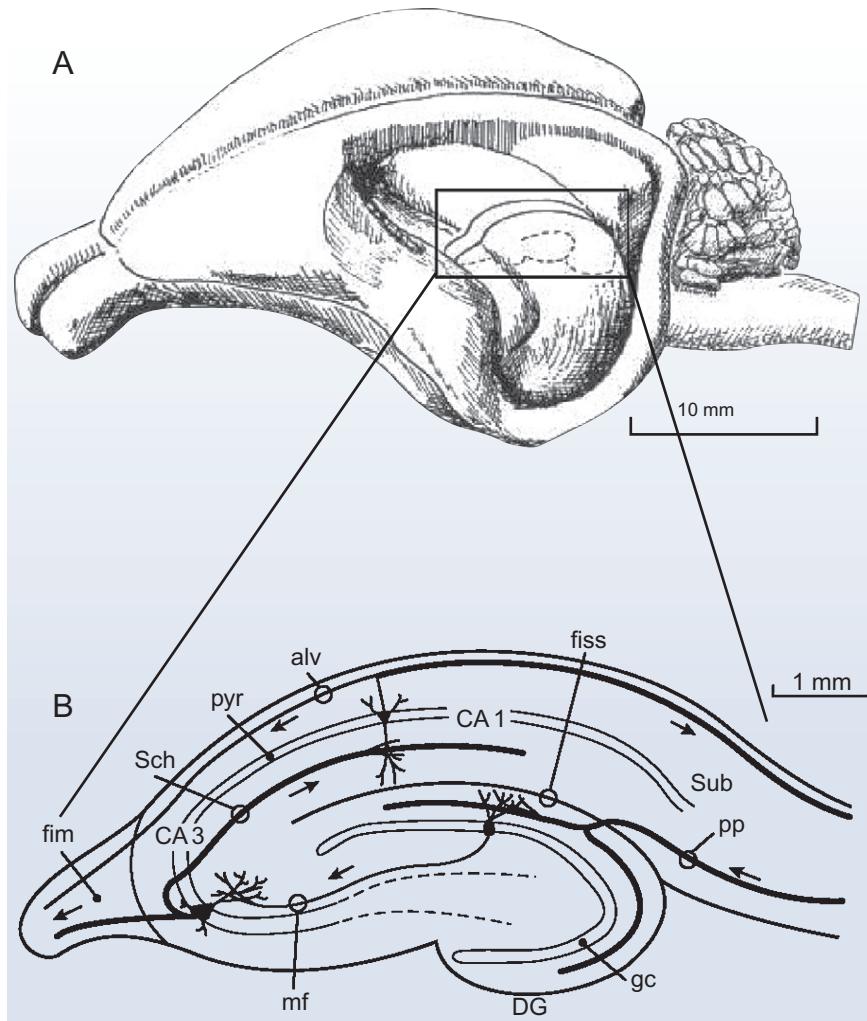
## IONOTROPIC AND METABOTROPIC GLUTAMATE RECEPTORS ARE PRINCIPAL PROTEINS AT THE POSTSYNAPTIC DENSITY

The classical glutamatergic synapse is a point of communication between a presynaptic nerve terminal and a postsynaptic dendritic spine (axo-dendritic synapses) or another nerve terminal (axo-axonal synapses). However, even astrocytes, oligodendrocytes and microglia express some types of glutamate receptor that may be stimulated by glutamate released from nerve terminals. Whereas glutamatergic terminals typically contact dendritic spines, GABAergic receptors are often located directly on dendritic shafts or on the soma. Glutamatergic synapses are easily recognized at high magnification as 'asymmetric': the postsynaptic membrane appears thicker than the presynaptic membrane (Fig. 17-4A). This postsynaptic density, or PSD, is on average 50nm thick, and may contain as many as 100 different proteins, among them the glutamate receptors (Fig. 17-4B).

Glutamate receptors belong to one of two main categories: Ionotropic receptors are cation channels whose opening is favored (over the closed state) when glutamate binds to the receptor. Metabotropic receptors do not conduct ion fluxes; instead they activate intracellular enzymes through G proteins when they bind glutamate. (G proteins are discussed in Ch. 21.)

## THREE CLASSES OF IONOTROPIC GLUTAMATE RECEPTORS ARE IDENTIFIED

For ionotropic glutamate receptors, the agonist binding sites and associated ion channel are incorporated into the same macromolecular complex. Agonist binding forces a conformational change in the receptor that increases the probability of channel opening. The three classes of ionotropic receptor were originally named after reasonably selective agonists — N-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (KA). The affinity for glutamate is different for the different glutamate receptors. The EC<sub>50</sub> for glutamate at NMDA receptors is approximately 1  $\mu$ mol/l, while at AMPA receptors it is approximately 400  $\mu$ mol/l (Traynelis et al., 2010). Several endogenous molecules may activate NMDA receptors, and some of them are,



**FIGURE 17-3** Hippocampus. (A) Its position beneath the parietal cortex in the rabbit brain. (With permission from Andersen, P. et al., *Exp. Brain Res.* 13: 222–238, 1971; copyright Springer Verlag.) (B) The main pathways of the hippocampus as seen in a hippocampal slice. (With permission from Skrede, K. et al., *Brain Res.* 35: 589–593, 1971.) The main input to the hippocampus is the perforant path (*pp*), which originates in the entorhinal cortex and traverses the hippocampal fissure (*fiss*) to the granule cell layer (*gc*) of the dentate gyrus (*DG*). The perforant path axons traverse the middle part of the dendritic trees of the granule cells, making synapses with several granule cells as they pass through (synapses ‘*en passage*’). The axons of the granule cells make up the mossy fibres (*mf*) that make large synapses with pyramidal cells (*pyr*) of the CA3 region, also of the *en passage* type. The axons of the CA3 pyramidal cells have two branches: one exits the hippocampus and enters fimbria (*fim*) to reach septum; the other branch, the Schaffer collateral (*Sch*), makes synapses *en passage* with CA1 pyramidal cells. The CA1 pyramidal cells exit the hippocampus as the alveus and project mainly to the subiculum (*Sub*) and more sparsely through fimbria to the septum.

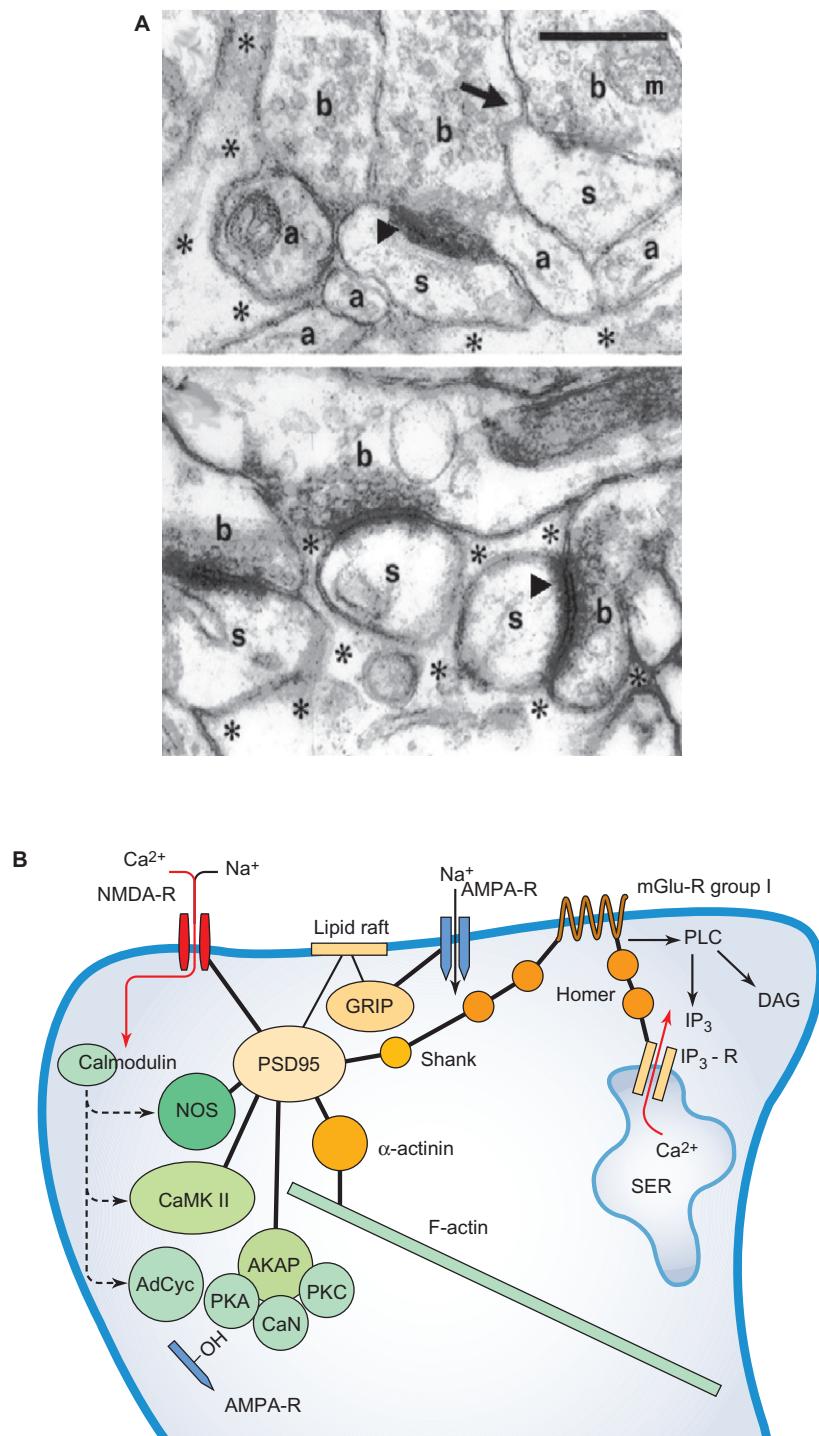
just like glutamate itself, acidic amino acids, e.g., aspartate and homocysteate.

### Seven functional families of ionotropic glutamate receptor subunits can be defined by structural homologies

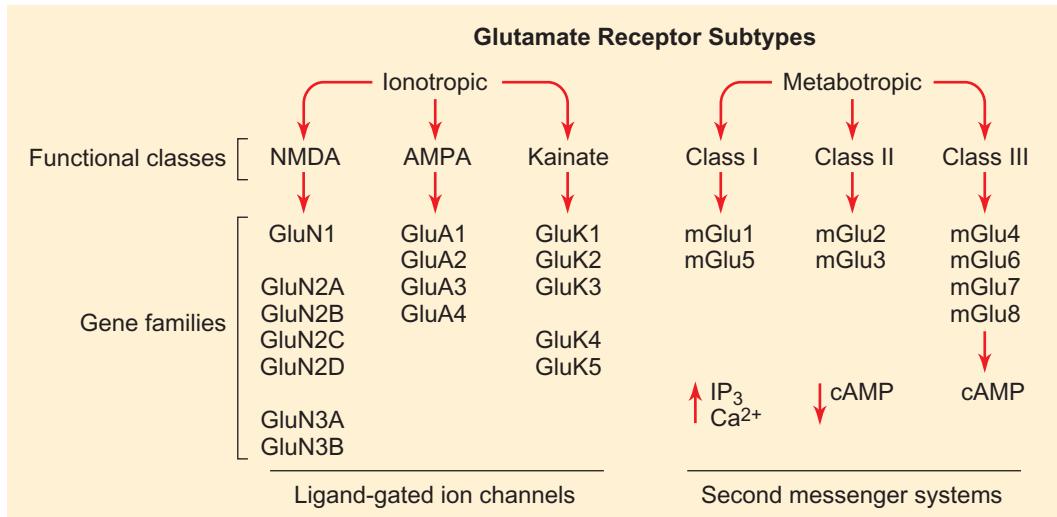
Since the cloning of the first glutamate receptor, GluA1, in 1989 (Hollmann et al., 1989), 18 mammalian genes that encode structurally related proteins have been identified. Currently, seven families of ionotropic glutamate receptor subunit have been described. The subunits assemble into functional receptors as shown in Figure 17-5. Within a given family, members

show at least 75% identity at the amino acid level over the 400 amino acid stretch of membrane-spanning regions. Between families, however, a lower degree of identity exists (55% or less). The function of the seventh family, consisting of GluD1 and GluD2 (not shown in Figure 17-5), is unknown although GluD2 can be activated by D-serine.

The ligand-gated ion channel receptors are tetrameric assemblies of the individual subunits. A significant feature of the glutamate receptors is that different subunit combinations produce functionally different receptors as described below. *In situ* hybridization and immunohistochemistry have highlighted regional differences in expression of subunits encoding glutamate receptors. These differences illustrate the heterogeneity of glutamate receptors throughout the CNS.



**FIGURE 17-4** Organization of the postsynaptic membrane. (A) Electron micrographs of glutamatergic axodendritic synapses in hippocampus CA1 (upper panel; stratum radiatum) and cerebellum (lower panel; molecular layer). The terminals (or boutons: *b*) are recognizable by their vesicles, and the postsynaptic spines (*s*) have the characteristic postsynaptic density (arrowhead). Note that glial processes (\*) surround synapses and axons (*a*), but that one of the terminals (*b*) is in close contact with a neighboring synaptic cleft, which could allow for spillover of glutamate from one synapse to another. *m*: a mitochondrion in a nerve terminal. Scale bar, 400 nm. (With permission from Lehre, K. P. et al., *J. Neurosci.* 18: 8751–8757, 1998; copyright Society for Neuroscience.) (B) Schematic representation of a dendritic spine of a glutamatergic synapse with some proteins of the postsynaptic density (PSD). Lipid rafts, scaffolding proteins (such as PSD95, GRIP, shank and homer) and cytoskeletal proteins (F-actin) help to concentrate and stabilize effector proteins at the spine. Effector proteins are glutamate receptors (AMPA-R, NMDA-R, mGlu-R), protein kinases and protein phosphatases, enzymes for the production of second messengers (nitric oxide, cAMP). AdCyc, adenylate cyclase; AKAP, (protein kinase) A-kinase anchoring protein; AMPA-R, AMPA receptor; CaMK II, Ca<sup>2+</sup>/calmodulin-dependent kinase II; CaN, calcineurin (protein phosphatase 2B); DAG, diacylglycerol; F-actin, filamentous actin; GRIP, glutamate receptor-interacting protein; IP<sub>3</sub>, inositol-1,4,5-triphosphate; mGlu receptor, metabotropic glutamate receptor; NMDA-R, NMDA-receptor; NOS, nitric oxide synthase; PKA and PKC, protein kinase A and C respectively (activated by cAMP and Ca<sup>2+</sup>/DAG respectively); PLC, phospholipase C; PSD95, postsynaptic density protein with a molecular weight of 95 kDa; SER, smooth endoplasmic reticulum.



**FIGURE 17-5 Molecular families of glutamate receptors.** Each of the two main glutamate receptor divisions comprises three functionally defined groups (classes) of receptor. These are made up of numerous individual subunits, each encoded by a different gene.

Together with differences in electrophysiological properties of different subunit combinations expressed in cell lines or oocytes, diverse patterns of subunit expression throughout the CNS support the existence of multiple subtypes of AMPA, kainate and NMDA receptors.

### AMPA and kainate receptors are both blocked by quinoxalinediones but have different desensitization pharmacologies

AMPA receptors are widespread throughout the CNS; they serve as receptors for fast excitatory synaptic transmission mediated by glutamate. AMPA receptor subunits are GluA1–GluA4, kainate receptor subunits are GluK1–GluK5. Glutamate receptors are approximately 900 amino acids long, compared to about 480 amino acids for nicotinic, GABA<sub>A</sub> or glycine receptor subunits. The extra length of AMPA receptors is due to an unusually large N-terminal extracellular domain. GluA1–GluA4 subunits co-assemble with one another to form tetramers with the pharmacologic profile of AMPA receptors (Fig. 17-6). Thus, when *Xenopus* oocytes are injected with mixtures of GluA1–GluA4 mRNAs, receptors are formed that can be blocked specifically by certain quinoxalinediones, notably 6-nitro-7-sulphamobenzo[f] quinoxaline-2,3-dione (NBQX). NBQX is a potent and selective competitive antagonist of AMPA receptors but has a weak or no effect on other receptors.

Subunits of the GluK1–GluK3 family, on the other hand, co-assemble with GluK4 or GluK5 into functional kainate receptors when studied in heterologous expression systems. Results of experiments with radioligands demonstrate that homomeric GluK1 and GluK3 receptors expressed in mammalian cell lines bind [<sup>3</sup>H]kainate with low affinity ( $K_d$  80–100 nmol/l). Such homomeric receptors may correspond to the ‘low-affinity’ kainate binding sites identified earlier in studies of plasma membranes from brain. Homomeric GluK2, GluK4 and GluK5 receptors, on the other hand, bind kainate with an affinity

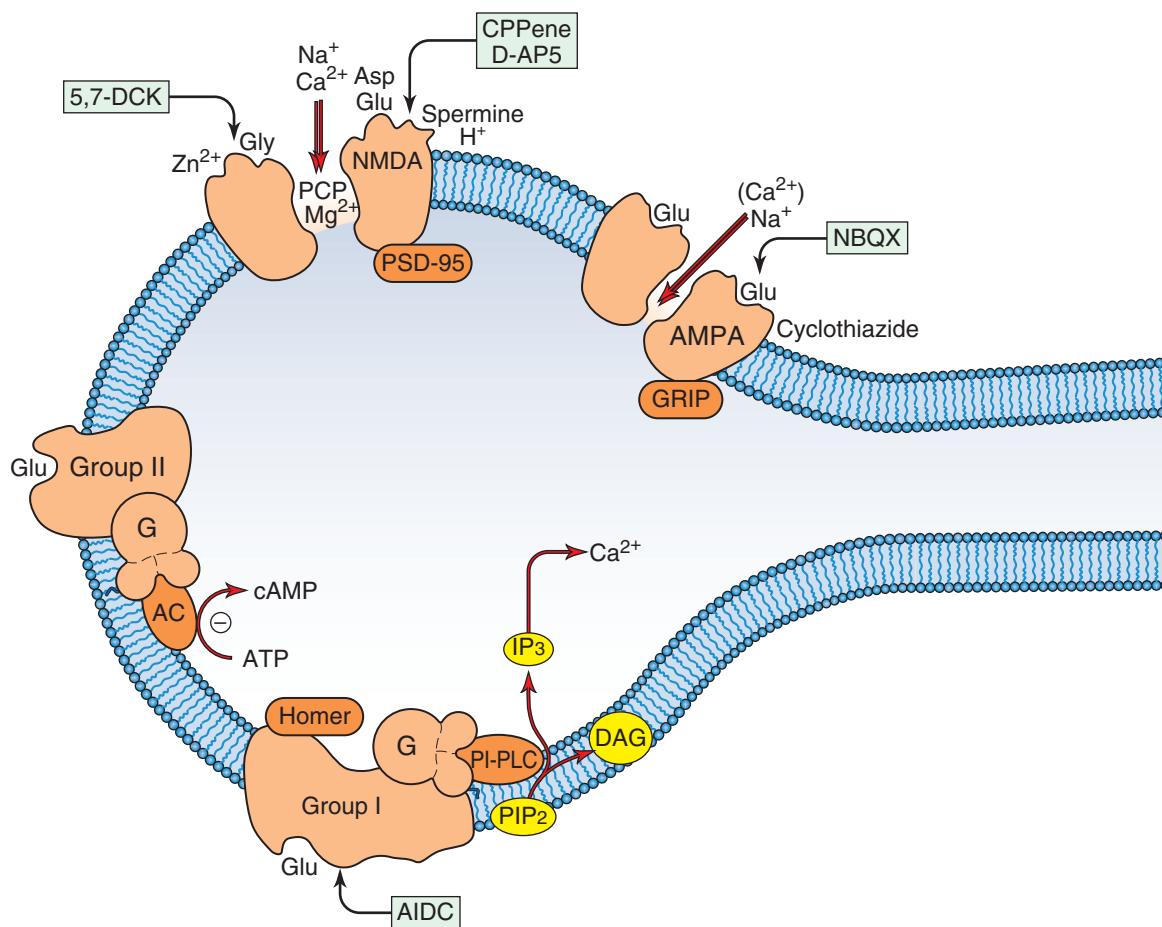
between 4 and 15 nmol/l respectively and may contribute to the ‘high-affinity’ kainate binding sites in brain. GluK4 and GluK5 are virtually inactive as ion channels when expressed alone and might therefore be thought of as modulatory subunits. Although none of the homomeric receptors are sensitive to AMPA, heteromeric complexes of GluK2 and GluK5 do respond to AMPA with a desensitizing current.

AMPA receptors desensitize within milliseconds upon exposure to AMPA, and kainate receptors likewise upon exposure to kainate. AMPA and kainate receptors can be securely distinguished from one another by their response to two drugs, cyclothiazide and the lectin concanavalin A (Fletcher & Lodge, 1996). Cyclothiazide relieves AMPA receptor desensitization without affecting kainate receptors. Conversely, concanavalin A relieves desensitization of kainate receptors, presumably via interaction with surface sugar chains, but has insignificant effects on AMPA receptors.

An important class of noncompetitive antagonists selective for AMPA receptors is represented by the 2,3-benzodiazepines such as GYKI 53655. These compounds act at sites different from those acted on by cyclothiazide and are useful tools for isolating synaptic responses mediated by kainate receptors. These compounds also show some promise as neuroprotective drugs for treating ischemic neuronal injury.

### N-methyl-D-aspartate (NMDA) receptors have multiple regulatory sites

To date, three NMDA receptor subunit families have been identified, one represented by a single gene (GluN1, encoding a protein of ~900 amino acids), the second by four genes (GluN2A–GluN2D, ~1450 amino acids) and the third by two known genes (GluN3A–GluN3B, about 1000 amino acids) (Fig. 17-5). Most if not all NMDA receptors in the brain are heteromeric receptors, as is likely to be the case with AMPA and kainate receptors. The mRNAs encoding most NMDA



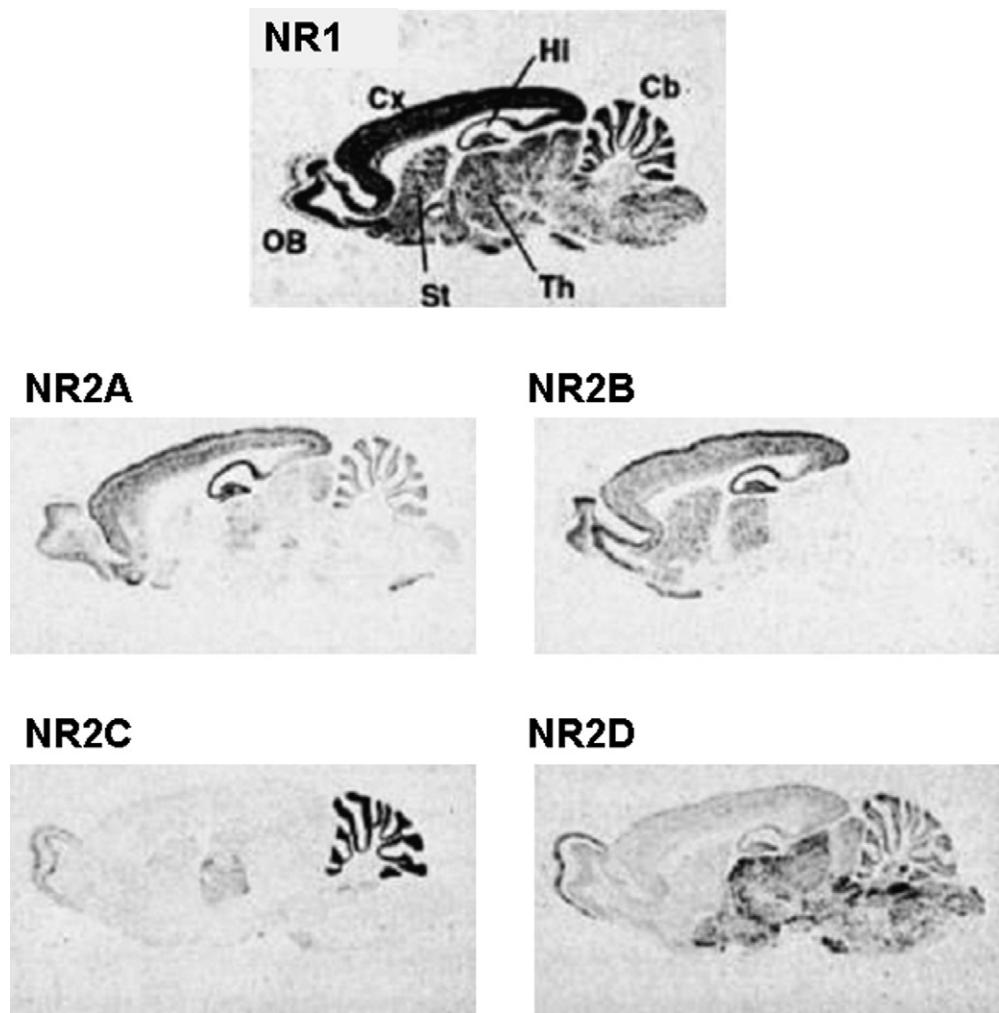
**FIGURE 17-6** Schematic views of four types of glutamate receptor. Two heteromeric ionotropic receptors are shown, the NMDA and AMPA receptors, as well as group I and group II metabotropic receptors. Competitive antagonists of each receptor are boxed. The NMDA receptor channel is additionally blocked by Mg<sup>2+</sup> and phencyclidine (PCP). Zn<sup>2+</sup> is both a negative and a positive modulator. Protons suppress NMDA receptor activation, and polyamines, such as spermine, relieve the proton block. Cyclothiazide removes desensitization of AMPA receptors. Both classes of metabotropic receptor are coupled via G proteins (G) to intracellular enzymes, phosphoinositide-specific phospholipase C (PI-PLC) for group I receptors and adenylate cyclase (AC) for group II receptors. PI-PLC catalyzes the production of inositol-1,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>). The resulting increase in cytoplasmic IP<sub>3</sub> triggers release of Ca<sup>2+</sup> from intracellular stores. Activation of group II metabotropic glutamate receptors typically results in inhibition of AC. The cytosolic proteins PSD-95, GRIP and Homer anchor the receptors to the PSD complex. AIDC, 1-aminoindan-1,5-dicarboxylate; 5,7-DCK, 5,7-dichlorokynurenic acid; D-AP5, D-2-amino-5-phosphonopentanoic acid; CPPene, 3-(2-carboxypiperazin-4-yl)1-propenyl-1-phosphoric acid; NBQX, 6-nitro-7-sulfamobenzo[f] quinoxaline-2,3-dione.

receptor subunits are differentially distributed, as are those of other glutamate receptors. Expression of GluN1 mRNA is nearly ubiquitous in the CNS. In contrast, the four GluN2 subunits show differential patterns of expression (Fig. 17-7). GluN2A is present throughout the forebrain and the cerebellum. GluN2B and GluN2C, however, have a more limited distribution. GluN2B is expressed in highest levels in the forebrain and GluN2C in the cerebellum where GluN2B mRNA is not detected. GluN2D expression seems virtually complementary to that of GluN2A in being high in the midbrain and hindbrain but low in the forebrain. GluN3A is expressed in the spinal cord and cortex, whereas GluN3B is found mainly in motor neurons in the spinal cord, pons and medulla.

NMDA receptors are some of the most tightly regulated neurotransmitter receptors. There are at least six distinct binding sites for endogenous ligands that influence the probability

of ion channel opening (Fig. 17-6). These consist of recognition sites for two different agonists (glutamate and glycine) and a polyamine regulatory site, all of which promote receptor activation, and separate recognition sites for Mg<sup>2+</sup>, Zn<sup>2+</sup> and H<sup>+</sup> that act to inhibit ion flux through receptors that have bound agonists. The redox state of the receptor also affects NMDA-receptor-mediated responses. One of the three pairs of cysteine residues may either be reduced (which enhances NMDA receptor-mediated currents) or oxidized to form disulfide bridges (which reduces currents).

NMDA receptor agonists are typically short-chain dicarboxylic amino acids such as glutamate, aspartate and NMDA. Acting at a site on the GluN2 subunit, glutamate is the most potent endogenous agonist in the mammalian brain. NMDA itself, although a very selective agonist at these receptors, is 30 times less potent than glutamate in electrophysiological



**FIGURE 17-7** Regional distribution of mRNAs encoding the five NMDA receptor genes in adult rat brain, by *in situ* hybridization. OB, olfactory bulb; Cx, cortex; Hi, hippocampus; Cb, cerebellum; Th, thalamus; St, striatum. (With permission from Nakanishi, 1992.)

assays. Competitive antagonists of the glutamate recognition site are formed from the corresponding agonists by extending the carbon chain, sometimes in a ring structure and often including replacement of the w-carboxyl group with a phosphonic acid group (Traynelis et al., 2010). Numerous competitive antagonists of this recognition site are available, notably d-2-amino-5-phosphonopentanoic acid (D-AP5) and 3-(2-carboxypiperazin-4-yl)1-propenyl-1-phosphonic acid (2R-CPPene), the latter having a  $K_d$  of approximately 40 nmol/l in binding and functional studies. These compounds are polar and penetrate the blood–brain barrier only poorly, although recently several NMDA receptor blockers have been developed that have better access to the brain from the blood.

The NMDA receptor is unique among all known neurotransmitter receptors in its requirement for the simultaneous binding of two different agonists for activation. In addition to the conventional agonist binding site typically occupied by glutamate, the binding of glycine to a site on the GluN1 sub-unit is required for receptor activation (Traynelis et al., 2010; Kleckner & Dingledine, 1988). Because neither glycine nor

glutamate acting alone can open this ion channel, they are referred to as ‘coagonists’ of the NMDA receptor (Kleckner & Dingledine, 1988). The glycine site on the NMDA receptor is pharmacologically distinct from the classical inhibitory glycine receptor (see Box, Chap. 12) in that it is not blocked by strychnine and is not activated by  $\beta$ -alanine. Several small analogs of glycine, including serine and alanine, also act as agonists at this site. In both cases the D-isomer is 20–30 fold more potent than the L-isomer. D-serine, formed by serine racemase, is a potent endogenous agonist at the glycine site (Wolosker et al., 1999). Bicyclic compounds and many derivatives of either kynurenic acid or quinoxalinedicarboxylic acid are competitive antagonists at the glycine site. Interestingly, most glycine site antagonists in these two series also block competitively the glutamate recognition site of AMPA receptors, suggesting possible structural similarities in the two ligand-recognition sites. Halogenation of both ring structures typically induces a large increase in potency, with 5,7-dichlorokynurenic acid (5,7-DCK) being a potent ( $K_d$  = 60 nmol/l) and highly selective glycine-site antagonist.

## NEURONAL AUTOIMMUNE ENCEPHALITIS

**George J. Siegel**

*Neuronal autoimmune encephalitis* comprises a group of autoantibody-mediated inflammatory processes of the CNS attributable to the binding of autoantibodies to neuronal cell antigens. This group includes *limbic encephalitis* (LE) with or without intractable seizures and often with other neurologic symptoms. The correct diagnosis of autoimmune encephalitis depends on awareness and is extremely important because often the encephalitis is completely or partially reversed by immunotherapy with steroids, IV IgG, or plasmapheresis (Davies et al., 2010). This discussion is focused on antibodies to glutamate receptors. However, antibodies related to GABA<sub>B</sub> and glycine receptors, GAD, as well as to VGKC and VGCC channel proteins (Malter et al., 2010), neoplasms and paraneoplastic syndromes have also been found causative of encephalitis. See Graus et al. for a detailed review (Graus et al., 2010).

Antibodies associated with encephalitis syndromes usually recognize antigenic neuronal epitopes at pre- or postsynaptic locations, particularly receptors and ion channels. The pathogenicity of these antibodies is proved by (1) demonstrating their binding to specific antigens in brain tissue, (2) response of symptoms to immunotherapy, and (3) correlation between antibody titers in serum or CSF with the neurologic outcome.

The glutamate receptor antigens associated with encephalitis so far discovered include NR1/NR2 subunits of the NMDA receptor, and GluR1/2 and GluR3 subunits of the AMPA receptor. Anti-NMDA GluR[epsilon]2 (same as NR2) has also been found in chronic epilepsy partialis continua (Takahashi et al., 2003). In addition, antibodies against mGluR1 have been found in two cases of cerebellar ataxia and Hodgkin's lymphoma.

### Mechanisms of Injury

Antibodies against glutamate receptors may interfere with their protein–protein interactions in the membrane with co-localized gelatinase, which is involved with synaptic and dendritic remodeling. The antibody binding may lead to alterations in dendritic plasticity and synaptic density (Gawlak et al., 2009). Antibodies to AMPA receptor found in LE produce alterations in synaptic receptor location. Application of these antibodies to cultures of neurons decreased the number of GluR2-containing AMPA receptor clusters at synapses and a decrease in AMPAR cluster density. These effects were reversed after antibody removal (Lai et al., 2009). Other possible mechanisms of injury are that antibodies may induce release of microglial factors that enhance NMDA receptor currents and toxicity (Moriguchi et al., 2003), bind to GluR on T cells to trigger inflammatory responses (Gonor et al., 2003), or be induced by elements of cell debris after T-cell-mediated damage (Takahashi et al., 2003) or apoptosis (Muñoz et al., 2010). In addition, GluR antibodies may block their activation or prevent their closing. In Rasmussen's encephalitis with intractable seizures, for example, serum antibodies to AMPA receptor GluR3 were found to maintain the receptors in a prolonged open state (Twyman et al., 1995), thus leading to intractable seizures and neuronal death (Levite et al., 1999).

### Potential Therapy

In rat brain, neuroinflammation can be reduced by antagonism of glutamatergic transmission or inhibition of COX2 activity (Willard et al., 2000). In this view, inhibitors of NMDA receptors, iNOS or COX2 may be potential routes of therapy in these encephalitides.

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Thus, glutamate and glycine act in concert to open NMDA receptor ion channels. In contrast, a very important brake on NMDA receptor activation is provided by extracellular Mg<sup>2+</sup>, which exerts a voltage-dependent block on the open ion channel (Nowak et al., 1984). Other voltage-dependent blockers of NMDA receptor channels include MK-801, the anesthetic ketamine and the recreational drug of abuse phencyclidine (PCP). These blockers (and Mg<sup>2+</sup>) exhibit varying degrees of voltage dependence and therefore probably recognize somewhat different domains in the channel of the NMDA receptor. Interestingly, the GluN3A/GluN3B subunits also possess a glycine binding site. Incorporation of a GluN3 subunit into NMDA receptors reduces Ca<sup>2+</sup> permeability of the receptor channel.

Another important endogenous allosteric inhibitor of NMDA receptor activation is H<sup>+</sup>. Protons reduce the frequency of channel opening at GluN2B-containing receptors over the physiological pH range, with a midpoint at pH 7.4. At pH 6.0, receptor activation is nearly completely suppressed (Traynelis et al., 1995). This suggests that an ionizable histidine or cysteine may play a key role in receptor activation. This regulatory mechanism may be especially important in acute stroke and other conditions that lead to accumulation of protons (tissue acidosis). One or more modulatory sites that bind polyamines such as spermine and spermidine are also found on NMDA receptors. Occupancy of one of the polyamine sites relieves tonic proton block and thus potentiates NMDA receptor activation in a pH-dependent manner (Traynelis et al., 1995). At higher concentrations, however, polyamines produce a voltage-dependent block of the ion channel and thus inhibit ion flux through activated receptors.

In addition to the regulatory mechanisms discussed above, an interesting form of Ca<sup>2+</sup>-dependent inactivation of NMDA receptors is brought about by calmodulin. Activated by Ca<sup>2+</sup> entry, calmodulin interacts with the C-terminal domain of the GluN1 subunit (Fig. 17-4B); this interaction causes inactivation of the receptor manifested by reduced channel opening frequency and reduced channel open time (Ehlers et al., 1996). The Ca<sup>2+</sup>-calmodulin-dependent phosphatase, calcineurin, was also shown to inactivate NMDA receptors (Tong et al., 1995), suggesting a two-step process for modulation involving dephosphorylation of the NMDA receptor followed by binding of the Ca<sup>2+</sup>-calmodulin complex.

### The transmembrane topology of glutamate receptors differs from that of nicotinic receptors

Knowledge about which segments of a receptor are intracellular, extracellular and transmembrane is necessary for identifying the ligand recognition sites, for understanding the mechanism by which ligand binding leads to channel opening, for interpreting mutagenesis data and for identifying potential targets for drug development. A variety of experimental approaches indicate that nicotinic receptor subunits have four transmembrane domains, with both N- and C-termini facing the extracellular fluid (Ch. 13). However, analysis of the AMPA receptor subunit GluA3 indicated only three transmembrane domains (Traynelis et al., 2010; Bennett & Dingledine, 1995). Most interestingly, the channel-lining M2 domain, which harbors the Q/R site (see below), was found to be a re-entrant

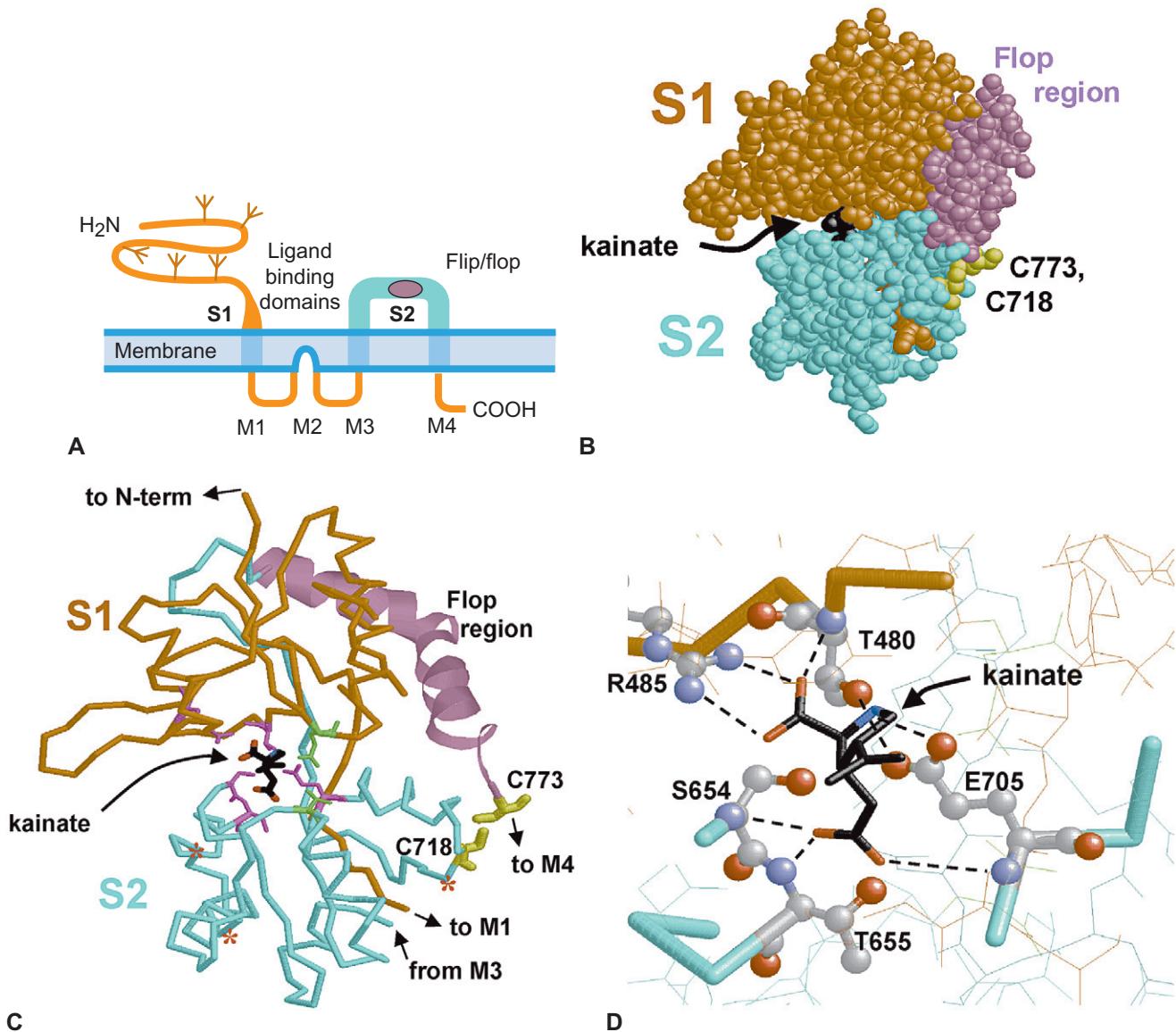
pore loop, with both ends facing the cytoplasm (Fig. 17-8A). Subsequent studies of NMDA receptor subunits provided evidence for a similar transmembrane topology (Traynelis et al., 2010; Kuner et al., 1996), suggesting that the glutamate receptor family has a different topology from that of other ligand-gated ion channels typified by the nicotinic acetylcholine receptors.

### Structure of the agonist-binding site has been analyzed

Homology mapping of the glutamate receptors onto the crystal structure of bacterial amino acid-binding proteins and functional evaluation of GluA3-GluK2 chimeras suggested that agonist binding requires portions of both the large N-terminus and a short region between M3 and M4. An important step forward was provided by Gouaux and collaborators, who crystallized a soluble fusion protein consisting of these two binding domains of the GluA2 subunit connected by a short amino acid linker (Armstrong et al., 1998). The agonist binding pocket lies at the hinge region of a clamshell-like gorge (Fig. 17-8B). Much has been learned about the mechanism of receptor activation by studying the structure of these two binding domains crystallized with different agonists and antagonists. Agonists contact residues on both upper and lower lobes of the clamshell (Figs. 17-8C,D), and binding causes partial closure of the clamshell. The resulting torque on the receptor is thought to be transmitted mechanically to the pore region, flipping the channel into the open conformation (reviewed in Traynelis et al., 2010; Mayer & Armstrong 2004). Interestingly, partial agonists appear to cause less domain closure than full agonists, and competitive antagonists work by binding in the pocket and preventing domain closure by a foot-in-the-door mechanism. The structure of the glycine binding site of the GluN1 subunit has also been solved and, like the GluA2 binding pocket, the glycine binding site lies at the base of a cleft between the two large extracellular lobes. A comparison of the GluA2 and GluN1 binding pockets explains clearly the basis of agonist and antagonist selectivity. Since these pioneering studies appeared, the structures of many other glutamate subunit domains have been solved.

### Genetic regulation via splice variants and RNA editing further increases receptor heterogeneity: the flip/flop versions and the Q/R site

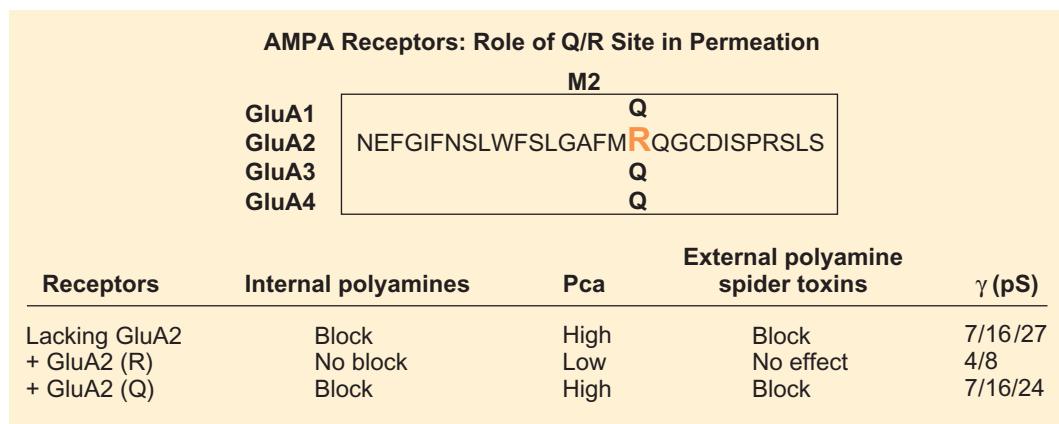
Splice variants that impart functional differences and/or different cellular expression patterns have been found for most of the glutamate receptor subunits. The first splice variants to be described were the so-called 'flip' and 'flop' versions of the AMPA receptor subunits GluA1-GluA4 (Sommer et al., 1990). The messenger RNA (mRNA) encoding each of these subunits exists in two versions differing by a 115bp segment. Within this encoded cassette of 38 amino acids that lie in the extracellular loop between the M3 and M4 segments (Fig. 17-8), the two alternative versions differ by only 9–11 amino acids, mostly conservative substitutions. The flip and flop splice variants give rise to receptors that differ in desensitization rate and in their regional distribution in the brain. C-terminal splice



**FIGURE 17-8** Transmembrane topology and binding pocket of glutamate receptors. (A) Schematic of a glutamate receptor subunit with the two domains that contain agonist-binding residues colored in orange (S1) and turquoise (S2). The flip/flop region is indicated in violet. (B) Space-filled representation of the kainate-bound S1 and S2 domains of GluA2 joined by an 11-residue linker peptide, with coloration the same as in (A). The flop domain is helical and located on a solvent-exposed face of the protein. The position of a single-kainate agonist molecule (black) within a deep gorge of the protein is indicated; the two disulfide-bonded cysteines (C718 and C773) are shown in yellow. (C) Backbone representation of the subunit, with kainate (black) docked into its binding site. The kainate-binding residues are shown as stick figures in magenta, the two cysteines in yellow and the flop helix structure in violet. The two green residues (E402 and T686) do not directly bind to kainate but instead interact with each other, helping to hold the clamshell in the closed conformation. (D) Close-up view of the ligand-binding pocket. The binding residues are in space-filled representation, with atoms colored conventionally (gray carbon, light blue nitrogen, red oxygen). (Courtesy of E. Gouaux; from [Traynelis et al., 2010](#).)

isoforms of many glutamate receptor subunits also exist and control the linkage to cytoskeletal proteins and their anchoring in the subsynaptic membrane. Alternative exon selection within the large N-terminal extracellular domain of GluN1 influences many modulatory sites, including those sensitive to pH,  $Zn^{2+}$  and polyamines (e.g., spermine). Thus, alternative exon selection is used by neurons to fine-tune the properties of all three classes of ionotropic glutamate receptor.

An important form of regulation is achieved by editing of the primary RNA transcripts of the AMPA and kainate receptor subunits. AMPA receptors that contain the GluA2 subunit are much less permeable to  $Ca^{2+}$  than those assembled without GluA2. This important feature of GluA2 was traced by site-directed mutagenesis techniques to a single amino acid within the second membrane-associated domain ([Hume et al., 1991](#)). A glutamine (Q) resides in this position in GluA1, GluA3 and



**FIGURE 17-9 Location and role of Q/R site in ion permeation through AMPA receptors.** The amino acid sequences of GluA1–GluA4 are identical within the M2 pore loop (Fig. 15-8A) except for the Q/R site, as shown (Q, glutamine; R, arginine). The phenotypes of AMPA receptors containing edited or unedited GluA2 or lacking GluA2 are summarized. Pca, calcium permeability;  $\gamma$ (pS), single channel conductance in picosiemens.

GluA4, but an arginine (R) is present in GluA2 (Fig. 17-9); this site has thus been named the 'Q/R site'. In addition to  $\text{Ca}^{2+}$  permeability, the Q/R site influences single channel conductance and the sensitivity of the activated receptor to block by polyamine spider toxins and cytoplasmic polyamines. Voltage-dependent block by cytosolic polyamines gives rise to inward rectification of AMPA receptors with low GluA2 abundance. Interestingly, the genomic DNA sequence has a glutamine codon in the Q/R position, even for the GluA2 subunit in which the mature mRNA has an arginine codon in this site. A small family of double-stranded RNA adenosine deaminases are employed as 'editors' to control the amino acid encoded by this critical codon (Higuchi et al., 1993). RNA editing of the Q/R and other sites, with associated changes in ionic permeability, have also been demonstrated for some kainate receptor subunits. The conditions under which neurons utilize RNA editing to regulate the permeability properties of their glutamate receptors remain to be demonstrated.

Given this combination of internal and C-terminus splice variants and site-specific RNA editing, it appears that four to eight or more mature RNAs might be made from each of the 18 known genes encoding mammalian ionotropic receptor subunits. Thus, neurons have a massive degree of flexibility in constructing a potentially huge number of receptors. The actual degree of glutamate receptor heterogeneity utilized by neurons remains a major unanswered question.

### The permeation pathways of all ionotropic glutamate receptors are similar, but vive la difference

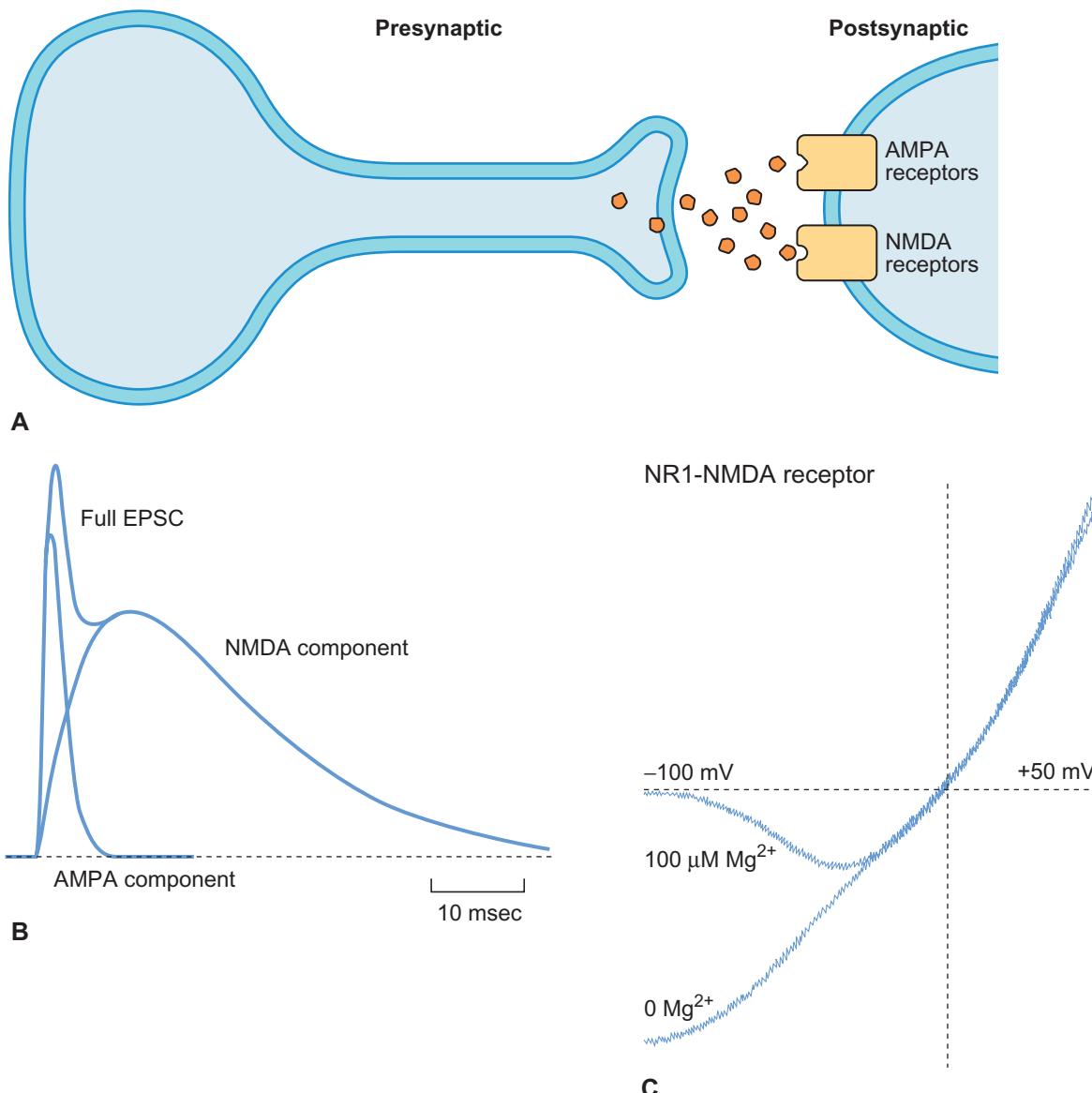
AMPA, kainate and NMDA receptors contain ion channels that conduct fluxes of  $\text{Na}^+$  and  $\text{Ca}^{2+}$ , but ion selectivity depends on the subunit composition of the receptor. Current flow through AMPA receptors containing GluA2 is normally largely carried by the movement of  $\text{Na}^+$  from the extracellular face to the intracellular compartment; these receptors have very low  $\text{Ca}^{2+}$  permeability. However, receptors that lack GluA2 are three to five times more permeable to  $\text{Ca}^{2+}$  than to

$\text{Na}^+$ , because of the absence of an arginine in the Q/R site, as mentioned above. An asparagine residing in the homologous site of all NMDA receptor subunits confers high  $\text{Ca}^{2+}$  permeability on these receptors (Wollmuth et al., 1996). Replacement by site-directed mutagenesis of this asparagine with an arginine produces NMDA receptors with very low  $\text{Ca}^{2+}$  permeability, similar to that of GluA2-containing AMPA receptors. An arginine or glutamine residing in the Q/R site of all known kainate receptor subunits also reduces the degree of  $\text{Ca}^{2+}$  permeability. Thus it is likely that the critical portion of the pore that forms the cation selectivity filter is similar among all glutamate receptors.

The permeation pathway of NMDA receptors has a property that sets them apart from other conventional ligand-gated receptors. At membrane potentials more negative (hyperpolarized) than about 50 mV, the concentration of  $\text{Mg}^{2+}$  in the extracellular fluid of the brain is sufficient to virtually abolish ion flux through NMDA receptor channels even in the presence of the coagonists glutamate and glycine (Nowak et al., 1984) (Fig. 17-10C). Thus, although glutamate and glycine are bound to their receptive sites and the channel is 'activated', the entry of  $\text{Mg}^{2+}$  into the channel pore blocks the movement of ions through the channel. In the presence of  $\text{Mg}^{2+}$  ions NMDA receptor channels exhibit a characteristic J-shaped current–voltage relationship. As the membrane potential is made less negative or even positive, the affinity of  $\text{Mg}^{2+}$  for its binding site decreases and the block becomes ineffective. Depolarization-induced relief from voltage-dependent channel block by  $\text{Mg}^{2+}$  is at the root of several of the most interesting aspects of NMDA receptor function (see below).

### GLUTAMATE PRODUCES EXCITATORY POSTSYNAPTIC POTENTIALS

Both NMDA and AMPA receptor components of excitatory postsynaptic potentials (EPSPs) are produced by the brief (1 ms) appearance of free transmitter in the synaptic cleft (Fig. 17-10A). Synaptically released glutamate thus results in a



**FIGURE 17-10** Biophysical properties of synaptic AMPA and NMDA receptors. (A) Diagram showing a postsynaptic cluster of NMDA and AMPA receptors and release of glutamate into the synaptic cleft. (B) Excitatory postsynaptic currents (EPSCs) of hippocampal neurons comprise both AMPA- and NMDA-receptor components. EPSCs are upward-going at positive membrane potentials. Treatment with the NMDA receptor blocker D-2-amino-5-phosphonopentanoic acid (D-AP5) or the AMPA receptor antagonist 6-nitro 7-sulfamobenzo[f] quinoxaline-2,3-dione (NBQX) reveals the other component in isolation, as shown. The EPSC is composed of a brief AMPA receptor component and a more prolonged NMDA component. (C) Current voltage relationships for NMDA receptors. Receptors can be expressed in *Xenopus* oocytes by micro-injection of *in vitro* transcribed RNA encoding various glutamate-receptor subunits. Oocytes were voltage-clamped to permit measurement of ionic currents flowing across the plasma membrane following exposure to agonists. In the presence of an agonist, the membrane potential was slowly ramped from -100 to +50 mV and the membrane current recorded. The horizontal dashed line shows zero membrane current. Receptors formed from homomeric GluN1 subunits exhibit a roughly linear current–voltage relationship in the absence of Mg<sup>2+</sup>, but, in the presence of Mg<sup>2+</sup>, current flux through NMDA receptor channels becomes progressively smaller as the membrane potential is made more negative. At 100 mV, the NMDA-induced current is virtually abolished by Mg<sup>2+</sup>.

two-component excitatory postsynaptic current (EPSC) upon binding to AMPA and NMDA receptors at most central synapses (Fig. 17-10B). Activation of AMPA receptors mediates a component that has rapid onset and decay, whereas the component mediated by NMDA receptor activation has a slower rise time and a decay lasting up to several hundred milliseconds. Rapid desensitization of AMPA receptors may control the time

course of EPSPs at many synapses. The long time course of NMDA receptor activation, by contrast, provides more opportunity for temporal and spatial summation of multiple inputs. The resulting summed depolarization may allow other synaptic inputs or nonsynaptic membrane channels to initiate action potential firing. The decay time of the NMDA receptor component is approximately 100 times longer than the mean open

time of the channel, so before the bound ligands dissociate, the receptor cycles through the open and closed states many times. The more prolonged activation of NMDA receptors is thought to be due to the higher affinity of glutamate for NMDA than AMPA receptors; high affinity often results from a slow dissociation of the agonist from its receptor. Postsynaptic kainate receptors also contribute to the EPSC at many synapses.

### Genetic knockouts provide clues to ionotropic receptor functions

In most neurons, full editing of the GluA2 subunit mRNA ensures that synaptic AMPA receptors will permit only insignificant  $\text{Ca}^{2+}$  influx. However, mice engineered to harbor a GluA2 gene that cannot be edited at the mRNA level expressed AMPA receptors with increased calcium permeability (Brusa et al., 1995). These mice develop seizures and die by 3 weeks of age, demonstrating that GluA2 editing is essential for normal brain development. Moreover, deletion of the GluA2 editing enzyme, RAD1, by gene targeting is also lethal, and this phenotype can be rescued by expression of a GluA2 subunit that has arginine hard-coded into the Q/R site. These results indicate that GluA2 mRNA itself is the most important target of the RAD1 editor. Surprisingly, complete deletion of the GluA2 allele, which also increases  $\text{Ca}^{2+}$  permeability of AMPA receptors in the targeted mouse neurons, neither induced seizures nor proved lethal in homozygous mice. Rather,  $\text{Ca}^{2+}$  entry through GluA2-lacking synaptic AMPA receptors could produce a form of LTP (Jia et al., 1996). These two genetic manipulations might have had such different outcomes because of different genetic backgrounds of the mice in the two studies. Alternatively, it is possible that a complete absence of GluA2 impairs AMPA receptor assembly, because the density of synaptic AMPA receptors appeared to be low in the latter study.

Conventional gene targeting of the NMDA receptor GluN1 subunit interferes with breathing and is lethal within a few hours of birth. However, mouse strains in which the GluN1 gene knockout is restricted to the CA1 region of the hippocampus survive and grow normally (Tsien et al., 1996). Remarkably, LTP is impaired in the CA1 but not in other hippocampal regions, and these mice exhibit impaired spatial memory in a water maze task. These findings demonstrate the essential role of NMDA receptors in LTP in the CA1 region, and also strongly suggest that LTP in the CA1 region is necessary for the acquisition of spatial memory.

## METABOTROPIC GLUTAMATE RECEPTORS MODULATE SYNAPTIC TRANSMISSION

### Eight metabotropic glutamate receptors (mGlu receptors) have been identified that embody three functional classes

Metabotropic glutamate receptors are so named because they are linked by trimeric G proteins to cytoplasmic enzymes (Conn, 2003). To date eight mGlu receptors have been cloned and named mGlu1–mGlu8. The corresponding genes encode

proteins that are thought to span the plasma membrane seven times and, like the ionotropic receptors, they possess an unusually large N-terminal extracellular domain preceding the membrane-spanning segments. mGlu receptors have been grouped into three functional classes based on amino-acid sequence homology, agonist pharmacology and the signal transduction pathways to which they are coupled (Fig. 17-5). Members of each class share ~70% sequence homology, with about 45% homology between classes. Alternatively spliced variants have been described for mGlu1, mGlu4, mGlu5 and mGlu7.

Glutamate itself activates all of the recombinant mGlu receptors, but with widely varying potencies ranging from 2 nmol/l (mGlu8) to 1 mmol/l (mGlu7). Highly selective agonists for each of the three groups have been identified. 3,5-dihydroxyphenylglycine (DHPG) appears to be a selective group I agonist, 2R,4R-4-aminopyrrolidine-2,4-dicarboxylate (APDC) is highly selective and reasonably potent (400 nmol/l) agonist for group II, whereas l-amino-4-phosphonobutyrate (l-AP4) is a selective agonist of group III mGlu receptors. Some phenylglycine derivatives are mGlu receptor antagonists but highly group-selective antagonists remain to be identified.

### mGlu receptors are linked to diverse cytoplasmic signaling enzymes

The classification of mGlu receptors into three groups is further supported by a consideration of their signal transduction mechanisms. Group I mGlu receptors stimulate phospholipase C activity to produce  $\text{IP}_3$ , which causes the release of  $\text{Ca}^{2+}$  from cytoplasmic stores (Fig. 17-6). The ability to increase intracellular  $\text{Ca}^{2+}$  levels differs between the members of this class and their splice variants, a property probably attributable to the different affinities each receptor has for its G protein. Activation of phospholipase C leads not only to the formation of  $\text{IP}_3$  but also diacylglycerol, which in turn activates protein kinase C (see Chapter 23). Activation of group II and group III mGlu receptors results in the inhibition of adenylate cyclase (Fig. 15-6). This response is blocked by pertussis toxin, which suggests that a G protein of the  $\text{G}_i$  family is involved (Chs 21 and 22).

### Postsynaptic mGlu receptor activation modulates ion channel activity

mGlu receptors located on the postsynaptic membrane modulate a wide variety of ligand- and voltage-gated ion channels expressed on central neurons, as would be expected if receptor activation is coupled to multiple effector enzymes (ion channels are discussed in Ch. 4). Activation of all three classes of mGlu receptor has been shown to inhibit L-type voltage-dependent  $\text{Ca}^{2+}$  channels, and both group I and II mGlu receptors inhibit N-type  $\text{Ca}^{2+}$  channels. mGlu receptor activation also decreases a high-threshold  $\text{Ca}^{2+}$  current in spiking neurons of *Xenopus* retina. Activation of mGlu receptors closes voltage-dependent,  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels in hippocampal and other cortical neurons, leading to slow depolarization and consequent neuronal excitation. The exact mechanism of modulation of  $\text{K}^+$  currents by mGlu receptors is at present unclear but

could occur through activation of CaMKII. In cerebellar granule cells, mGlu receptor activation increases the activity of  $\text{Ca}^{2+}$ -dependent and inwardly rectifying  $\text{K}^+$  channels, leading to a reduction in excitability.

A large number of ligand-gated channels are also modulated by mGlu receptor activation including NMDA and kainate receptors as well as dopamine, GABA<sub>A</sub> and norepinephrine receptors. Whether activation of mGlu receptors acts to inhibit or potentiate an ionotropic receptor depends on what component of the signal transduction mechanism is affected and this is often tissue specific. For example, in hippocampal pyramidal cells, group I mGlu receptor activation potentiates currents through NMDA receptors. This effect is reduced by inhibitors of either protein kinase C or Src kinase, and may proceed through dual signaling pathways. In contrast, in cerebellar granule cells, mGlu receptor activation inhibits NMDA receptor-induced elevations of intracellular calcium, also by a mechanism thought to involve PKC.

### Presynaptic mGlu receptor activation can lead to presynaptic inhibition

Immunohistochemical studies at both the light and electron microscopic level have firmly placed a number of mGlu receptors on the presynaptic terminals of central neurons. Activation of presynaptic mGlu receptors blocks both excitatory glutamatergic and inhibitory GABAergic synaptic transmission in a variety of central structures. For example, activation of mGlu2 located on presynaptic granule cell terminals blocks EPSPs evoked in CA3 hippocampal pyramidal neurons by mossy fiber stimulation. In contrast, transmission at synapses between Schaffer collaterals and CA1 pyramidal cells is resistant to presynaptic mGlu2 activation but is blocked by activation of mGlu4 or 7, as suggested by the effect of L-AP4, an agonist of all group III mGlu receptors. The actual mechanism of how mGlu receptor activation regulates synaptic transmission is unclear. However, because all three mGlu receptor groups are known to inhibit voltage-dependent  $\text{Ca}^{2+}$  channels it seems probable that presynaptic  $\text{Ca}^{2+}$  channels are targets for mGlu receptor modulation. A direct demonstration of this has been shown in presynaptic terminals of the calyx of Held in the brain stem, where mGlu receptor agonists suppress a high-voltage-activated P/Q-type  $\text{Ca}^{2+}$  conductance, thereby inhibiting transmitter release at this glutamatergic synapse (Takahashi et al., 1996).

### Genetic knockouts provide clues to mGlu receptor functions

The ability to ascertain the precise physiological functions of mGlu receptor has been hampered, in part, by the lack of sufficiently potent and selective pharmacological agents. The use of alternative strategies for the study of mGlu receptors is therefore clearly warranted. To address this problem, several research groups have used gene targeting to produce 'knockout' mice that are devoid of the mGlu receptor of interest. These experiments point directly to a physiological role for a number of mGlu receptors. Establishment of specific neuronal connections in the mature nervous system occurs by a process in which redundant connections formed during

development are eliminated. In the adult cerebellum, each Purkinje cell (PC) is innervated by a single climbing fiber (CF) that originates from the inferior olive of the medulla oblongata. This one-to-one relationship is preceded by a developmental stage in which each PC is innervated by multiple CFs. Massive elimination of synapses formed by CFs occurs postnatally, and a monosynaptic relationship is established at around postnatal day 20 in the mouse. Mice that lack the mGlu1 gene show symptoms of cerebellar dysfunction such as ataxic gait, intention tremor and dysmetria, and they are impaired in motor coordination and motor learning (Kano et al., 1997). These mutant mice are deficient in long-term depression (LTD) at CF-PC synapses; LTD in the cerebellum is thought to be a cellular basis for motor learning. In these mGlu1 mutant mice, innervation of multiple CFs onto a PC persists into adulthood, suggesting that the precise sculpting of these synaptic connections requires activation of mGlu1 during development.

The strong expression of mGlu2 in dentate gyrus granule cells has suggested a role for this receptor in presynaptic regulation of transmission at the mossy fiber-CA3 pyramidal neuron synapses as described above. In mice lacking the mGlu2 gene, basal synaptic transmission was indistinguishable from wild-type responses. In contrast, presynaptic inhibition at mossy fiber synapses induced by the mGlu2 agonist DCG-IV was markedly reduced in the mutant mice, confirming a role for mGlu2 receptors in the presynaptic regulation of neurotransmission. Interestingly, this mGlu2 deficiency was without effect on the magnitude of LTP induced at these synapses, but LTD was significantly impaired (Yokoi et al., 1996). In a variety of CNS structures including the hippocampus, olfactory tract, spinal cord and thalamus, activation of presynaptically located Group III mGlu receptors by L-AP4 inhibits synaptic transmission. One member of this group, mGlu4, is preferentially expressed in the cerebellum and has a role in the presynaptic regulation of synaptic transmission. Targeted elimination of the mGlu4 gene reveals that mGlu4 is essential in providing a presynaptic mechanism for maintaining synaptic efficacy during repetitive activation, and suggests that the presence of mGlu4 at the parallel fiber-PC synapse is required for maintaining normal motor function (Pekhletski et al., 1996). Likewise, mGlu8 seems to be the exclusive mediator of presynaptic inhibition at the lateral perforant path in the mouse.

## GLUTAMATE RECEPTORS DIFFER IN THEIR POSTSYNAPTIC DISTRIBUTION

NMDA and AMPA receptors are spread across the postsynaptic density (PSD), whereas metabotropic glutamate receptors (except mGlu7) are located along the periphery of the PSD (Fig. 17-2). NMDA receptors appear to be present at most or all glutamatergic synapses whereas the content of AMPA receptors is variable—from 0 to about 50 receptors per PSD (Kennedy et al., 2005). Some synapses are 'silent', meaning that activation of them does not elicit AMPA receptor currents when the plasma membrane is hyperpolarized and  $\text{Mg}^{2+}$  blocks NMDA receptors. Such silent synapses contain only NMDA receptors.

However, AMPA receptors are recruited from the cytosol to the PSD to activate such silent synapses in LTP.

## PROTEINS OF THE POSTSYNAPTIC DENSITY MEDIATE INTRACELLULAR EFFECTS OF GLUTAMATE RECEPTOR ACTIVATION

The PSD is a multiprotein complex with many functions (Fig. 17-4B). From the perspective of glutamatergic neurotransmission it may be viewed as a signal transduction machine that converts the extracellular glutamate signal into various intracellular signals. Apart from glutamate receptors, the PSD contains ion channels, glycolytic enzymes, transporters (e.g., a lactate transporter), proteins of intracellular signaling pathways, cell adhesion molecules and scaffolding proteins that link the proteins of the PSD together. Some of the proteins of the PSD are made lipophilic by the binding of two palmitic acid residues. Therefore they have an affinity for so-called lipid rafts, small areas of the plasma membrane that are rich in cholesterol and sphingomyelin (Hering et al., 2003). Such rafts are present in dendritic spines and help to concentrate proteins of the PSD at the spines (Fig. 17-4B). In the following paragraphs we focus on a few of the proteins of the PSD to illustrate some of the mechanisms that regulate synapse stability, function, and plasticity.

### A major scaffolding protein of the PSD is PSD95

Two N-terminal cysteines of this protein bind palmitic acid residues, which anchor PSD95 to lipid rafts (Kano et al., 1997). PSD95 contains several domains that bind other proteins: three so-called PDZ domains (short for PSD95/disc large/zona occludens-1), an Src homology (SH3) domain, and a guanylate kinase (GK) domain. This family of proteins is called MAGUKs (membrane-associated guanylate kinases), although the guanylate kinase domain is catalytically inactive. PDZ domains are stretches of about 90 amino acids that bind C-termini of other proteins.

Neuroligin is a protein that binds to the PDZs of PSD95. Neuroligin reaches into the synaptic cleft and interacts with  $\beta$ -neurexin, a protein that reaches into the synaptic cleft from the presynaptic side;  $\beta$ -neurexin is anchored presynaptically to the PDZ domain of another MAGUK, CASK. This arrangement connects the pre- and postsynaptic elements and stabilizes the synapse mechanically.

The GluN2 subunit of NMDA receptors binds to PDZ domains of PSD95 with its cytoplasmic C-terminus. PSD95 also binds  $\alpha$ -actinin, which again binds to filamentous actin (F-actin), a main cytoskeletal protein in dendritic spines. In this manner PSD95 anchors the NMDA receptor to the cytoskeleton of the dendritic spine.

PSD95 also couples the NMDA receptor to intracellular signaling systems that mediate intracellular effects of NMDA receptor activation. Calcium ions that enter the postsynaptic cell through the NMDA receptor bind to calmodulin probably

at all glutamatergic synapses. Calmodulin with bound  $\text{Ca}^{2+}$  activates a protein kinase,  $\text{Ca}^{2+}$ /calmodulin-dependent kinase II (CaM kinase II). CaM kinase II is one of the most abundant proteins at the PSD; it connects to  $\alpha$ -actinin via the scaffolding protein densin. Activated CaM kinase II can phosphorylate a host of other proteins. One such protein is the GluA1 subunit of the AMPA receptor, which is phosphorylated on a serine residue (ser 831). Phosphorylation of GluA1 by CaM kinase II increases the conductance of the AMPA receptor, which is one mechanism that contributes to potentiation of glutamatergic synapses.

The  $\text{Ca}^{2+}$ -calmodulin complex may also activate nitric oxide synthase (NOS), which binds to a PDZ domain of PSD-95. Activated NOS produces NO from arginine; NO, in turn, activates guanylate cyclase, the enzyme that catalyzes the conversion of GTP to the intracellular messenger cGMP, which activates protein kinase G (PKG).

AKAP (A-kinase anchor protein) is another scaffolding protein of the PSD. AKAP binds PKA (protein kinase A), PKC (protein kinase C), and the protein phosphatase calcineurin (PP2B) as well as PSD95 (at its SH3 and GK domains). This arrangement ensures a close proximity of NMDA receptors to PKA. Calcium influx through the NMDA receptor causes  $\text{Ca}^{2+}$ -calmodulin activation of adenylate cyclase and formation of cAMP. cAMP activates PKA, which, among other things, phosphorylates GluA1 on serine 845; this phosphorylation is necessary for recruitment of AMPA receptors to the plasma membrane of the dendritic spine. In fact, AMPA receptors are to a variable degree located intracellularly in recycling endosomes, whence they may be recruited to the postsynaptic membrane to strengthen the synapse during induction of LTP. PKC in the PSD has an important role in phosphorylating other serines on GluA1 (S816 and S818). Once phosphorylated here, GluA1 can bind to an actin-binding protein (Protein 4.1N), and travel along actin filaments to the plasma membrane driven by the motor protein myosin V (Lin et al., 2009; Correia et al., 2008). Long-term depression of glutamatergic synapses, which is also mediated by NMDA receptor activation, involves  $\text{Ca}^{2+}$ -dependent activation of the phosphatase calcineurin, which causes the dephosphorylation of GluA1 on serine 845 (Collingridge et al., 2010). The result of this dephosphorylation is the relocation of AMPA receptors from the plasma membrane to the cytosol.

AMPA receptors do not appear to bind directly to PSD95, but may do so indirectly through other proteins, such as TARPs (trans-membrane AMPA-R regulatory proteins), and this interaction appears necessary for the recruitment of AMPA receptors from the intracellular pool to the PSD (Kato et al., 2010). The C termini of AMPA receptors bind directly to other PSD proteins with PDZ domains: GRIP (glutamate receptor-interacting protein), PICK1 (protein interacting with C kinase 1), ABP (AMPA receptor-binding protein) and SAP97 (synapse-associated protein of 97 kDa). GRIP is another protein that is palmitoylated and therefore has an affinity for lipid rafts (Hering et al., 2003).

GRIP, PICK1 and PSD95 also interact with kainate receptor subunits GluK1 and GluK2. PKC, which binds to PICK1, may phosphorylate these subunits, thereby causing them to anchor more stably at the synapse, a mechanism that could strengthen kainate receptor-mediated transmission.

Metabotropic glutamate receptors are anchored to the periphery of the PSD by the scaffolding proteins homer and shank (Fig. 17-4). Homer is a cytoplasmic protein that may multimerize and link proteins that have homer-binding motifs, such as mGlu receptors and shank. Shank binds to the GK domain of PSD95 and thus links mGlu receptors to PSD95. Through homer, mGlu receptors also connect to the IP<sub>3</sub> receptor (1,4,5-trisphosphate receptors) (Correia et al., 2008); the activation of the IP<sub>3</sub> receptor by IP<sub>3</sub> causes the release of Ca<sup>2+</sup> from the smooth endoplasmic reticulum (see Chap. 23). The proximity of mGlu receptors and IP<sub>3</sub> receptors ensures that the IP<sub>3</sub> that is formed upon stimulation of type I mGlu receptors reaches the IP<sub>3</sub> receptor. Similar arrangements occur presynaptically, where mGlu7a binds to the PDZ domain of PICK1.

### Small GTP-binding proteins (GTPases) mediate changes in gene expression upon NMDA receptor activation

Synaptic plasticity manifesting as LTP or LTD depends in part on formation of new proteins, which requires transcription of specific genes. Activation of NMDA receptors during LTP may cause more than 100 genes to be transcribed, resulting in the formation of mRNAs. Some genes are turned on immediately upon NMDA receptor activation, others are activated later. The genes encode transcription factors, AMPA receptor subunits, cytoskeletal proteins, intracellular signaling molecules, ion pumps, and enzymes of energy metabolism (Hong et al., 2004) (see also Ch. 27).

The intracellular mechanisms that relay glutamate receptor activation to transcription constitute a much-studied field. Small, monomeric GTPases are important activators or silencers of signaling pathways that lead to changes in gene transcription. A final step in these pathways is the activation of transcription factors that cause initiation of transcription.

Ras is a small GTPase that is central in mediating alteration of gene transcription upon NMDA receptor activation. Ras stimulates several intracellular signaling pathways, including the Raf-MEK-ERK pathway and the PI3 kinase (phosphoinositide 3-OH kinase) pathway, the latter leading to activation of protein kinase B (Akt/PKB) as well as the MEKK-JNKK-JNK pathway (see Ch. 23 and 25). Phosphorylated (activated) ERK translocates from the cytosol to the nucleus, where it activates the transcription factors Elk and CREB (Ch. 27). Akt/PKB activation leads to the activation and nuclear translocation of transcription factors NF<sub>κ</sub>B and CREB. Activation and nuclear translocation of JNK lead to the activation of the transcription factors c-Jun, c-Fos and ATF2.

Small GTPases, such as Ras, cycle between an active GTP-bound state and an inactive GDP-bound state (Ch. 21). They are regulated by activating GEFs (guanyl nucleotide exchange factors) and inhibitory GAPs (GTPase-activating proteins). GEFs stimulate the exchange of GDP for GTP, which enables the GTPases to bind to their effector proteins. GAPs stimulate the GTPases to hydrolyze their bound GTP to GDP, which causes them to lose signaling activity. GEFs and GAPs may become activated through binding of calcium ions, Ca<sup>2+</sup>/calmodulin and/or diacylglycerol. A Ras-specific GAP, synGAP (synaptic GAP), binds to PSD95. Ras GEFs have been identified that associate

with NMDA receptor subunit NR2A and couple NMDA receptor activation to ERK activation (Jin et al., 2010). Thus, glutamate receptor activation may activate both GAPs and GEFs to modulate the activity of Ras and its downstream effectors.

An enigma of LTP research has been how the neuron comes to strengthen just the activated synapse and not all its synapses, since the mRNA made in the cell body will tend to become distributed throughout the cell. An answer seems to be that the LTP-inducing stimulus, in addition to activating genes in the cell body, marks the synapse where the mRNAs are to be translated into protein. The 'mark' may be the activation of cytoplasmic polyadenylation element binding protein (CPEB) by phosphorylation (Si et al., 2003). CPEB, a component of the PSD that binds to the 3' end of translationally dormant mRNAs, is phosphorylated upon NMDA receptor activation and initiates a process that causes elongation of the mRNA poly (A) tail. This polyadenylation of the mRNAs triggers their translation into protein only at the potentiated synapse.

### DENDRITIC SPINES ARE MOTILE, CHANGING THEIR SHAPE AND SIZE IN RESPONSE TO SYNAPTIC ACTIVITY WITHIN MINUTES

Intense synaptic activity leads to enlargement of the dendritic spine and the PSD. Although the pathways that regulate spine morphology in response to synaptic activity are not fully known, influx of calcium ions through the NMDA receptor is an important first signal and modulation of actin is a final result. The spines are highly motile structures. Most of the actin of the dendritic spine is broken down and rebuilt within a minute. Small GTPases of the Rho family regulate actin morphology (Kennedy et al., 2005). Among them, Rac and Cdc42 promote outgrowth of the actin cytoskeleton to create or enlarge spines, whereas activation of Rho itself has been shown to cause a loss of spines. The balance between Rac and Cdc42 on the one hand and Rho on the other is therefore important in controlling spine morphology in response to NMDA receptor activation. Rho GEFs have been identified that bind to PSD-95. Citron, a downstream effector of Rho, also binds to PSD95. In addition, both Rac and Cdc42 may be activated through Ras-dependent pathways.

### SODIUM-DEPENDENT SYMPORTERS IN THE PLASMA MEMBRANES CLEAR GLUTAMATE FROM THE EXTRACELLULAR SPACE

The area of the synaptic cleft is approximately 0.1 μm<sup>2</sup> and the width is 20 nm, which gives a volume of approximately  $2 \times 10^{-18}$  liter, 100 times greater than that of a synaptic vesicle. Therefore, the concentration of glutamate in the cleft when a vesicle has emptied its content will be in the low millimolar range. Rapid removal of glutamate from the extracellular space is important for the synapse to function properly on a millisecond time scale and to avoid overexcitation of the

postsynaptic cell. This is accomplished by diffusion of glutamate out of the synaptic cleft; however, to maintain a gradient for diffusion glutamate must be actively removed from the extracellular fluid.

Removal of glutamate occurs by both high-affinity transporters (with  $K_m$ s in the low micromolar range) and low-affinity transporters (with  $K_m$ s around 0.5 mmol/l). Only the former have been characterized on a molecular level. Glutamate removal has two components: binding of glutamate to the transporters and translocation to the interior of the cell. Binding is an important component, as the following calculation will show: The density of glutamatergic synapses in gray matter is roughly  $1/\mu\text{m}^3$ . A bouton may release the content of about 2–5 vesicles per second. With a content of 1,200 molecules of glutamate per vesicle, the number of glutamate molecules released per second would be 2,500–6,000. In the same volume 15,000–20,000 glutamate transporters may be present (Danbolt, 2001). Therefore, binding of glutamate to transporters is a rapid form of inactivation. The translocation of glutamate to the interior of the cell may take only a few additional milliseconds.

The high density of glutamate transporters and the tendency for astrocytic processes to cover the synaptic cleft (Fig. 17-2) controls the degree of spillover of glutamate into neighboring synapses. Spillover of glutamate from one synapse to another does occur, however, and has been shown to modulate transmission at nearby synapses, including glutamatergic, GABAergic and monoaminergic. Modulation of GABAergic and monoaminergic neurotransmission through spillover of glutamate occurs through activation of presynaptic metabotropic glutamate receptors on nerve terminals.

Five high-affinity glutamate transporters have been identified. They are termed EAAT (for excitatory amino acid transporter) 1–5. EAAT2 is the principal transporter in the forebrain; it is present in both astrocytes and nerve terminals (Fig. 17-2). EAAT1 is only present in astrocytes; it is abundant in cerebellum but is also expressed in the forebrain. EAAT3 is expressed in neurons in the whole brain and is found presynaptically on GABAergic nerve terminals, whereas EAAT4 is largely expressed on dendrites of cerebellar Purkinje cells. EAAT5 is expressed in retina. In the rat EAAT1–3 are termed GLAST, GLT and EAAC1, respectively; EAAT4 and 5 have the same names in man and rat.

The high density of glutamate transporters means that they may compete with glutamate receptors for binding of transmitter glutamate and thus modulate the glutamate signal. The density of glutamate transporters at the cell membrane as well as their affinity for glutamate are probably subject to regulation. Activation of PKC leads to a loss of EAAT2 from the cell membrane but causes an increase of EAAT3 in the cell membrane. Glutamate transporter-associated proteins (GTRAPs) have been identified that bind to and regulate the affinity of transporters for glutamate.

Electron microscopic studies with immunocytochemical detection of glutamate transporters show that the majority of the transporters are localized in astrocytic cell membranes, and it is generally assumed that most transmitter glutamate gets taken up by astrocytes as a part of the glutamine cycle (see above). However, when brain slices are exposed to the glutamate analog D-aspartate, this compound is found in nerve

terminals, not primarily in astrocytes. Therefore, there is some uncertainty regarding the quantitative importance of astrocytes versus neurons for uptake of transmitter glutamate.

The uptake of glutamate occurs against its concentration gradient and so needs a driving force, which is provided primarily by the sodium electrochemical gradient across the plasma membrane. Glutamate enters cells together with three sodium ions and one proton; one potassium ion is counter-transported (Danbolt, 2001). Uptake of glutamate is therefore electrogenic, resulting in a net movement of positive charge across the plasma membrane. The glutamate transporters also act as chloride channels, but the chloride conductance is not coupled to the transport of glutamate.

Because glutamate uptake is driven by cation gradients it is a secondarily active process that relies on the activity of the sodium-potassium ATPase (Ch. 3). For each molecule of glutamate that is taken up, one molecule of ATP is spent on the extrusion of the three sodium ions in exchange for two potassium ions. In addition, one molecule of ATP is spent if glutamate is converted into glutamine in astrocytes by the ATP-dependent glutamine synthetase. Several studies suggest that this energy expenditure is covered by increased glycolysis. Glycolysis gives two molecules of ATP per molecule of glucose that is converted to lactate. Excess lactate exits the astrocytes to the extracellular fluid and enters neurons where it is used as an energy source (Voutsinos-Porche et al., 2003) (see also Ch. 11).

## SODIUM-DEPENDENT GLUTAMINE TRANSPORTERS IN PLASMA MEMBRANES MEDIATE THE TRANSFER OF GLUTAMINE FROM ASTROCYTES TO NEURONS

Astrocytes convert much of transmitter glutamate into glutamine, and glutamine is exported to the extracellular space from which neurons accumulate it. Both astrocytes and neurons have transporters that are sodium driven and that concentrate glutamine inside the cells. The astrocytic and neuronal transporters are called system N transporters (SN) and system A transporters (SA), respectively (Fig. 17-2). They are closely related, but the astrocytic SN transporter exchanges a proton for sodium and does therefore not carry a net charge into the cell. It therefore depends entirely on the chemical component of the sodium gradient. This feature allows for transporter reversal when the sodium gradient becomes reduced, as would occur when astrocytes depolarize, so that some glutamine may leak out of the astrocytes into the extracellular fluid. An increase in intracellular glutamine or pH (reduction in  $[\text{H}^+]$ ) would contribute in the same direction. System A transporters do not exchange a proton for sodium and therefore carry a net charge into the neuron. Therefore system A transporters are driven by both the sodium gradient and the membrane potential, which makes them less likely to leak glutamine to the extracellular fluid (Chaudhry et al., 2002). The net effect of SN being more leaky than SA is a flux of glutamine from astrocytes to neurons.

## EXCESSIVE GLUTAMATE RECEPTOR ACTIVATION MAY MEDIATE CERTAIN NEUROLOGICAL DISORDERS

### Glutamate and its analogs can be neurotoxins and cause excitotoxicity

A paradox of glutamatergic neurotransmission is that activation of glutamate receptors may promote growth and survival of neurons (a trophic effect) on the one hand and cause degeneration and cell death (a toxic effect) on the other. One view has been that the degree of receptor activation determines how the stimulated cell will respond: moderate activation is trophic, but excessive activation kills the neuron by flooding it with calcium ions through NMDA receptor activation. An alternative view is that activation of synaptic (NMDA) receptors has a trophic effect by activating intracellular pathways that stimulate cell development and survival. In contrast, activation of extrasynaptic NMDA receptors, receptors that lie outside the synaptic area, on the spine shafts or on the dendrites themselves, engages intracellular pathways that lead to apoptosis and cell death (Hardingham & Bading, 2010) (see Ch. 37). The latter mechanism may be important in situations and disease states when the extracellular level of glutamate becomes very high, such as in stroke or traumatic brain injury (see below). In such situations glutamate may flood the entire extracellular space and activate extrasynaptic receptors.

Glutamate and structurally related ligands, such as NMDA and kainate, in addition to their powerful excitatory effects at glutamate receptors, are potent neurotoxins (see also Ch. 35). The toxic effect of glutamate and its analogs is related to their excitatory properties at glutamate receptors, and this type of toxicity is therefore called excitotoxicity. That glutamate and other amino acids act as neurotoxins was first realized in the 1970s when these agents were given orally to immature animals. Acute neurodegeneration was observed in those areas not well protected by the blood-brain barrier, notably the arcuate nucleus of the hypothalamus. The mechanisms of neurodegeneration are divergent, and activation of all classes of ionotropic glutamate receptor has been implicated.

Injection of kainic acid into the ventricles of the brain has been shown to result in a well-characterized pattern of neuronal cell damage. In the hippocampus, kainic acid causes a selective lesion of the CA3 pyramidal neurons, an area rich in GluK4 and GluK2 receptors. The lesion does not compromise passing axons, which is why this type of (excitotoxic) lesion is often referred to as 'axon-sparing'. Kainic acid injection into the hippocampus also leads to epileptiform discharges in cells normally innervated by the damaged pyramidal neurons.

### Some dietary neurotoxins may cause excessive glutamate receptor activation and cell death

Some rare food contaminants are glutamate analogs and cause excessive glutamate receptor activation, leading to neuronal cell death. Domoic acid is a shellfish poison that potently activates kainate receptors and produces seizures

and death or, if the patient survives, chronic memory impairment because of damage to the hippocampus. Amyotrophic lateral sclerosis (ALS) is a disorder that entails degeneration of upper and lower motor neurons (in the motor cortex and spinal cord/medulla oblongata respectively). While 90% of cases are sporadic, 10% are hereditary. The high incidence of ALS in the Pacific island of Guam could be due to the dietary ingestion of the cycad *Cycas circinalis*. This seed contains an amino acid,  $\beta$ -N-methylamino-L-alanine (BMAA). In the presence of bicarbonate, BMAA becomes excitotoxic through a mechanism that involves the activation of AMPA and NMDA receptors. The action of BMAA can be blocked by the NMDA receptor antagonist D-AP5. Another disorder that also primarily affects upper and lower motor neurons is neurolathyrism, a disorder occurring in East Africa and India. It is associated with the dietary consumption of the chickpea *Lathyrus sativus*. The glutamate-like excitant  $\beta$ -N-oxalylamino-L-alanine (BOAA) has been identified as the toxin in this plant. The action of BOAA at AMPA receptors may be responsible for the observed degeneration of motor neurons (see Motor Neuron Diseases, Ch. 45).

A mold that can contaminate sugar cane in China produces nitropropionic acid, a toxin that causes destruction mainly of the basal ganglia if ingested. Nitropropionic acid is not a glutamate analog, but an inhibitor of succinate dehydrogenase of the TCA cycle. The toxin causes ATP depletion and secondary loss of ion gradients that drive the glutamate transporters; the result is extracellular accumulation of glutamate and excessive stimulation of glutamate receptors, which leads to neuronal death. Such overstimulation of glutamate receptors caused by energy failure is typical even in hypoxia and ischemia, as discussed below.

### Abnormal activation of glutamate receptors in disorders of the central nervous system

Excessive or abnormal activation of glutamate receptors is probably a feature of most of the main disorders of the central nervous system, be they infection, inflammation, hypoxia and ischemia, traumatic injury, tumors, slowly progressive neurodegenerative states, or psychiatric disorders. It is typical of these disorders that they involve several pathogenic mechanisms, and the relative importance of glutamatergic neurotransmission probably varies a great deal from one disorder to another.

In bacterial infections that cause formation of abscesses (pus-filled cavities), there is a corresponding loss of brain tissue. Infections may destroy nerve cells by the direct effects of bacteria or their toxins or through the activity of leukocytes that are attracted to the site of infection. However, damage to neuronal cell membranes leads to leakage of glutamate from the cells, potentially causing toxic extracellular levels of glutamate. In abscesses the level of glutamate may remain high over time. This extracellular reservoir of glutamate probably predisposes individuals to epileptic attacks, which are frequent in patients with brain abscesses. (See Ch. 40 for a comprehensive presentation of epilepsy.)

Infection of the brain with human immunodeficiency virus 1 (HIV-1) may cause cognitive decline. The viral protein tat (trans-activator of transcription), which is released to the extracellular fluid, can activate the NMDA receptor and cause loss of dendritic spines or cell death, which may contribute to cognitive symptoms in persons with acquired immunodeficiency syndrome.

Glutamate toxicity may have a role in multiple sclerosis, an autoimmune inflammatory disease in which focal inflammation of CNS white matter causes damage to oligodendrocytes and demyelination of axons. In animal models of this disease, AMPA- or NMDA-receptor antagonists reduce tissue damage. This finding is consistent with the localization of AMPA receptors on oligodendrocytic cell bodies and NMDA receptors in the myelin itself. The source of excess glutamate in inflammatory states could be leukocytes that are attracted to the site of inflammation, or reactive astrocytes. Another autoimmune disease is (limbic) encephalitis caused by antibodies against neuronal proteins. One target of such antibodies is the NMDA receptor. Symptoms may include cognitive decline, personality changes, and seizures, which agree with the inflammation being most active in the temporal lobes. It is not known whether the symptoms are caused by a specific interaction of the antibodies with the receptor, or if they are caused (nonspecifically) by the inflammation per se.

In vascular disease that causes occlusion of brain arteries and stroke there is a rapid (within seconds) reduction in energy production due to lack of oxygen and glucose. With the ensuing loss of ATP and ion gradients the driving force is lost for glutamate uptake from the extracellular fluid. In this situation the transporters are reversed by the glutamate gradient, and glutamate leaves the cells, causing toxic concentrations of glutamate extracellularly. In animal studies treatment with AMPA- or NMDA-receptor antagonists greatly limits infarct development. Unfortunately, such antagonists have not yet proved useful in humans, partly because the infarct volumes tend to be greater and more variable in humans and the antagonists do not penetrate readily into the ischemic tissue, and partly because some glutamate antagonists cause side effects, such as hypotension, ataxia and cognitive disturbances, that have sidelined their clinical development.

Trauma may cause comprehensive injuries to the brain, including mechanical damage, hemorrhage, and inflammation. Extracellular levels of glutamate may remain high for a long time and contribute to excessive activation of receptors, high metabolic demand, and epileptic activity. One mechanism behind the high levels of glutamate is the loss of glutamate transporters in the injured tissue.

Some malignant tumor cells tend to release glutamate to the extent that the extracellular concentration of glutamate becomes toxic. It has been suggested that one effect of glutamate release from brain tumors is to provide room for tumor growth by killing neighboring neurons; in the brain this has a special significance, since the rigidity of the skull prevents much volume expansion. Glutamate may have a trophic effect on tumors. Tumor cells have been shown to express both AMPA and NMDA receptors. In animal experiments pharmacological inhibition of glutamate receptors reduces tumor growth and enhances animal survival. Similarly, knocking

down GluA1 subunits in tumor cells reduces growth. A similar effect has been achieved by increasing the expression of glutamate transporter EAAT2 in tumor cells, which presumably augments removal of glutamate from the extracellular fluid, reducing glutamate receptor activation.

A primary role for glutamate toxicity in chronic neurodegenerative diseases has been difficult to ascertain, but it is likely that altered glutamatergic neurotransmission plays a part in both the degenerative processes and in the symptomatology of the diseases. Huntington's disease, a heritable disease that affects the basal ganglia and causes movement disorders and dementia, is caused by an expansion of a CAG triplet repeat in the huntingtin gene (see Chap. 48). The resulting huntingtin protein has a longer than normal glutamine repeat. The main downstream effect of this mutation is not established, but it has been shown that glutamate reuptake is reduced in both experimental animals that carry an abnormally long huntingtin CAG repeat and in human Huntington's disease patients. Such a reduction could cause high levels of glutamate in synaptic clefts, leading to excessive activation of glutamate receptors and excitotoxicity.

Among the neurodegenerative diseases, Alzheimer's disease is one of the most common and a leading cause of dementia. Structures such as the entorhinal cortex and hippocampus are damaged early in the disease process (see Chap. 46). These structures are crucial for cognition, and they rely on glutamatergic neurotransmission for their function (see section on hippocampus). However, a primary role for glutamate receptors or glutamate toxicity has so far not been found. The low affinity NMDA receptor channel blocker, memantine, is being used as treatment in Alzheimer's disease, mainly because it may modulate the behavior of the demented patient, not because it has been shown to slow disease progression.

Historically, the neurochemistry of schizophrenia has been inferred from how drugs may cause or ameliorate psychosis. Thus, 'the dopamine hypothesis of schizophrenia' stems from the psychosis-precipitating effect of amphetamine and the therapeutic effect of dopamine D2 receptor blockers on the 'positive' symptoms of schizophrenia (psychosis and delusion). The serotonergic hypothesis derives from the hallucinogenic effect of LSD or mescaline and the therapeutic effect of 5-HT<sub>2A</sub> serotonin receptor blockers. The glutamate hypothesis of schizophrenia originates from the effect of NMDA receptor blockers (ketamine, phencyclidine), which produce a withdrawn, passive state that resembles the 'negative' symptoms of schizophrenia (passivity and anhedonism). The glutamate hypothesis posits that schizophrenia is linked to NMDA receptor hypofunction. On this basis augmenting activation of NMDA receptors with glycine site agonists (glycine or atypical antipsychotic clozapine) or glycine reuptake inhibitor sarcosine has been attempted clinically, and it seems that this strategy may reduce negative schizophrenic symptoms (see Chap. 58).

Finally, several clinical trials have shown efficacy for NMDA receptor antagonists in treatment-resistant major depression, and this may well become the next clinical use of glutamate receptor antagonists.

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