

## MINIREVIEW

# Control of Assembly and Function of Glutamate Receptors by the Amino-Terminal Domain

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### ABSTRACT

The extracellular amino-terminal domains (ATDs) of the ionotropic glutamate receptor subunits form a semiautonomous component of all glutamate receptors that resides distal to the membrane and controls a surprisingly diverse set of receptor functions. These functions include subunit assembly, receptor trafficking, channel gating, agonist potency, and allosteric modulation. The many divergent features of the different ionotropic glutamate receptor classes and different subunits within a class may stem from dif-

ferential regulation by the amino-terminal domains. The emerging knowledge of the structure and function of the amino-terminal domains reviewed here may enable targeting of this region for the therapeutic modulation of glutamatergic signaling. Toward this end, NMDA receptor antagonists that interact with the GluN2B ATD show promise in animal models of ischemia, neuropathic pain, and Parkinson's disease.

### Introduction

Glutamate-gated cation-selective channels, referred to as ionotropic glutamate receptors, mediate excitatory synaptic transmission throughout the central nervous system. Thus, these receptors are fundamental to the normal brain functions of learning, memory, and motor control. Glutamate receptors are membrane-spanning proteins with a tetrameric arrangement of four subunits that form a central cation-selective pore. The relationship between structure and function of this receptor class has been intensely studied for the past 20 years since the isolation of cDNAs encoding the first glutamate receptor subunits. The overwhelming majority of these studies have focused on the nature of the ion-conducting pore and the agonist binding domains. However, more recent work has cast a spotlight onto the amino-terminal domain as a component of the receptor that critically controls

a wide range of functional and structural properties. In this review, we focus on the structure and function of this versatile region of the glutamate receptors.

Ionotropic glutamate receptors can be grouped into four functional classes based on pharmacology and sequence homology: the AMPA receptors (GluA1–GluA4), kainate receptors (GluK1–GluK5), NMDA receptors (GluN1, GluN2A–D, GluN3A–B), and  $\delta$  subunits (GluD1 and GluD2). Sequence similarity among all subunits suggests that these different functional classes of glutamate receptors share a similar architecture (Traynelis et al., 2010). Each glutamate receptor subunit, regardless of subtype, possesses a modular structure with four distinct semiautonomous domains that include the extracellular amino-terminal domain (ATD), the extracellular ligand binding domain, the transmembrane domain, and an intracellular carboxyl-terminal domain (Fig. 1A). Of these domains, the extracellular ATD is most diverse among subunits, typically sharing 20 to 35% residue identity at a given position for all subunits within functional classes and only 0.2% sequence identity for all subunits. However, the sequence identity of ATDs can be higher between individual subunits or subgroups within functional classes (e.g., GluK1–3 or GluK4–5 kainate receptor subunits). Not surprisingly, the ATD mediates a diverse range of functions, including trans-synaptic protein-protein interactions and

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**ABBREVIATIONS:** AMPA,  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; NMDA, *N*-methyl-D-aspartate; mGluR, metabotropic glutamate receptor; ATD, amino-terminal domain; CP-101,606, traxoprodil mesylate; PDB, Protein Data Bank.

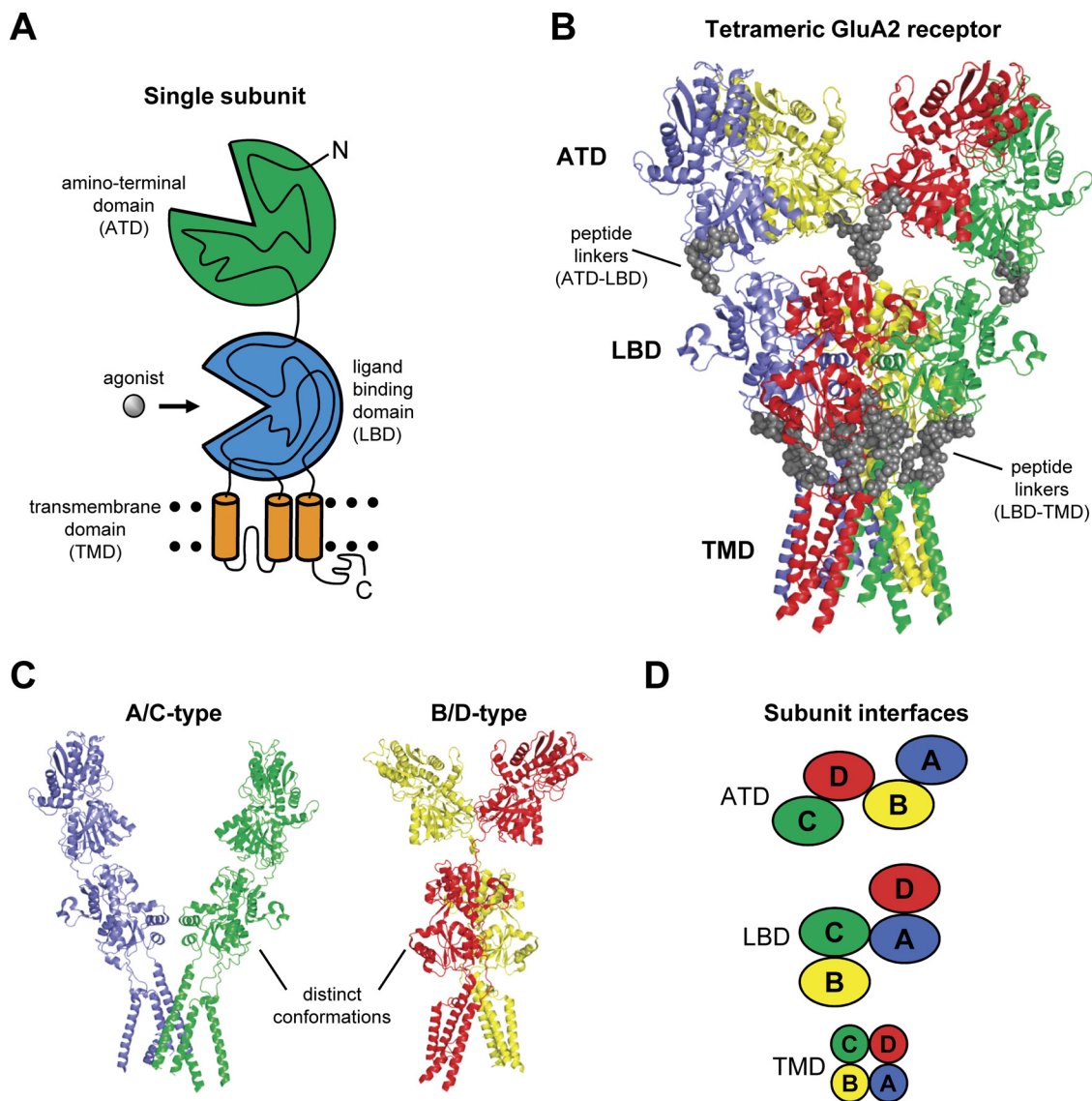
receptor assembly, as well as controlling receptor open probability, agonist potency, and response time course. Perhaps most interesting, however, are the findings that the ATD in some subunits harbors a binding site for allosteric modulators. This finding identified the ATD as a candidate drug target at least within the NMDA receptor family, with multiple classes of ligands that act on the GluN2B NMDA receptor subunit ATD currently undergoing clinical trials (Mony et al., 2009a; Traynelis et al., 2010).

### Structure of the Glutamate Receptor Amino-Terminal Domain

Subsequent to the signal peptide, which targets each receptor to the membrane and is removed by proteolysis, the first ~400 residues in all mammalian glutamate receptor subunits fold

into a semiautonomous bilobed structure that comprises the ATD. The glutamate receptor ATDs have modest sequence and structural similarity to the extracellular ligand binding domain of the metabotropic glutamate receptor mGluR1, and show weak sequence similarity to several bacterial periplasmic amino acid binding proteins (O'Hara et al., 1993; Wo and Oswald, 1995; Paas et al., 1996, 1998; Masuko et al., 1999; Kunishima et al., 2000). The similarity between the ATD and these other ligand binding proteins supports the idea that the ATD could bind endogenous and/or xenobiotic ligands within a pocket located in a cleft within a clamshell-like structural arrangement.

Crystallographic studies describing the structure of a membrane-spanning tetrameric AMPA receptor (Fig. 1B) have provided a structural model for all glutamate receptors



**Fig. 1.** Structure of the tetrameric ionotropic glutamate receptor. A, ionotropic glutamate receptor subunits possess a modular structure with four distinct semiautonomous domains that include the extracellular ATD, the extracellular ligand binding domain (LBD), the transmembrane domain (TMD), and an intracellular carboxyl-terminal domain. B, structure of the membrane-spanning tetrameric GluA2 AMPA receptor [Protein Data Bank (PDB) code 3KG2]. Peptide linkers that connect the semiautonomous domains are shown as gray spheres. C, glutamate receptors have two conformationally distinct subunits within the tetrameric receptor, which have been denoted A/C-type and B/D-type subunits. D, the extracellular ATDs and ligand binding domains are organized as dimer of dimers with approximately 2-fold symmetry, whereas the pore-forming transmembrane domain is organized with 4-fold symmetry. The A-type subunit forms an ATD dimer with the B-type subunit, but the ligand binding domain of the A-type subunit forms a ligand binding domain dimer with the D-type subunit. The “subunit crossover” creates an opportunity for both extensive intersubunit as well as intrasubunit interactions that involve the ATD.

(Sobolevsky et al., 2009). The structure of homomeric GluA2 receptors, which lacks the carboxyl-terminal domain, reveals an approximate 2-fold symmetry with the extracellular ATDs and ligand binding domains organized as dimer of dimers. The extracellular part of the tetrameric receptor is arranged with a 2-fold symmetry, whereas the pore-forming transmembrane domain shows striking similarity to certain potassium channels with 4-fold symmetry (Sobolevsky et al., 2009). The structure of a membrane-spanning tetrameric AMPA receptor surprisingly revealed two conformationally distinct subunits within the tetrameric receptor. That is, although the GluA2 amino acid sequence is identical in each of the four subunits in the homomeric channel, the subunits fold into two different conformations (i.e., different conformers). As a result of this unexpected feature of the glutamate receptor structure, the two subunits that assemble to form an ATD dimer do not also form a ligand-binding domain dimer, as one might expect. The two different conformers in the structure of the homomeric GluA2 receptor have been denoted A/C-type and B/D-type subunits (Fig. 1C). The A-type subunit forms an ATD dimer with the B-type subunit, but the ligand binding domain of the A-type subunit forms a ligand binding domain dimer with the D-type subunit. This “subunit crossover” creates an opportunity for both extensive intersubunit as well as intrasubunit interactions that involve the ATD. Furthermore, the existence of two conformationally distinct subunits within the tetrameric glutamate receptor raises the possibility that the A/C subunits couple to channel gating differently from the B/D subunits. Figure 1 illustrates the subunit arrangement for the GluA2 AMPA receptor structure. Cross-linking experiments have recently shown that the subunit crossover observed for AMPA receptors is also present in kainate receptors, confirming that kainate and AMPA receptors have a conserved extracellular architecture (Das et al., 2010).

Crystallographic studies have provided detailed structures of the ATDs from AMPA, kainate, and NMDA receptor subunits (Clayton et al., 2009; Jin et al., 2009; Karakas et al., 2009; Kumar et al., 2009; Sobolevsky et al., 2009). These structural data together with physiological experiments allow an initial evaluation of the relationship between the ATD structure and glutamate receptor function. Moreover, these structural data support the idea that the ATD from NMDA receptors can bind a wide range of molecules and ions and show that structural divergence accompanies the sequence differences of the ATD. However, it remains unclear whether the AMPA and kainate ATDs participate in small molecule binding.

The ATD forms two distinct types of subunit-subunit contacts within the tetrameric GluA2 structure (Sobolevsky et al., 2009). An extensive contact surface exists between A/B and C/D subunits, which is a feature that is also observed in the structures of the isolated GluA2 and GluK2 ATD dimers (Clayton et al., 2009; Jin et al., 2009; Kumar et al., 2009). A second contact surface resides at the 2-fold symmetry axis between B and D subunit conformers (Fig. 1C). The extent of the dimer interface as well as the dimer stability may influence receptor assembly and function (see below).

The subunit crossover between the ATD and the ligand binding domain levels of the receptor is mediated by the linker regions that connect the ATD to the ligand binding domain, referred to as the ATD-LBD linkers. For example, the ATD-LBD linkers of the A/C subunit conformers adopt a

more compact conformation than the ATD-LBD linkers of the B/D subunit conformers, which exist in an extended conformation. This structural difference in the ATD-LBD linkers of A/C and B/D conformers is intriguing, and functional studies have shown that the ATD-LBD linker of GluN2 NMDA receptor subunits can influence the deactivation kinetics and open probability of NMDA receptors (Gielen et al., 2009; Yuan et al., 2009a). The ATD-LBD linker also influences the actions of GluN2C/GluN2D-selective allosteric potentiators (Mullasseril et al., 2010).

The GluN2B ATD is also a clamshell-like bilobed structure composed of two halves, referred to as R1 (distal to the membrane) and R2 (proximal to the membrane) (Fig. 2) (Karakas et al., 2009). The N terminus is located at the top of the R1 lobe, whereas the bottom of the R2 lobe connects to the ligand binding domain through the ATD-LBD linker. The GluN2B ATD has a structural fold similar to that of non-NMDA receptor ATDs (GluA2/GluK2) but with a strikingly different conformation. The GluN2B ATD also has some structural similarity to the ligand binding domain of the metabotropic glutamate receptor mGluR1, which can bind the neurotransmitter glutamate within the cleft between the R1 and R2 lobes (Kunishima et al., 2000). One key feature of the GluN2B ATD structure is that the position of the R1 lobe is rotated  $\sim 50^\circ$  relative to the R2 lobe compared with the mGluR1 ligand binding domain and the other ATDs from AMPA and kainate receptors (Karakas et al., 2009). This “twisted” R1-R2 orientation of GluN2B ATD results from the lack of a helix-loop motif present in both AMPA and kainate receptors as well as mGluR1, which hold R1 and R2 lobes together in a “nontwisted” configuration. The lack of a helix-loop motif is a general feature of NMDA receptor subunits; thus, it is highly likely that ATDs from other NMDA receptor subunits have a similar “twist” as well. Most importantly, the “twisted” R1-R2 orientation of NMDA receptor ATD implies that the subunit arrangement at ATD is different from the ones observed in GluA2 or GluK2. This view is supported by the fact that superposition of the GluN2B ATD structure onto either the GluA2 or the GluK2 ATD dimer at the R1 lobe results in clash of the R2 lobes in the modeled GluN2B ATD dimer (Fig. 2).

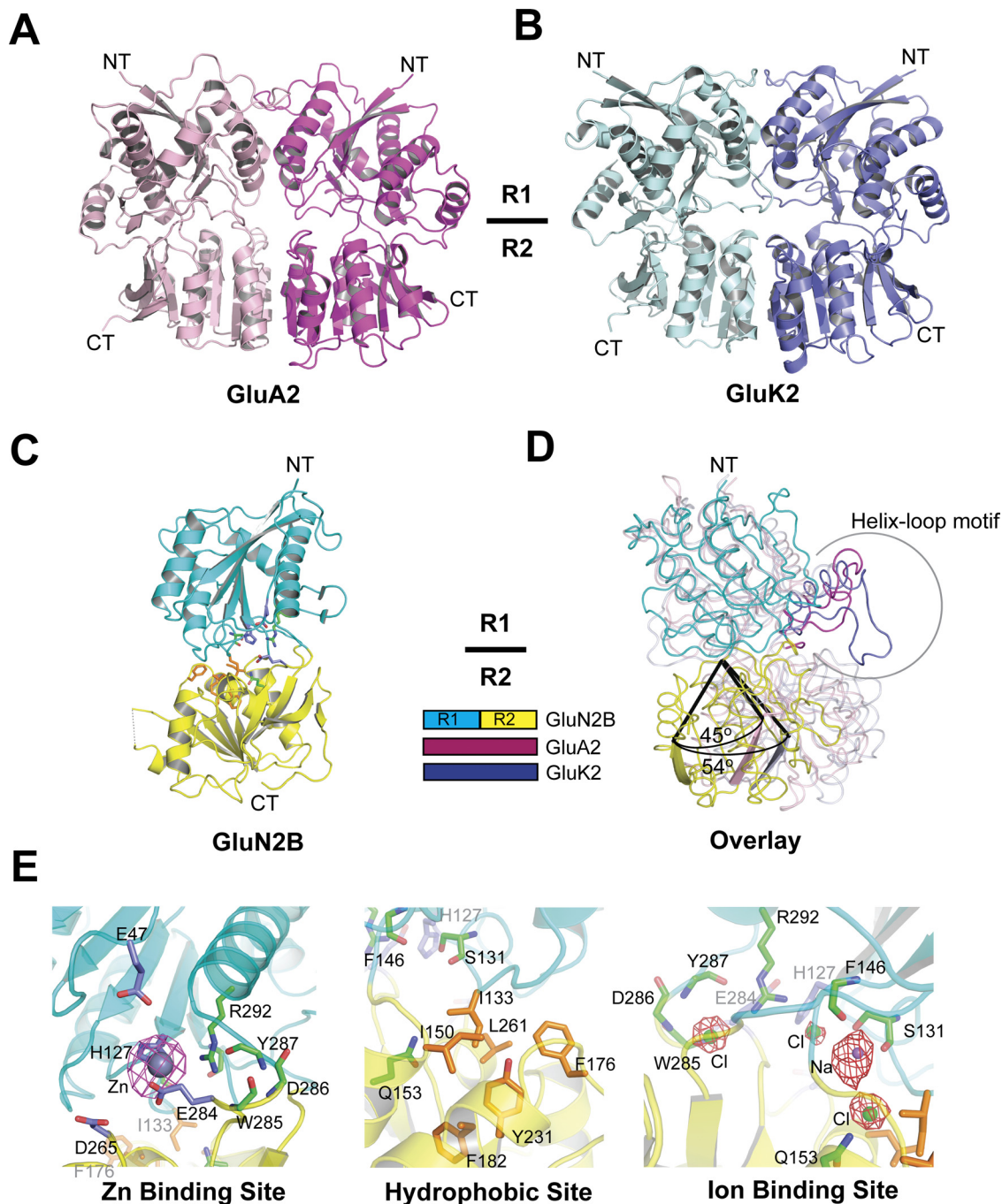
Evaluation of the GluN2B ATD suggests potential modulatory binding sites (Fig. 2). The cleft between the R1 and R2 lobes contains a hydrophilic pocket with polar residues that participate in  $Zn^{2+}$  coordination in GluN2B and a hydrophobic pocket that resides deeper in the cleft and contains some (but not all) residues critical for activity of the GluN2B-selective antagonist ifenprodil, which will be discussed in a subsequent section (Karakas et al., 2009).

### The Amino-Terminal Domain and Receptor Assembly

Functional glutamate receptors are formed exclusively by assembly of subunit members of the same pharmacological/structural receptor class (Partin et al., 1993; Kuusinen et al., 1999; Leuschner and Hoch, 1999; Ayalon and Stern-Bach, 2001; Ayalon et al., 2005). Multiple lines of evidence support the idea that AMPA receptors assemble as dimers of dimers, creating 2-fold extracellular symmetry. The interaction between the AMPA and kainate ATDs is of sufficient energy to allow isolated ATDs from kainate and AMPA receptor subunits to form stable dimers in solution (Clayton et al., 2009; Jin et al., 2009; Kumar et al., 2009). Moreover, the ATD

dimer interactions presumably mediate the initial steps in subunit-dimer formation, followed by ligand binding domain dimer formation, with additional subunits to create the subunit crossover arrangement revealed by structural analysis

of GluA2 (Greger et al., 2007). Formation of the tetrameric arrangement (i.e., assembly of two subunit dimers) occurs through multiple interactions of the ligand binding domains together with formation of ion channel pore in the transmem-



**Fig. 2.** Structures of ionotropic glutamate receptor ATDs. A, structure of the AMPA receptor GluA2 ATD dimer (PDB code 3H5V). The ATD structure adopts a bilobed structure composed of two halves, referred to as R1 (distal to the membrane) and R2 (proximal to the membrane), with extensive intersubunit R1-R1 and R2-R2 contacts. The N terminus is located at the top of the R1 lobe, whereas the bottom of the R2 lobe connects to the ligand binding domain through the ATD-S1 linker. B, the structure of the kainate receptor GluK2 ATD (PDB code 3H6G) is similar to that of the GluA2 ATD. C, the GluN2B ATD is also a clamshell-like bilobed structure composed of two halves (R1 in blue and R2 in yellow) (PDB code 3JPY). D, structural alignment of the ATD structures from GluN2B and non-NMDA receptors (GluA2/GluK2). The GluN2B ATD has a structural fold similar to that of non-NMDA receptor ATDs but with a strikingly different conformation. The position of the R1 lobe in the GluN2B ATD is rotated  $\sim 50^\circ$  relative to the R2 lobe compared with the ATDs from AMPA and kainate receptors. This “twisted” R1-R2 orientation of GluN2B ATD results from the lack of a helix-loop motif present in both AMPA and kainate receptors, which hold R1 and R2 lobes together in a “nontwisted” configuration. E, evaluation of the GluN2B ATD structure reveals possible modulatory binding sites. The cleft between the R1 and R2 lobes contains a hydrophilic pocket with polar residues that participate in  $\text{Zn}^{2+}$  coordination, a hydrophobic pocket that resides deeper in the cleft containing some (but not all) residues critical for activity of the GluN2B-selective antagonist ifenprodil, and an ion binding site that accommodates one  $\text{Na}^+$  and three  $\text{Cl}^-$  ions. Density for  $\text{Zn}^{2+}$  is represented by anomalous difference Fourier map at  $6\sigma$  whereas that for  $\text{Na}^+$  and  $\text{Cl}^-$  is represented by  $F_o - F_c$  omit map at  $5.5\sigma$ .

brane domain (Ayalon and Stern-Bach, 2001; Mansour et al., 2001; Ayalon et al., 2005). Receptor assembly occurs in the endoplasmic reticulum, where various mechanisms monitor correct protein folding and subunit assembly. Some of these quality control mechanisms may require ligand binding and gating before trafficking, because dominant-negative mutations can block trafficking to the surface (Greger et al., 2002, 2006; Fleck et al., 2003; Grunwald and Kaplan, 2003; Mah et al., 2005; Valluru et al., 2005; Priel et al., 2006; Penn et al., 2008). ATD may assist in the process of functional class specificity, such that AMPA receptor ATD dimer formation prevents kainate or NMDA receptor subunits from entering a newly forming AMPA receptor complex (Leuschner and Hoch, 1999; Ayalon and Stern-Bach, 2001; Ayalon et al., 2005).

The NMDA receptors differ from AMPA and kainate receptors in that at least two distinct classes of subunits assemble in a fixed relationship; the main subtypes of NMDA receptors are most likely assembled from two GluN1 and two GluN2 subunits. Although NMDA receptors can also contain GluN3 subunits, the expression and stoichiometry of native GluN3-containing NMDA receptors are still unresolved. Several conceptual models of the steps required for NMDA receptor subunit assembly have been proposed. For example, one model of NMDA receptor assembly suggests that GluN1-GluN1 and GluN2-GluN2 homodimers initially form and then coassemble to form the tetrameric receptor (Meddows et al., 2001; Schorge and Colquhoun, 2003; Papadakis et al., 2004; Qiu et al., 2005). An alternative model invokes initial formation of a GluN1-GluN1 homodimer to which GluN2 monomers are sequentially added to form the complete tetrameric NMDA receptor complex (Atlason et al., 2007). A third model suggests that initial GluN1-GluN2 heterodimer formation is followed by formation of a tetrameric arrangement (Schüler et al., 2008). There are insufficient data to fully evaluate these models at present, but as with AMPA receptors, the NMDA receptor ATD has been proposed to initiate dimer formation (Meddows et al., 2001; Papadakis et al., 2004). In addition to a role in subunit assembly, data also suggest that the ATD may influence receptor trafficking. The GluN2A ATD has been shown to contain a retention signal that prevents exit from the endoplasmic reticulum, unless it is specifically masked by assembly with the GluN1 ATD (Qiu et al., 2009).

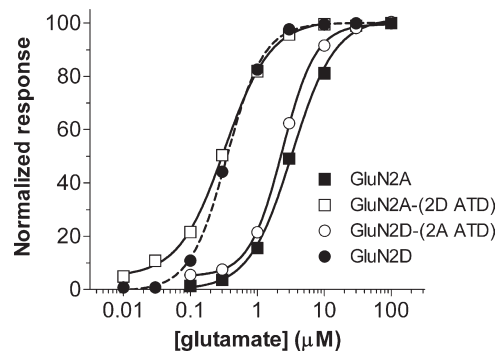
### Role of the Amino-Terminal Domain in Regulation of NMDA Receptor Function

A number of studies have revealed an unexpected degree to which the ATD can affect function of glutamate-gated channels. In particular, a great deal of information has been obtained from NMDA receptors constructed from chimeric GluN2 subunits. The GluN2 subunit defines the temporal signaling properties of NMDA receptors as well as agonist potency. For example, GluN2A-containing recombinant receptors show a rapid deactivation ( $\tau \sim 35$  ms) of the response after removal of glutamate, compared with an unusually slow time course for deactivation ( $\tau > 2$  s) of recombinant (Monyer et al., 1994; Vicini et al., 1998; Yuan et al., 2009a) and some native GluN2D-containing NMDA receptors (Misra et al., 2000). Moreover, recombinant GluN2D-containing receptors rarely open even when the agonist binding site is occupied, showing an open probability of approximately 0.01 (Yuan et al., 2009a). By contrast, recombinant GluN2A-containing NMDA receptors have an open prob-

ability 50 times higher than GluN2D ( $\sim 0.5$ ) when agonist is bound (Popescu and Auerbach, 2003; Erreger et al., 2005; Yuan et al., 2009a). Thus, glutamate is not a full agonist at any NMDA receptors, because a full agonist in theory would activate a channel with an open probability near 1.0. In addition, the GluN2 subunit influences both glutamate and glycine agonist potency (Kutsuwada et al., 1992; Ishii et al., 1993; Erreger et al., 2007; Chen et al., 2008; Hansen et al., 2008).

The role of the ATD in mediating the distinct properties conferred by the different GluN2 subunits was initially revealed in studies of GluN2 chimeric receptors. Exchange of the ATD between GluN2A and GluN2D, two subunits with the most divergent properties, shifts the open probability, response time course, and agonist potency in the direction of the subunit contributing the ATD (Yuan et al., 2009a) (Figs. 3 and 4). Similar results have been observed for open probability and agonist potency between GluN2A and GluN2B ATD-chimeric receptors (Gielen et al., 2009). It is noteworthy that the short ATD-LBD linker region (16 amino acids) of GluN2 seems to be critically involved in some of these actions (Gielen et al., 2009; Yuan et al., 2009a). This region shows divergent sequence among GluN2 subunits and occupies an extended conformation in the structure of the closely related GluA2 homomeric receptor. Although it is unclear how ATD controls all of these different functions, it may reflect a combination of allosteric interactions between the ATD and the ligand binding domain (e.g., Kew and Kemp, 1998; Erreger and Traynelis, 2005; see below) as well as effects of the ATD configuration on ligand binding domain dimer stability (Gielen et al., 2008, 2009).

Removal of the ATD from the GluN2A subunit of the NMDA receptor reduces open probability, whereas removal of the ATD from GluN2D increases open probability (Yuan et al., 2009a). That is, for NMDA receptors, removal of the ATD seems to bring several properties (such as open probability and agonist potency) to a similar common level for the remaining core receptor. One apparent conclusion from these data are that the ligand binding domain core and the transmembrane region of the receptor composed of very different GluN2 subunits share somewhat similar properties in the absence of the GluN2 ATD and the ATD-LBD linker (Yuan et al., 2009a).



**Fig. 3.** The NMDA receptor subunit GluN2 ATD influence agonist potency. Exchange of the ATD between GluN2A and GluN2D, two subunits with the most divergent properties, shifts the potency of glutamate in the direction of the subunit contributing the ATD. The chimeric GluN2A-(2D ATD) is GluN2A with the ATD replaced by the ATD of GluN2D, and GluN2D-(2A ATD) is GluN2D with the ATD replaced by the ATD of GluN2A. The glutamate potency at GluN2A-(2D ATD) is similar to that of wild-type GluN2D, and the potency of GluN2D-(2A ATD) is similar to that of wild-type GluN2A. Wild-type and chimeric GluN2 subunits were coexpressed with GluN1 in *Xenopus laevis* oocytes and glutamate potencies were determined by two-electrode voltage-clamp electrophysiology. See also Fig. 4. Data are from Yuan et al. (2009a).

It is noteworthy that bacterial glutamate receptors lack the ATD, and mammalian receptors function even when the ATD is removed (Fayyazuddin et al., 2000; Meddows et al., 2001; Pasternack et al., 2002; Horning and Mayer, 2004; Matsuda et al., 2005; Rachline et al., 2005; Madry et al., 2007; Gielen et al., 2009; Yuan et al., 2009a). That is, truncated subunits assemble into functional receptors that can be correctly trafficked to the plasma membrane at levels sufficient to allow experimental evaluation of their function.

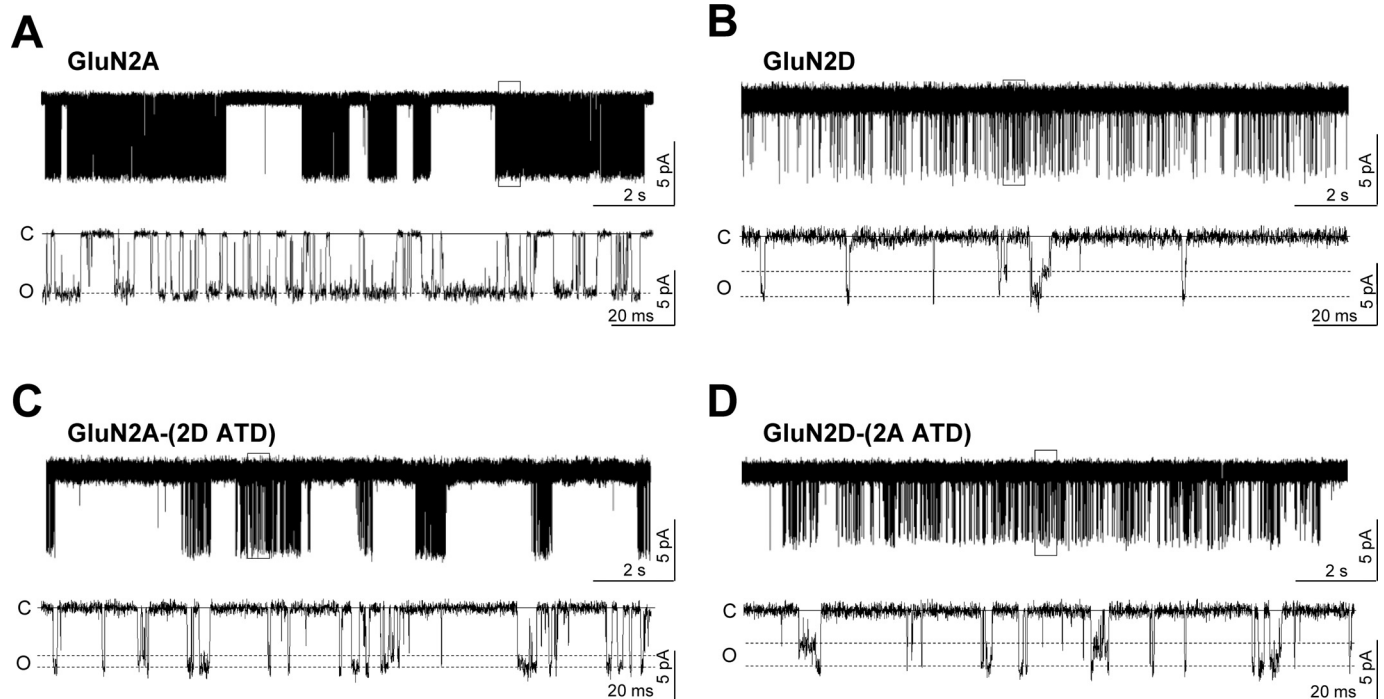
A key feature of ATD function may involve the relative separation between the upper and lower ends of the bilobed domain (Gielen et al., 2009). This is consistent with the proposed role of the ATD as a binding site for modulators, which may alter the relative orientation of the bilobed ATD. For example, the ATD for the glutamate receptor family harbors binding sites for proteins, extracellular ions, and small organic molecules.  $Zn^{2+}$ ,  $Ni^{2+}$ , polyamines, lectins, and di-aryl organic small molecules such as phenylethanolamines are all known to interact with the ATD of various glutamate receptor subunits to modulate function (Traynelis et al., 2010). The signals initiated by these ligands range from modification of desensitization to inhibition of receptor function. A number of these modulators have been hypothesized to bind within the cleft of the bilobed ATD.

#### The GluN2A Amino-Terminal Domain Harbors a High-Affinity $Zn^{2+}$ Binding Site

Extracellular  $Zn^{2+}$  potently inhibits native (Peters et al., 1987; Westbrook and Mayer, 1987) and recombinant NMDA receptors (Williams, 1996; Chen et al., 1997; Paoletti et al., 1997; Traynelis et al., 1998), showing biphasic inhibition

curves for recombinant GluN1/GluN2A receptors that reflect high-affinity voltage-independent inhibition ( $IC_{50}$  5–80 nM) and low-affinity voltage-dependent channel block ( $IC_{50}$  20–80  $\mu$ M). Voltage-dependent channel block by extracellular  $Zn^{2+}$  seems to share molecular determinants with  $Mg^{2+}$  block, being reduced by both GluN1 (Kawajiri and Dingle, 1993) and GluN2A mutations on the re-entrant M2 pore loop that alter  $Mg^{2+}$  sensitivity (Paoletti et al., 1997). The rate constants governing entry and exit from the intrapore  $Zn^{2+}$  binding site are too fast to allow individual blockages to be resolved by the patch-clamp recording (Christine and Choi, 1990).

High-affinity  $Zn^{2+}$  inhibition of GluN2A-containing receptors is voltage-independent and incomplete even at saturating  $Zn^{2+}$  concentrations. The  $Zn^{2+}$  binding site has been proposed to involve three histidine residues that reside in the cleft of the GluN2A bilobed ATD (Choi and Lipton, 1999; Fayyazuddin et al., 2000; Low et al., 2000). Deletion of the GluN2A ATD by mutagenesis or cleavage by the serine protease plasmin at Lys317 reduces or eliminates voltage-independent high-affinity  $Zn^{2+}$  inhibition (Gielen et al., 2009; Yuan et al., 2009a,b). Extracellular  $Zn^{2+}$  can also inhibit GluN2B-containing receptors in a similar dual voltage-independent and -dependent manner (Williams, 1996; Traynelis et al., 1998; Choi and Lipton, 1999; Rachline et al., 2005). Crystallographic structures for the GluN2B ATD reveal a binding site for  $Zn^{2+}$  within the cleft of the R1-R2 clamshell (Karakas et al., 2009) (Fig. 2). Mutagenesis of the residues that interact with  $Zn^{2+}$  suggests that this binding site accounts for the voltage-independent inhibition of GluN2B. As hypothesized for high-affinity binding of  $Zn^{2+}$  to the GluN2A



**Fig. 4.** The NMDA receptor subunit GluN2 ATD influences open probability. Exchange of the ATD between GluN2A and GluN2D shifts the open probability in the direction of the subunit contributing the ATD without changing the channel conductance. Replacement of the GluN2A ATD with that of GluN2D decreases open probability and mean open duration. By contrast, replacement of the GluN2D ATD with that of GluN2A increases open probability and mean open duration. Wild-type and chimeric GluN2 subunits were coexpressed with GluN1 in human embryonic kidney cells and characterized by recordings from single channels in outside-out patches in the presence of saturating glutamate and glycine. See also Fig. 3. [Reproduced from Yuan H, Hansen KB, Vance KM, Ogden KK, and Traynelis SF (2009) Control of NMDA receptor function by the NR2 subunit amino-terminal domain. *J Neurosci* 29:12045–12058. Copyright © 2009 The Society for Neuroscience. Used with permission.]

ATD (Paoletti et al., 2000), these crystallographic data are consistent with the idea that  $Zn^{2+}$  binding stabilizes a closed-cleft conformation within the bilobed ATD through direct interactions with residues His127 and Glu284 of the GluN2B R1 and R2 domains, respectively (Karakas et al., 2009).

The GluN2A and GluN2B ATDs have been hypothesized to exist as bilobed structures with both closed- and open-cleft conformation. Although this idea is consistent with crystallographic data on  $Zn^{2+}$  binding to GluN2B ATD (Karakas et al., 2009), no unliganded (*apo*) structure with an open-cleft conformation has been described that can serve as a basis for determination of the degree of domain closure in the ATD. Nevertheless, the hypothesized ATD cleft closure associated with  $Zn^{2+}$  binding has been proposed to destabilize the interface of the GluN1-GluN2 ligand binding domain dimer (Gielen et al., 2008). Although speculative,  $Zn^{2+}$  binding to the ATD might induce inhibition by a rearrangement of the ligand binding domain dimer interface by a mechanism analogous to desensitization of AMPA and kainate receptors (Sun et al., 2002; Armstrong et al., 2006; Weston et al., 2006).

A number of experimental observations support the idea that the effects of  $Zn^{2+}$  binding in some manner involves opening and closing of the ATD clamshell, the GluN1-GluN2 subunit interface at the ATD, and rearrangement of the ligand binding domain dimer interface. First, although the  $Zn^{2+}$  binding site is located in the GluN2 ATD, deletion of the GluN1 ATD eliminates voltage-independent  $Zn^{2+}$  inhibition, consistent with the idea that intersubunit ATD interface is important for receptor modulation (Madry et al., 2007). Second, cross-linking of mutant receptors harboring cysteine residues at the ligand binding domain dimer interface can reduce the inhibitory effects of negative modulators, such as  $Zn^{2+}$ , binding to the GluN2A ATD (Gielen et al., 2008). Third, mutations that destabilize the ligand binding domain dimer interface enhance  $Zn^{2+}$  sensitivity (Gielen et al., 2008). Fourth, covalent modification of a cysteine residue introduced within the ATD cleft of GluN2B with different size reagents has been hypothesized to pry open the ATD cleft to enhance receptor function in a manner correlated with the size of the modifying group (Gielen et al., 2009). That is, the larger the modifying group (and presumably more open the cleft), the more active the receptor. These data support the intriguing idea that the ATD can regulate receptor function through ATD cleft closure upon modulator binding and rearranging the dimer interface of ligand binding domains that are directly tethered to ATD. However, more work is needed to verify this hypothesis, which, as it stands, rests heavily on the precedent in AMPA and kainate receptors that ligand binding domain dimer interface critically controls receptor function and the assumption that the ligand binding domain dimer interface will similarly control NMDA receptor function.

Protons inhibit all glutamate receptors in a voltage-independent fashion without changing the ionization or  $EC_{50}$  values of the activating agonist (Christensen and Hida, 1990; Giffard et al., 1990; Tang et al., 1990; Traynelis and Cull-Candy, 1990; Vyklický et al., 1990; Wu and Christensen, 1996; Ihle and Patneau, 2000; Lei et al., 2001; Mott et al., 2003; Banke et al., 2005). Among NMDA receptors, proton  $IC_{50}$  for inhibition varies with the GluN2 subunit, with  $IC_{50}$  values near physiological pH for GluN2A, GluN2B, and

GluN2D (7.0–7.4), leading to the idea that these receptors are under tonic inhibition by physiological levels of protons (Traynelis et al., 1995; Gielen et al., 2009). Multiple lines of evidence at the macroscopic and single-channel levels suggest that  $Zn^{2+}$  binding to the GluN2A ATD shifts the proton inhibition curve leftward, consistent with the idea that  $Zn^{2+}$  binding increases proton affinity and thus the proportion of protonated, nonfunctional receptors at physiological pH (Choi and Lipton, 1999; Low et al., 2000; Erreger and Traynelis, 2005, 2008). Mutations at the ligand binding domain dimer interface and elsewhere in the receptor that alter  $Zn^{2+}$  inhibition also strongly influence proton inhibition (Traynelis et al., 1998; Low et al., 2003; Gielen et al., 2008), suggesting a functional link between these two forms of modulation. It is noteworthy that protons have previously been reported to modify AMPA receptor desensitization (Ihle and Patneau, 2000; Lei et al., 2001), a process that also involves rearrangement at the ligand binding domain dimer interface (reviewed by Traynelis et al., 2010).

GluA2 and GluK2 ATDs form dimers in solution as well as in crystals (Clayton et al., 2009; Jin et al., 2009; Kumar et al., 2009; Sobolevsky et al., 2009). Likewise, the isolated GluA1 and GluA4 ATD also form dimers in solution (Kuusinen et al., 1999; Wells et al., 2001; Jin et al., 2009). These results suggest that the dimer interface for AMPA and kainate receptors is stable under a variety of conditions, in contrast to GluN2B (and presumably  $Zn^{2+}$ -binding GluN2A), which can exist as a monomer in solution. Comparison of the R1 and R2 lobes of GluA2 and GluK2 ATDs with the corresponding domains of mGluR1 reveals that the GluA2 and GluK2 ATDs adopt an intermediate conformation relative to the open- and closed-cleft states observed for the ligand binding domain of mGluR1. In addition, the extensive interactions between the two ATD protomers within the dimer for GluA2 and GluK2 involve multiple contacts (Clayton et al., 2009; Jin et al., 2009; Kumar et al., 2009; Sobolevsky et al., 2009). The extensive interactions between the R2 lobes are mostly, but not exclusively, hydrophobic residues, which are conserved or conservatively substituted between AMPA and kainate receptors. In contrast, there is no sequence conservation between AMPA/kainate receptors and GluN2B. Moreover, the “twisted” orientation of the R1 and R2 lobes presumably makes R2-R2 interactions in the NMDA receptor subunit ATD dimers less extensive compared with those of AMPA and kainate receptors and dimerization of the NMDA receptor ATDs is therefore probably less stable. This would be consistent with the idea that modulator binding to the NMDA receptor ATD can trigger ATD cleft-closure and perhaps rearrangement of some ATD interfaces (Gielen et al., 2008, 2009). This could also suggest that the existence of modulators that bind at the NMDA ATD cleft reflects not only the availability of a binding pocket but also the ability of binding-induced cleft closure to impart significant rearrangement of the domains. That is, there may be an adequate binding site within the ATD of AMPA and kainate receptor that can accept ligands, yet the stability of the dimer interface may be such that ligand binding cannot induce a measurable change in conformation and thus cannot alter receptor function. This is because separation at the kainate or AMPA receptor R2–R2 ATD interface would expose the R2 hydrophobic region to water, which would be energetically unfavorable. By contrast, the lower energy driving proposed

dimerization of the ATDs of the NMDA receptor could better allow closure of the R1-R2 clamshell and enable separation or rearrangement of some as-yet-unidentified ATD interfaces, which may be the trigger for allosteric modulation of the ion channel. To date no ions or small molecules have been identified that bind within the AMPA or kainate receptor ATD cleft to modify receptor function.

Another feature of  $Zn^{2+}$  modulation of GluN2A is the  $Zn^{2+}$ -induced desensitization first reported by Chen et al. (1997). Subsequent studies, which built on the mechanism of glycine-dependent and glutamate-dependent desensitization (Mayer et al., 1989; Nahum-Levy et al., 2001), showed that a positive allosteric interaction between glutamate binding and  $Zn^{2+}$  binding occurred in which each ligand enhanced the binding of the other (Zheng et al., 2001). Multiple lines of investigation support the idea that glutamate binding enhances  $Zn^{2+}$  binding, causing a glutamate-induced relaxation to a new equilibrium as subsaturating extracellular  $Zn^{2+}$ , when present, binds to the GluN2A ATD in a concentration-dependent fashion (Zheng et al., 2001; Erreger and Traynelis, 2005). Thus, the  $Zn^{2+}$ -induced desensitization actually reflects the time course for  $Zn^{2+}$  association with its ATD binding site in the GluN2A subunit after a glutamate-induced shift of the  $Zn^{2+}$  binding site into a high-affinity state. This relatively rapid time course of  $Zn^{2+}$  association and subsequent inhibition probably reflects a component of desensitization observed in any preparation in which GluN2A receptors are active and ambient or contaminant  $Zn^{2+}$  was not removed by addition of a chelator.

### The GluN2B Amino-Terminal Domain Harbors a Binding Site for Di-Aryl Ligands

After the initial identification that the vasodilator ifenprodil is a noncompetitive NMDA receptor antagonist (Carter et al., 1988), it was subsequently shown that ifenprodil acts as a subunit-selective antagonist for NMDA receptors, with more than 100-fold higher potency at NMDA receptors that contain the GluN2B subunit than other receptors (Williams, 1993). After this finding, ifenprodil has been used in an exceptionally wide range of studies to explore the role of GluN2B-containing NMDA receptors in development, normal brain function, and disease (reviewed by Mony et al., 2009a). These studies have identified a wide range of normal processes and neurological diseases that involve GluN2B receptors (Wang and Shuaib, 2005; Mony et al., 2009a; Traynelis et al., 2010) and have stimulated a vigorous medicinal chemistry effort that has led to the identification of a large family of diverse organic molecules that can selectively inhibit GluN2B receptors (Table 1 and Fig. 5). A number of known di-aryl ligands (Tamiz et al., 1998) of targets other than the glutamate receptors show structural similarity to ifenprodil and also act as (sometimes potent) noncompetitive GluN2B-selective NMDA receptor antagonists. In addition, a multitude of new molecular scaffolds that show high potency and increased subunit selectivity have emerged from systematic medicinal chemistry programs (Table 1). Several GluN2B-selective compounds have progressed into clinical trials for a wide range of conditions (Mony et al., 2009a; Traynelis et al., 2010). Among the clinical trials performed, studies evaluating the use of traxoprodil mesylate (CP-101,606) for traumatic brain injury (Yurkewicz et al., 2005), neuropathic pain, and treatment resistant depression (Preskorn et al.,

2008) have provided intriguing and in some cases promising results.

Molecular studies of ifenprodil and several prototypical analogs suggest that these compounds interact with a binding site on the GluN2B ATD (Gallagher et al., 1996; Perin-Dureau et al., 2002; Wong et al., 2005; Han et al., 2008; Ng et al., 2008). Scanning mutagenesis experiments further support the idea that ifenprodil and related analogs bind between the two lobes of the clamshell-like structure and provide a detailed map of residues at which mutations can perturb the inhibitory actions of ifenprodil (Perin-Dureau et al., 2002). Molecular modeling and ligand docking studies are consistent with this possibility and suggest that a hydrophobic pocket exists that could accommodate at least part of the typically di-aryl GluN2B-selective ligands (Marinelli et al., 2007; Mony et al., 2009b). This pocket resides deep within the bilobed cleft in the crystal structure of the GluN2B ligand-binding domain (Karakas et al., 2009), although the cleft appears to be closed and occupied by  $Zn^{2+}$  together with  $Na^+$  and  $Cl^-$ . It is noteworthy that GluN1 ATD mutations and deletion can perturb ifenprodil inhibition (Masuko et al., 1999; Madry et al., 2007), supporting the idea that the GluN2B and GluN1 ATDs interact closely, as predicted by structural studies of the related homomeric GluA2 receptor (Sobolevsky et al., 2009).

Extensive structure-activity relationship data exist on a wide range of noncompetitive antagonists that are thought to bind to the GluN2B ATD (Table 1). The broad SAR governing these compound classes has typically involved two aromatic rings separated by a carbon linker that often contains nitrogen (Chenard and Menniti, 1999; Tamiz et al., 1999; Nikam and Meltzer, 2002; Borza and Domány, 2006; Layton et al., 2006). A variety of chemically distinct linkers and substituted aryl compounds retain activity, leading to considerable diversity in ligand structure. However, with few exceptions, there remains a lack of comprehensive glutamate receptor subunit-selectivity data for most ligands. Although the structural diversity of active ligands suggests that the binding site can accommodate different molecular scaffolds in different binding poses, few data exist with which to evaluate the idea that the compounds may share some overlapping, but not all, atomic contacts within GluN2B (Malherbe et al., 2003; Marinelli et al., 2007; Mony et al., 2009b).

Many of the early GluN2B-selective noncompetitive antagonists share a set of off-target actions that include binding to  $\alpha 1$  adrenergic receptors (ifenprodil is a vasodilator) (Carron et al., 1971), human *ether-a-go-go*-related gene channels, sigma receptors,  $Ca^{2+}$  channels, and other targets (e.g., Keiser et al., 2009). This off-target activity probably hindered early efforts to move GluN2B antagonists forward in the clinic. However, more recent medicinal chemistry efforts have led to new classes of molecules that retain potent and selective inhibition at GluN2B but circumvent some of these off target limitations, including  $\alpha 1$  adrenergic receptors and human *ether-a-go-go*-related gene channels (Liverton et al., 2007; e.g., Kawai et al., 2007; Mosley et al., 2009). It is possible that these newer compounds will provide a clearer path to clinical evaluation of efficacy in several indications.

GluN2B-selective noncompetitive antagonists inhibit NMDA receptor function by a complex mechanism. Ifenprodil binding to the GluN2B ATD is voltage-independent and associated with an enhancement in glutamate/NMDA binding, as



TABLE 1

IC<sub>50</sub> values for noncompetitive NMDA receptor antagonists acting at the GluN2B ATDIC<sub>50</sub> values are micromolar and are from rat recombinant receptors, except for ifenprodil, which has IC<sub>50</sub> values from recombinant human receptors. Chemical structures of the compounds are shown in Fig. 5.

|   | Method   | GluN1/GluN2A     | GluN1/GluN2B      | GluN1/GluN2C     | GluN1/GluN2D     |
|---|----------|------------------|-------------------|------------------|------------------|
|   |          | $\mu\text{M}$    |                   |                  |                  |
| Known compounds   |          |                  |                   |                  |                  |
| Ifenprodil <sup>a</sup>   | TEVC     | 40               | 0.11 <sup>a</sup> | 29               | 76               |
| Nylidrin <sup>b</sup>   | TEVC     | 32               | 0.18              | 42               |                  |
| AM-92016 <sup>c</sup>   | TEVC     | >30 <sup>d</sup> | 0.15 <sup>d</sup> | >30 <sup>d</sup> | >30 <sup>d</sup> |
| Eliprodil <sup>e, f</sup>   | TEVC     | >100             | 1.0               | >100             |                  |
| Clobenpropit <sup>g</sup>   | TEVC     | 14               | 1.0               | 7.3              | 11               |
| Trifluoperidol <sup>h</sup>   | TEVC     | 69               | 1.2               | 390              |                  |
| Haloperidol <sup>h</sup>  | TEVC     | >300             | 3.1               | >300             | >300             |
| Capsazepine <sup>g</sup>  | TEVC     | 66               | 8.1               | 44               | 42               |
| Novel antagonists   |          |                  |                   |                  |                  |
| Ro 25-6981 <sup>i</sup>   | TEVC     | 52               | 0.0090            |                  |                  |
| Ro 63-1908 <sup>j</sup>   | TEVC     | >100             | 0.003             |                  | >10              |
| Traxoprodil (CP 101,606) <sup>k</sup>   | TEVC     | >100             | 0.039             | >100             | >100             |
| MK-0657 <sup>l</sup>  | N.A.     | 30               | 0.0036            |                  |                  |
| Merck 20j <sup>m</sup>  | CF, WCPC | >30              | 0.0056            | >30              | >30              |
| Radiprodil (RGH-896) <sup>n</sup>   | TEVC, RB | >10              | 0.003–0.01        |                  |                  |
| Besonprodil (CI-1041) <sup>o</sup>  | CI       | >50              | 0.008             |                  |                  |
| <i>N</i> -(2-(3,4-Dichlorophenyl amino)ethyl)-2-(4-(methyl sulfonamido)phenoxy) acetamide (compound 52) <sup>p</sup>                                      | TEVC     | 82               | 0.057             | 58               | 107              |
| <i>N</i> -[2-(4-Hydroxyphenyl ethyl)-5-phenylpentyl amine (compound 20) <sup>q</sup>  | TEVC     | 12               | 0.008             | 39               |                  |
| 2-(4-(4-Fluorobenzyl)pi-peridin-1-yl)-2-oxo- <i>N</i> -(2-oxo-2,3-dihydro-1 <i>H</i> -benzo[ <i>d</i> ]imidazol-5-yl)acetamide (compound 3a) <sup>r</sup> | CI       | >15              | 0.002             |                  |                  |
| 2-(4-Benzylpiperidin-1-yl)-2-oxo- <i>N</i> -(2-oxo-2,3-dihydrobenzo[ <i>d</i> ]oxazol-6-yl)acetamide (compound 3d) <sup>r</sup>                           | CI       | >15              | 0.003             |                  |                  |
| 2-[[4-(2-Fluorobenzyl)pi-piperidin-1-yl]methyl] benzimidazol-5-ol (compound 37a) <sup>s</sup>   | CI, WCPC | >10              | 0.097             | >10              | >10              |
| <i>N</i> -(4-Phenylbutyl)-4-hydroxycinnamide (compound 16) <sup>t</sup>   | TEVC     | >100             | 0.077             | >100             |                  |
| 5-[3-(4-Benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-one (compound 46b) <sup>u</sup>  | TEVC     | 35               | 0.005             | >100             |                  |
| ( <i>S</i> )-1-(4-Methanesulfonamidephenoxy)-3-(3,4-dichlorophenylethylamino)-2-propanol (compound 29) <sup>v</sup>                                       | TEVC     | >10              | 0.050             | >10              | >10              |

AM-92016, 1-(4-methanesulfonamidophenoxy)-3-(*N*-methyl-3,4-dichlorophenylethylamino)-2-propanol hydrochloride; CF, Ca<sup>2+</sup> flux assay; CI, fluorescent calcium imaging of fluo-4/AM loaded Ecr293 cells or fluo-3 loaded L(tk<sup>-</sup>) cells; MK-0657, (3*S*,4*R*)-4-methylbenzyl 3-fluoro-4-((pyrimidin-2-ylamino)methyl)piperidine-1-carboxylate; MK-801, 5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (dizocilpine maleate); N.A., methods not available; RB, radiolabeled binding studies using [<sup>3</sup>H]MK-801 or [<sup>3</sup>H]Ro-25-6981; Ro 25-6981, ( $\alpha$ *R*, $\beta$ *S*)- $\alpha$ -(4-hydroxyphenyl)- $\beta$ -methyl-4-(phenylmethyl)-1-piperidinepropanol maleate; Ro 63-1908, 1-[2-(4-hydroxyphenoxy)ethyl]-4-[(4-methylphenyl)methyl]-4-piperidinol hydrochloride; TEVC, two-electrode voltage-clamp recordings from *Xenopus laevis* oocytes; WCPC, whole-cell patch-clamp recordings.

<sup>a</sup> Hess et al., 1998. IC<sub>50</sub> values vary with stereoisomer; see Chenard et al., 1991.

<sup>b</sup> Whittemore et al., 1997a.

<sup>c</sup> Tahirovic et al., 2008.

<sup>d</sup> S. F. Traynelis, unpublished data; *n* = 3 to 4 oocytes for each receptor.

<sup>e</sup> Avenet et al., 1997.

<sup>f</sup> Whittemore et al., 1997b.

<sup>g</sup> Hansen et al., 2010.

<sup>h</sup> Ilyin et al., 1996.

<sup>i</sup> Fischer et al., 1997.

<sup>j</sup> Gill et al., 2002.

<sup>k</sup> Mott et al., 1998.

<sup>l</sup> Addy et al., 2009.

<sup>m</sup> Liverton et al., 2007.

<sup>n</sup> Mony et al., 2009a.

<sup>o</sup> Nagy et al., 2003.

<sup>p</sup> Mosley et al., 2009.

<sup>q</sup> Tamiz et al., 1998.

<sup>r</sup> Barta-Szalai et al., 2004.

<sup>s</sup> McCauley et al., 2004.

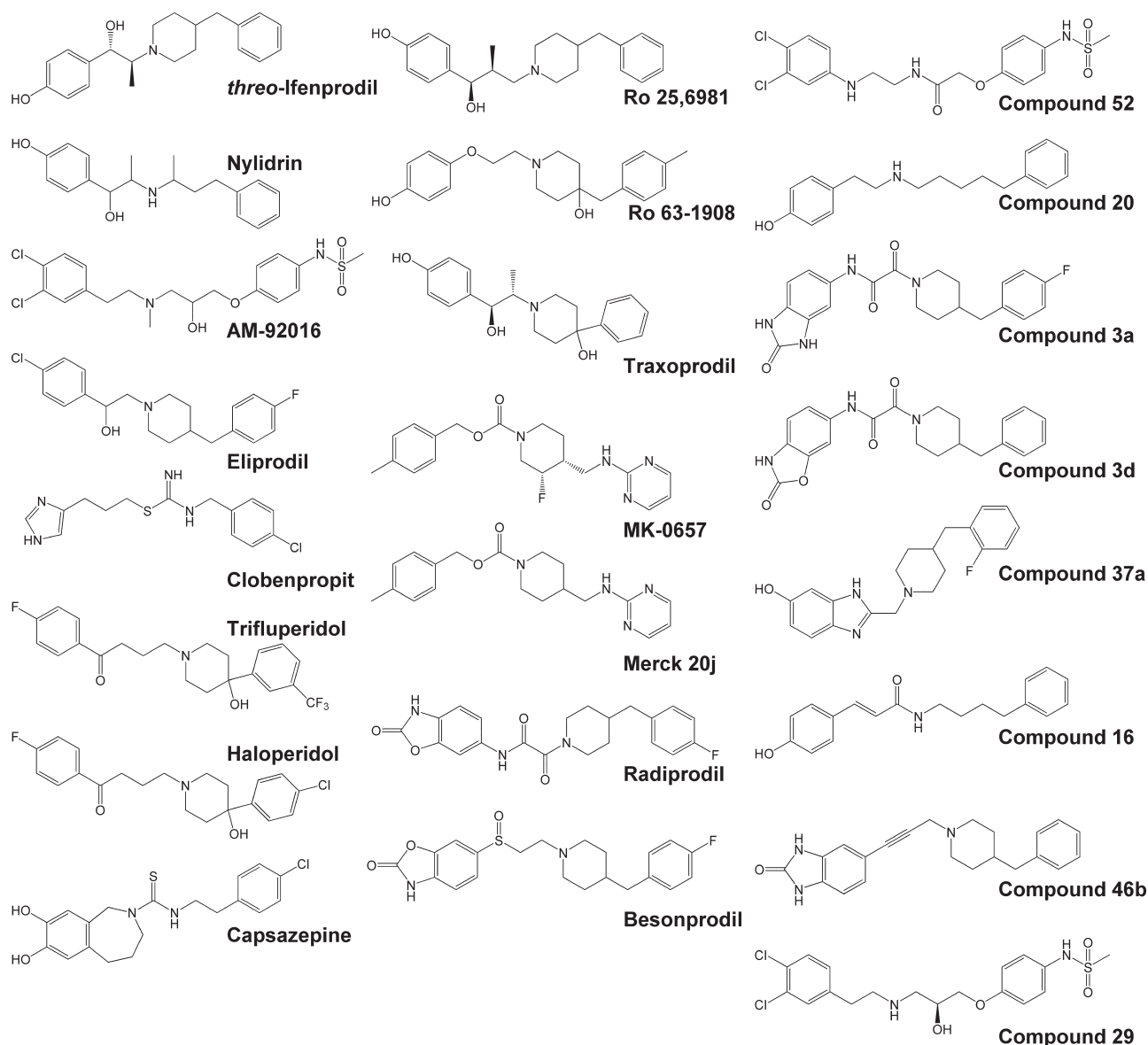
<sup>t</sup> Tamiz et al., 1999.

<sup>u</sup> Wright et al., 2000.

determined by a change in the functionally determined  $EC_{50}$  values and the glutamate deactivation time course (Kew et al., 1996; Zhang et al., 2000). This observation suggests that a positive allosteric interaction occurs between the ATD and the ligand binding domain within the GluN2B subunit, which produces a paradoxical enhancement of the NMDA receptor response by GluN2B ligands at low agonist concentrations (Kew et al., 1996). Ifenprodil potency was reduced at low concentrations of the agonist NMDA compared with higher concentration of NMDA (Kew et al., 1996). This feature of GluN2B antagonist action is shared with  $Zn^{2+}$  binding to its high affinity site within the GluN2A ATD.  $Zn^{2+}$  binding to the GluN2A ATD enhances the affinity for glutamate (but not glycine) binding, whereas glutamate binding enhances the affinity for  $Zn^{2+}$  binding (Zheng et al., 2001; Erreger and Traynelis, 2005). This positive allosteric interaction produces a fade in the response after a concentration jump to glutamate for GluN2A responses recorded in the

presence of subsaturating concentrations of extracellular  $Zn^{2+}$ . The basis for this is that glutamate binding rapidly increases  $Zn^{2+}$  affinity, allowing  $Zn^{2+}$  to relax to a new, higher level of binding, thereby producing a time-dependent fade in the current as more  $Zn^{2+}$  binds the receptor. A similar fade in the current response can be seen after a glutamate concentration jump in the presence of ifenprodil (e.g., Zheng et al., 2001), arguing for a shared mechanism of action of ligands that bind to the NMDA receptor ATD.

Ifenprodil binding to native NMDA receptors in rat hippocampal neurons reduces both channel opening frequency and open duration without an effect on channel conductance (Legendre and Westbrook, 1991). Inhibition is incomplete at saturating concentrations of ifenprodil, suggesting that ifenprodil binding reduces the maximal achievable response of the receptor to agonist binding (Legendre and Westbrook, 1991). The combination of GluN2B selectivity, a residual response in the presence of saturating concentration of



**Fig. 5.** Structures for noncompetitive NMDA receptor antagonists acting at the GluN2B ATD. See Table 1 for  $IC_{50}$  values for inhibition of NMDA receptor subtypes by these ligands. The *threo*-ifenprodil stereoisomers, one of which is shown, appear more potent than *erythro*-ifenprodil isomers (Chenard et al., 1991).

GluN2B-selective antagonists, and the allosteric interaction between glutamate and ifenprodil binding that produces an activity-dependence to ifenprodil's action may explain in part why GluN2B-selective antagonists in general are better tolerated *in vivo* than nonselective NMDA receptor antagonists (Kew et al., 1996; Dingledine et al., 1999).

Kinetic analysis of ifenprodil binding to NMDA receptors in rat cortical neurons suggests that ifenprodil binding shifts the receptor into a less active state (Kew et al., 1996, 1998). Ifenprodil and related analogs also seem to show an increased affinity for both the active and the desensitized state, which has been interpreted as evidence for a mechanism involving enhanced desensitization (Kew et al., 1996, 1998). This idea is in part consistent with a subsequently proposed interaction between the proton sensor and ifenprodil and related analogs (Pahk and Williams, 1997; Whittemore et al., 1997a; Mott et al., 1998). Ifenprodil binding enhances proton sensitivity, leading to the suggestion that ifenprodil inhibition reflects enhanced tonic proton inhibition that occurs at physiological pH. Protonation of the GluN2B receptors has recently been shown to promote entry of channels into an inactive state of the receptor (Banke et al., 2005) that is conceptually similar to the ifenprodil-bound state proposed by Kew et al. (1996). That is, the less active ifenprodil-bound state may reflect an ifenprodil-bound set of protonated and unprotonated states, with the protonated receptors being inactive. It is noteworthy that there is a differential pH sensitivity to the potency of different GluN2B-selective antagonists (Mott et al., 1998). If ligands could be developed that maximize potency at the acidic pH values observed in ischemic tissue, such compounds may have enhanced safety profile because they would act as context-dependent antagonists that block NMDA receptors in ischemic but not healthy brain tissue.

Biochemical and functional single-channel data suggest that some native receptors include two different GluN2 subunits (Traynelis et al., 2010). For example, coimmunoprecipitation studies suggest that GluN1/GluN2A/GluN2B triheteromeric receptors exist *in vivo* (Chazot and Stephenson, 1997; Luo et al., 1997), yet it remains unclear how the presence of two different GluN2 subunits influences receptor properties or pharmacology. The existence of receptors that contain GluN2A and GluN2B raises a question as to whether ifenprodil can inhibit receptors that contain only a single copy of GluN2B. To evaluate this question, Chazot et al. (2002) immunopurified NMDA receptors containing GluN1, GluN2A, GluN2B subunits, and evaluated the binding of radiolabeled GluN2B-selective antagonists. Their data suggested that [<sup>3</sup>H]Ro-25,6981 can bind with high affinity to triheteromeric receptors containing a copy of both the GluN2A and GluN2B, whereas no specific binding of [<sup>3</sup>H]CP-101,606 was observed. Functional data support this result, in that single-channel openings of GluN1/GluN2A/GluN2B receptors seem insensitive to CP-101,606 (Brimecombe et al., 1997). These data suggest that two classes of GluN2B-selective ligands exist, only one of which is capable of inhibiting receptors with a single copy of the GluN2B subunit. To further address this question, Hatton and Paoletti (2005) devised a strategy in which multiple mutations in the GluN2A subunit were used to reduce the contribution of NMDA receptors containing only GluN2A or GluN2B subunits when recording in the presence of extracellular Mg<sup>2+</sup>. Their data

suggest that under these conditions both ifenprodil and Zn<sup>2+</sup> can inhibit recombinant receptors containing a single active ATD for their respective binding sites. However, this interpretation relies on several assumptions inherent in their experimental design, such as the assumption that the contribution of receptors with two copies of the mutated GluN2A to residual currents recorded in Mg<sup>2+</sup> is negligible.

### Binding of the Amino-Terminal Domain to Extracellular Proteins

The ATD is the region of the receptor that is most distal from the membrane; as such, it is strategically placed as a locus for interactions with extracellular proteins and trans-synaptic contacts. It is noteworthy that the ATD seems to contain binding sites for extracellular proteins, such as *N*-cadherin (Saglietti et al., 2007) and neuronal pentraxins (NARP and NP1) for AMPA receptors (O'Brien et al., 1999; Xu et al., 2003; Sia et al., 2007), the ephrin receptor for NMDA receptors (Dalva et al., 2000; Takasu et al., 2002), and Cbln1 for the GluD2 receptor (Matsuda et al., 2010; Uemura et al., 2010). NARP and NP1 are secreted neuronal calcium-dependent lectins (i.e., pentraxins) that can form tight complexes via disulfide bonds and coiled-coil domains. NARP and NP1 have been shown to cluster AMPA receptors at the cell surface, increasing the number of dendritic AMPA receptor clusters, which could contribute to excitatory synaptogenesis (O'Brien et al., 1999; Xu et al., 2003; Sia et al., 2007). NARP can induce clustering of GluA1, GluA2, and GluA3 (but not GluA4) homomeric AMPA receptors in transfected human embryonic kidney cells (O'Brien et al., 1999). By contrast, NP1 can produce clustering of GluA4 homomeric receptors (Sia et al., 2007). Experiments using deletion mutants of the AMPA receptor subunits show that the ATD is responsible for the interaction with NARP and NP1 and that the ATD is also required for the regulation of excitatory synaptogenesis (Passafaro et al., 2003; Xu et al., 2003; Sia et al., 2007).

EphrinB binding to the EphB receptor tyrosine kinase enables an interaction between EphB and NMDA receptors (Dalva et al., 2000; Takasu et al., 2002). This interaction is mediated by the extracellular domains of EphB and GluN1, and the ATD (rather than the ligand binding domain) is presumably the primary interacting domain of GluN1 (Dalva et al., 2000). Kinase activity of EphB is not required for the interaction to occur, but EphrinB activation of EphB was shown to potentiate NMDA receptors in primary cortical neurons via NMDA receptor phosphorylation by the *Src* family of tyrosine kinases (Takasu et al., 2002). It is possible that ephrinB-dependent stimulation of EphB can modulate NMDA receptor function *in vivo* and thereby regulate synaptic function.

Interactions between the ATD and extracellular proteins have also been proposed to be trans-synaptic and to be involved in synapse development and maturation. The GluA2 ATD promotes dendritic spine formation and growth by directly interacting with the cell adhesion molecule *N*-cadherin, both in a *cis*-synaptic (i.e., expressed in the same neuron) and a *trans*-synaptic manner (Saglietti et al., 2007). This interaction decreases lateral diffusion of the AMPA receptors on the neuronal cell surface.

It was recently found that Cbln1, a protein secreted from cerebellar granule cells, can interact with the ATD of glutamate receptor-like GluD2 ( $\delta 2$ ) subunits and that this interaction is

necessary to induce new synapses in the adult cerebellum (Matsuda et al., 2010; Uemura et al., 2010). It is noteworthy that GluD2 can induce presynaptic terminal differentiation even without the ligand-binding domain (Kuroyanagi et al., 2009). The ligand-binding domain of GluD2 binds D-serine in a similar (but not identical) fashion to GluN1 (Naur et al., 2007). However, transgenic experiments have shown that insertion of a nonfunctional mutant GluD2 into GluD2(−/−) mice can rescue these mice from neurological deficits (Kakegawa et al., 2007a,b), raising the idea that GluD2 mediates important actions other than catalyzing a transmembrane ion flux. Indeed, no data have shown functional ionic currents in wild-type GluD2 receptors. It is interesting that the physiological role of the glutamate receptor-like GluD2 might be entirely nonionotropic, relying instead on specific interactions of the ATD with extracellular proteins to regulate synapse formation and function. Because the glutamate receptor ATDs can influence the arrangement of the ligand-binding domains and thereby receptor function, it is conceivable that agonist binding to the ligand binding domain can modulate the structural arrangement of the ATDs (e.g., see Nakagawa et al., 2005) and that these ligand-induced ATD rearrangements might change how the glutamate receptor interacts with extracellular proteins. D-Serine and calcium binding to the GluD2 ligand binding domain have opposing effects on the stability of the ligand binding domain dimer interface (Hansen et al., 2009), and thus these ligands could potentially affect the ATD arrangements of GluD2.

### Future Directions

Accumulating evidence supports a significant role of the ATD in regulating a diverse range of ionotropic glutamate receptor functions. Moreover, it is clear that the ATD participates in trans-synaptic protein-protein interactions as well as receptor assembly—two processes unrelated to conventional roles of ion channels, broadening the potential importance of the glutamate receptor class. Furthermore, the binding sites for allosteric modulators render the NMDA receptor ATD a therapeutic target for modulation of glutamatergic synaptic transmission. Indeed, multiple classes of ligands that selectively bind to the GluN2B NMDA receptor subunit ATD to modulate receptor function have been identified. Several of these compounds are currently undergoing clinical trials. In addition, the emerging structural information on glutamate receptors, particularly the ATD, has raised intriguing hypotheses regarding how modulators interact with the ATD as well as the mechanism of action of inhibitors that bind to the ATD. In particular, the role in modulator action of both the ATD dimer interface and the ligand binding domain dimer interface is ripe for further investigation. Likewise, the fascinating link between the inhibition by GluN2B-selective NMDA receptor antagonists and the proton sensor of the NMDA receptor could lead to novel therapeutic strategies, in addition to providing new insight into receptor gating. In summary, the growing understanding of ATD structure and function suggests that new information on the ATD could trigger exciting advances in multiple neurological processes and diseases.

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