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Citation for published version:

Digital Object Identifier (DOI):
10.1523/JNEUROSCI.1613-05.2005

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Journal of Neuroscience

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Mechanism of Partial Agonism at NMDA Receptors for a Conformationally Restricted Glutamate Analog

Kevin Erreger, Matthew T. Geballe, Shashank M. Dravid, James P. Snyder, David J. A. Wyllie, and Stephen F. Traynelis

The NMDA ionotropic glutamate receptor is ubiquitous in mammalian central neurons. Because partial agonists bind to the same site as glutamate but induce less channel activation, these compounds provide an opportunity to probe the mechanism of activation of NMDA-type glutamate receptors. Molecular dynamics simulations and site-directed mutagenesis demonstrate that the partial agonist homoquinolinate interacts differently with binding pocket residues than glutamate. Homoquinolinate and glutamate induce distinct changes in the binding pocket, and the binding pocket exhibits significantly more motion with homoquinolinate bound than with glutamate. Patch-clamp recording demonstrates that single-channel activity induced by glutamate or by homoquinolinate has identical single-channel current amplitude and mean open-channel duration but that homoquinolinate slows activation of channel opening relative to glutamate. We hypothesize that agonist-induced conformational changes in the binding pocket control the efficacy of a subunit-specific activation step that precedes the concerted global change in the receptor–channel complex associated with ion channel opening.

Key words: ion channel; glutamate; NMDA; molecular dynamics; structure; gating
Materials and Methods

Electrophysiological recording from human embryonic kidney 293 cells and Xenopus oocytes. Human embryonic kidney 293 (HEK293) cells were maintained and transiently transfected by the calcium phosphate method with cDNA encoding NR1-1a (GenBank accession numbers U11418 and U08261; pCIneo vector; hereafter NR1), NR2A (D13211; pCIneo), and green fluorescent protein at a ratio of 1:2:1 (0.2 μg/ml NR1) for 4–12 h, as described previously (Zheng et al., 1998). Currents from outside-out patches were digitally recorded with pClamp8 software using an Axopatch 200B amplifier (Molecular Devices, Union City, CA). Single-channel records were filtered at 5 kHz using an eight-pole Bessel filter (-3 dB); Frequency Devices, Haverhill, MA) and digitized at 40 kHz. Macroscopic currents were filtered at 2–3 kHz and digitized at 20 kHz. The extracellular solution consisted of (in mM) 150 NaCl, 10 HEPES, 0.5 CaCl₂, 3 KCl, and 0.01 EDTA supplemented with 50 μM glycine and 1 μM glutamate, unless noted otherwise (pH 7.3, 23°C). For most experiments, the agonist-containing extracellular solution was made from ultra-pure salts with an Mg²⁺ concentration of <0.2 μM measured using inductively coupled plasma-mass spectrometry by the Laboratory for Environmental Analysis at the University of Georgia (Athens, GA). Single-channel data recorded with normal salts was included in the final analysis because the measured Mg²⁺ concentration was <1 μM, and the mean open time was not significantly different in paired comparisons of normal and ultrapure salts in the same patches expressing NR1/NR2A (p > 0.05; n = 6). The internal solution consisted of the following (in mM): 110 Cs gluconate, 30 CsCl, 5 HEPES, 4 NaCl, 0.5 CaCl₂, 3 KCl, and 0.01 EDTA supplemented with 50 μM glycine and 1 μM glutamate, unless noted otherwise (pH 7.3, 23°C). For most experiments, the agonist-containing extracellular solution was made from ultra-pure salts with an Mg²⁺ concentration of <0.2 μM measured using inductively coupled plasma-mass spectrometry by the Laboratory for Environmental Analysis at the University of Georgia (Athens, GA). Single-channel data recorded with normal salts was included in the final analysis because the measured Mg²⁺ concentration was <1 μM, and the mean open time was not significantly different in paired comparisons of normal and ultrapure salts in the same patches expressing NR1/NR2A (p > 0.05; n = 6).

The internal solution consisted of the following (in mM): 110 Cs gluconate, 30 CsCl, 5 HEPES, 4 NaCl, 0.5 CaCl₂, 3 KCl. The extracellular solution contained (in mM) 90 NaCl, 10 HEPES, 0.01 EDTA, and 5 β-APTA, 2 Na-ATP, and 0.3 Na-GTP, pH adjusted to 7.35 with CsOH.

Results

Homoloquinolinate is a partial agonist for NR1/NR2A

We used two-electrode voltage-clamp recording of steady-state current responses from recombinant NR1/NR2A receptors expressed in Xenopus oocytes to quantify the potency and efficacy of homoloquinolinate relative to the maximal current evoked by a saturating concentration of glutamate. Our results indicate that homoloquinolinate is a candidate partial agonist when studied in oocytes, able to evoke ~70% of the maximal response produced by glutamate (n = 19) (Fig. 1A, Table 1). This summation response by 1 μM homoloquinolinate could be converted to a maximal response by coapplication of homoloquinolinate with increasing concentrations of glutamate (n = 5) (Fig. 1A), confirming that homoloquinolinate is a partial agonist. We subsequently evaluated the efficacy of rapidly applied homoloquinolinate relative to glutamate for activation of NR1/NR2A receptors in outside-out patches isolated from HEK293 cells (Fig. 1B). Comparison of the peaks responses for a maximally effective concentration of homoloquinolinate to the average of control and recovery responses to a maximally effective concentration of glutamate showed that homoloquinolinate evoked 63 ± 4% (n = 4) of the glutamate-evoked NR1/NR2A current response in mammalian cells. The relative efficacy of homoloquinolinate for peak currents in outside-out patches from HEK293 cells was similar to that for steady-state currents in oocytes. We have shown previously that NR1/NR2A receptors in outside-out patches have a peak open probability of...
The rise times are significantly slower for homoquinolinate-activated currents compared with glutamate (4.6 μM) was lower than for homoquinolinate (24.4 μM) (Table 1). From this measurement and the relative efficacy of homoquinolinate to glutamate, we estimate that the maximum open probability for homoquinolinate activation of NR1/NR2A receptors is 0.31. The EC$_{50}$ value for glutamate (4.6 μM) was lower than for homoquinolinate (24.4 μM) (Table 1).

**Activation rate of NR1/NR2A by homoquinolinate is slower than for glutamate**

To better understand the mechanism by which NR2A-containing receptors become activated by agonist, we performed recordings under voltage clamp of macroscopic NR1/NR2A current responses in excised outside-out patches. Current responses to prolonged application of both glutamate and homoquinolinate decayed with a mean time course that could be described by one or two exponential components, depending on the patch. However, it should be noted that NMDA channels in excised patches exhibit substantially greater desensitization (independent of glycine, calcium, or zinc) than NMDA channels recorded in whole-cell or perforated-patch recording mode (Zheng et al., 2001; Erreger et al., 2005). When a single exponential time constant is fitted to the relaxation for all patches, the mean time constant for glutamate (187 ± 64 ms; n = 14 patches) was not significantly different than for homoquinolinate (193 ± 20 ms; n = 14). The time course was significantly better fitted in the majority of patches (seven of nine patches tested) by the sum of two exponential components (p < 0.001). The averaged composite time course decayed with time constants (and relative amplitudes) of 60 ms (38%) and 257 ms (62%) for glutamate, which were similar to the time constants of 82 ms (28%) and 229 ms (72%) describing homoquinolinate-induced desensitization. In addition, the steady state-to-peak current ratio was not significantly different between glutamate (0.15 ± 0.03; n = 14) and homoquinolinate (0.13 ± 0.02; n = 12). These data suggest that the rate and degree of desensitization induced by the two agonists is similar. We also measured the time course for deactivation after a brief (5–15 ms) pulse of maximal concentration of agonist. We found that the time constant describing deactivation after brief glutamate application (36.1 ± 2.6 ms; n = 8) was significantly slower than that describing deactivation from homoquinolinate (21.7 ± 4.0 ms; n = 4; p = 0.011; unpaired t test). In addition, the 10–90% rise time of macroscopic currents (Fig. 1C) in response to rapid application of a saturating (1 mM, >40-fold EC$_{50}$ value) concentration of homoquinolinate (12.2 ± 0.8 ms; n = 14) was significantly slower than for saturating glutamate (6.8 ± 0.8 ms; n = 16; p = 0.0003; unpaired t test). Identical results were obtained in a subset of patches in which rise times were measured for both glutamate- and homoquinolinate-evoked currents in the same patch (homoquinolinate, 12.3 ± 1.4 ms; glutamate, 8.0 ± 0.7 ms; p = 0.016; paired t test; n = 8). These data are consistent with the hypothesis that the main determinant of partial agonism is not a difference in desensitization but rather a specific effect on conformational changes that precede or control channel opening.

**Modeling of glutamate and homoquinolinate interaction with the agonist binding pocket**

To better understand the nature of the reduced potency and reduced efficacy of homoquinolinate, we used MD applied to a homology model of the S1S2 domain of NR2A docked to either glutamate or homoquinolinate (see Materials and Methods). Figure 2A illustrates the alignment used to generate a homology model of the glutamate binding domain of NR2A (Chen et al., 2005) based on a crystal structure of the NR1 glycine binding domain (Furukawa and Gouaux, 2003). The α-helices and β-sheets were labeled according to the NR1 structure (Furukawa and Gouaux, 2003). Figure 2B–D superimposes the model of the NR2A S1S2 binding domain with either glutamate or homoquinolinate docked using MD. Figure 3A shows the glutamate α-carboxyl anchored by Arg499 plus a backbone nitrogen from Ser670 (Chen et al., 2005). Arg499 also makes an important hydrogen bond with the backbone carbonyl of Thr494 (data not shown). The α-amino group of glutamate is hydrogen bonded by the hydroxyl of Tyr742 (Fig. 3B) and has the potential for favorable interactions with the hydroxyls of Ser492 and Thr494 and the carboxylate of Asp712 (data not shown). The γ-carboxyl interacts with the hydroxyl groups from Ser670 and Thr671, with additional support from the Thr671 backbone nitrogen (Fig. 3C). Homoquinolinate is a conformationally restricted glutamate analog that contains two carboxyl groups and a secondary amine embedded in an aromatic ring with side-chain bond angles of 120° compared with 109° for glutamate. Figure 2, B and C, illustrates the superposition of the docked agonists, which show similar positioning for the three functional groups. However, several differences between simulated homoquinolinate and glutamate binding are apparent. For example, the aromatic ring of homoquinolinate takes up more space than glutamate, with resulting displacement of residues in the pocket. This can be visualized by the apparent displacement of Tyr742 by homoquinolinate (Fig. 3B). In addition, Ser670 changes its primary hydrogen-bonding
Table 1. Potency for glutamate and homoquinolinate at wild-type and mutant receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Glu EC₅₀ (µM)</th>
<th>Glu Hill slope</th>
<th>n</th>
<th>HQ EC₅₀ (µM)</th>
<th>HQ Hill slope</th>
<th>n</th>
<th>Iₘ₅₀ HQ/Glu</th>
<th>Glu EC₅₀ mut/wt</th>
<th>HQ EC₅₀ mut/wt</th>
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<tbody>
<tr>
<td>NR1/NR2A</td>
<td>4.6</td>
<td>1.4</td>
<td>11</td>
<td>24.4</td>
<td>1.2</td>
<td>19</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR1/NR2A-S670G</td>
<td>421</td>
<td>1.4</td>
<td>12</td>
<td>151</td>
<td>1.1</td>
<td>9</td>
<td>0.51</td>
<td>92</td>
<td>6</td>
</tr>
<tr>
<td>NR1/NR2A-T671A</td>
<td>2967</td>
<td>1.3</td>
<td>5</td>
<td>384</td>
<td>0.7</td>
<td>9</td>
<td>0.10</td>
<td>645</td>
<td>16</td>
</tr>
<tr>
<td>NR1/NR2A-S492A</td>
<td>146</td>
<td>1.2</td>
<td>6</td>
<td>202</td>
<td>1.2</td>
<td>6</td>
<td>0.67</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>

Parameters measured from steady-state recording of indicated subunits expressed in Xenopus oocytes are shown. n is the number of cells. Iₘ₅₀ HQ/Glu is the ratio of maximal current induced by homoquinolinate relative to glutamate at a saturating agonist concentration in the same cell. Glu, Glutamate; HQ, homoquinolinate; wt, wild type; mut, mutant.

differential effects on glutamate and homoquinolinate EC₅₀. As predicted by MD analysis, the receptors comprising NR2A(S492A) showed a 32-fold increase in glutamate EC₅₀ but only an 8-fold increase in homoquinolinate EC₅₀. These functional data are thus consistent with the results of MD modeling.

The bulkier size of homoquinolinate and its displacement of residues such as Tyr742 suggest that homoquinolinate may not be held as tightly in the pocket as glutamate. If this is the case, we predict that the EC₅₀ value determined from functional data and the molecular motion of side chains comprising the pocket will both increase. To evaluate this hypothesis, we analyzed the root mean square (rms) deviations of the atomic positions of 22 residues comprising the binding pocket over the final 150 ps of MD. With the exception of Tyr742, which fluctuates in and out of a hydrogen bond with the amino group of glutamate, these residues showed considerably more motion around docked homoquinolinate (mean ratio rmsₜₙₐₜ/ rmsₕₙₜₐₜ for each residue, 1.33) than around both glutamate-bound and apo structures during MD runs (Fig. 4) (apo data not shown). The greatest contribution to pocket motion came from the S2 residues 669–674 (rmsₜₙₐₜ/rmsₕₙₜₐₜ, 1.73) that secure the carboxylates of homoquinolinate and more distant residues that make up the top of helix F. This is the same region that shows wide variation in hydrogen binding between glutamate and homoquinolinate γ-carboxyl, which act as a bridge across the binding pocket (Figs. 3C, 5). Unlike glutamate, homoquinolinate binding in this region involves a water bridge to Asp712 that will increase intrapocket motion (Fig. 5C). Additional determinants of the increased rms values may also reflect increased side-chain motion that results from ongoing steric contacts between homoquinolinate and residues within the binding pocket. This increased motion suggests a decreased binding stability for homoquinolinate compared with glutamate. Increased motion for homoquinolinate also suggests an increased Kᵢ value, consistent with the experimentally determined increased EC₅₀ value for homoquinolinate compared with glutamate (Table 1).

Translation of agonist binding to channel opening

A number of interesting features of the S2 backbone helix F are apparent in our simulations. First, the displacement of helix F from helix G does not change considerably between the apo state and the glutamate-bound state (Fig. 5). In addition, ho-

Figure 2. MD simulation of glutamate or homoquinolinate docking to a homology model of the NR2A S1S2 agonist binding pocket. A, Alignment used for construction of the homology model for NR2A. B, Superposition of MD simulation of glutamate (orange) or homoquinolinate (gray) docked to S152. ATD, N-terminal domain; M1–M4, membrane domains 1–4. Superposition of glutamate and homoquinolinate shown without amino acid residue side chains emphasizes superposition of functional groups.

The partner from the γ-carboxyl of glutamate to the α-carboxyl in homoquinolinate (Fig. 3C). This change in hydrogen bonding of the γ-carboxyl identified by MD analysis, if correct, would be predicted to alter homoquinolinate sensitivity to mutation of S2 contact residues by comparison with glutamate. We directly tested this idea by generating concentration-response curves for contact residues by comparison with glutamate. We directly predicted to alter homoquinolinate sensitivity to mutation of S2, substituted residues within the binding pocket. This increased motion suggests a decreased binding stability for homoquinolinate compared with glutamate. Increased motion for homoquinolinate also suggests an increased Kᵢ value, consistent with the experimentally determined increased EC₅₀ value for homoquinolinate compared with glutamate (Table 1).
moquinolinate appears not only to displace helix F (Fig. 2D), compared with glutamate, but to open up more space within the binding pocket, suggesting that homoquinolinate can propagate longer range perturbations through the domain structure. Furthermore, contact between helix F and other elements of the secondary structure is lost with homoquinolinate in the pocket (Fig. 5B, C). These contacts are formed through three hydrogen bonds in the apo-NR2A that connect helix F to β-sheet 12 and a loop between β-sheets 5 and 6. The hydrogen bond between Ser670 and Asp712 is lost after glutamate or homoquinolinate binding. Homoquinolinate binding further disrupts the hydrogen bonds between Thr671 and the backbone oxygen of Ile710 as well as between the backbones of Asn668 and Gly467 (Fig. 5C). In the NR2A—glutamate complex, these contacts are essentially retained by preservation of the backbone hydrogen bond between Asn668 and Gly467 and a frequent hydrogen bond between the γ-carboxyl of glutamate and the backbone hydrogen of Asp712. The homoquinolinate system substitutes the latter interaction with a trio of water molecules (Fig. 5C). The direct contacts between helix F and the neighboring secondary structure are not found in the averaged structure with homoquinolinate. One intriguing, yet speculative, hypothesis is that this uncoupling of helix F to the rest of the protein may play a role in the reduced translation of binding energy to displacement of gating elements by homoquinolinate, which may in part contribute to its reduced ability to open the channel (see below).

Homoquinolinate alters NR1/NR2A single-channel properties

To examine the mechanism of activation by glutamate and homoquinolinate, single-channel recordings were made from outside-out patches. Channel activity was evoked by steady-state application of a maximally effective concentration of either glutamate (1 mM) or homoquinolinate (1 mM) (Fig. 6). The single-channel chord conductance and the mean channel open duration were the same for both agonists. These two measures suggest that the properties of the open-channel conformation are not directly dependent on the identity of the agonist. The primary difference among the functional properties of the single-channel activations caused by the two agonists was within the distribution of the shut times, which was described by at least four exponential components. There were no significant differences in the relative area of the fourth component of the shut-time distribution for either agonist (p > 0.05; paired t test; n = 7). The shortest shut-time component, which we estimate to be <0.1 ms, was too near the limit of our recording resolution to be measured precisely in some patches. The next fastest time constant (shut time τs) (Fig. 6, gray arrow) was not significantly different between agonists. Shut time τs (Fig. 6, black arrow) was significantly longer for homoquinolinate-induced activity than for glutamate-induced activity. A similar shift in the analogous shut-time component has been described previously with partial agonists for the glutamate site for NR1/NR2B and was hypothesized to reflect a slowing of a single conformational change within the NR2 subunit (Banke and Traynelis, 2003). Changes in the fourth shut-time component (τs) could not be interpreted, because this component likely contained time periods between closure of one channel and opening of another channel, given that the total number of channels contained within the patches we recorded from was unknown and was unlikely to be equal for all patches. The relative area of this fourth component of the shut-time distribution is substantial (glutamate, 10 ± 3%; homoquinolinate, 16 ± 3%; n = 7). Nonetheless, we suggest that this kinetic component reflects in part the time course of a desensitization-related process and not the fundamental mechanism of channel opening. We consider the fitting of shut-time distributions as a first-pass model-independent analysis of agonist-specific features of channel gating. We subsequently fitted the sequence of single-channel events with an explicit kinetic model to attempt to draw mechanistic conclusions from the data.

Homoquinolinate slows a specific pregating step

As indicated above, the only consistent feature of single-channel open and closed time histograms that changed when channel activity was evoked by homoquinolinate or glutamate was the

Figure 3. Comparison of glutamate and homoquinolinate binding. A, Glutamate and homoquinolinate docking are shown with α-carboxyl and γ-carboxyl hydrogen bond contacts. B, Displacement of Tyr742 illustrates the steric clash produced by the aromatic ring of homoquinolinate when docked into the glutamate binding pocket. C, Ser670 is reoriented to interact with the α-carboxyl of homoquinolinate compared with the γ-carboxyl of glutamate.

Figure 4. Increased atomic motion within the binding pocket for homoquinolinate. The SD (i.e., rms) of atomic position is shown in pseudocolor for residues that comprise the agonist binding pocket, with cold colors (blue) representing low rms and high colors (red) representing high rms. Results are shown after MD runs for glutamate and homoquinolinate. The range of rms values for residues comprising the binding pocket were 0.0257–0.1045 nm for glutamate and 0.0316–0.0823 nm for homoquinolinate.
value of the third exponential component in the shut-time distribution. Such a change could reflect any number of features of the NMDA receptor activation process. To circumvent the indirect nature of inferences made from histogram fitting, we used maximum likelihood fitting of explicit models that encapsulated our working hypothesis of NMDA receptor activation to single-channel records (Banke and Traynelis, 2003; Erreger et al., 2005). A number of previous studies have convincingly argued that at least two pregating kinetically distinct conformation changes are required before NMDA receptors can open (Gibb and Colquhoun, 1991; Wyllie et al., 1998; Banke and Traynelis, 2003; Popescu and Auerbach, 2003; Popescu et al., 2004; Erreger et al., 2005). We therefore used hidden Markov modeling of our single-channel data to explore whether differences exist between glutamate-evoked or homoquinolinate-evoked channel transition rates for the conformational changes that precede NMDA channel opening. Data were analyzed by subdividing records on the basis of a critical time (30 ms) that was calculated to allow identification of individual activations (Erreger et al., 2005). That is, the critical closed times were used to separate open and closed times within an activation from closed times between two different activations (see Materials and Methods). The idealized sequence of channel events was then fitted with a model postulating two pregating conformational changes (a fast step for NR1, a slow step for NR2), followed by rapid pore dilation. The hypothesized assignment of individual subunits to the kinetic steps is based on previous work demonstrating the pharmacological sensitivities of these steps to agonists acting at the glycine or glutamate site in NR1/NR2B receptors, as well as the sensitivity of these steps to the identity of the NR2 subunit (Banke and Traynelis, 2003; Erreger et al., 2005). Two directly interconverting open states were included to better describe the distribution of open-channel dwell times (Popescu et al., 2004).

We directly fit this explicit kinetic model to the specific sequence of channel openings and closings. Scheme 1 (Fig. 7) postulates that the NR1 and NR2 subunits activate independently and that both must be active before the channel pore opens. The rates “f” (fast) and “s” (slow) that are shown are for the hypothesized transition between an agonist-bound “inactive” state and an agonist-bound “active” state for the NR1 (f) and NR2 (s) subunits (Banke and Traynelis, 2003; Erreger et al., 2005). Because the recordings were performed in the continuous presence of a saturating concentration of both glutamate and glycine, no explicit binding steps are included and full occupancy of ligand binding sites is assumed. Desensitized states are also omitted because data were segmented with a critical shut time to remove desensitized shut durations. We fitted scheme 1 to the sequence of channel openings in each patch for sequential application of both glutamate and homoquinolinate and found that our working model fit data from each patch well, with limited variability between rate constants among patches. The average log likelihood (a measure of the quality of the fit of the model to the data) value was identical when the model was fitted to either single-
channel activations evoked by glutamate or homoquinolinate (log likelihood per event: glutamate, 4.97 ± 0.03; homoquinolinate, 4.94 ± 0.03). Table 2 compares the mean (±SEM) rate constants for activations by glutamate or homoquinolinate fitted to scheme 1. The fitted rate constants show that only the “slow” (hypothesized NR2-dependent) activation rate (Banke and Traynelis, 2003; Erreger et al., 2005) changes significantly for scheme 1, consistent with the hypothesis that the identity of the agonist bound to the NR2A subunit controls the rate of a slower conformational change required for gating.

Macroscopic NR1/NR2A current responses to glutamate or homoquinolinate

Partial agonists by definition produce reduced response compared with a full or prototypical agonist. If our analysis of single-channel records is correct to a first approximation, then we should be able to predict the relative degree of partial agonism from the rate constants derived from steady-state, single-channel records. To directly evaluate the ability of gating rate constants derived from fitted single-channel data to accurately describe peak open probability and the time course of macroscopic currents, we first recorded macroscopic currents in response to rapid application of glutamate or homoquinolinate to excised outside-out patches. Three sets of responses were generated for each agonist (Fig. 8B, C), including (1) saturating agonist concentration applied for 1 s, (2) saturating agonist concentration applied for 5–15 ms, and (3) subsaturating agonist concentration applied for 1 s. The response for each patch to prolonged application of the high (1 mM) agonist concentration response was normalized to the measured peak open probability of NR1/NR2A in response to glutamate in one channel patch (0.50) (Erreger et al., 2005), and responses averaged across patches for all three protocols.

Homoquinolinate-evoked responses in each patch were scaled to the relative open probability (0.31) calculated from the peak open probability to glutamate (0.50) and the relative efficacy of homoquinolinate (62%) determined in outside-out patches in response to maximal concentrations of glutamate and homoquinolinate (see above) (Fig. 1B), and averaged across patches. All three curves for each agonist were then fitted simultaneously with scheme 2 (Fig. 8A) with the gating rates fixed to those determined from single-channel analysis. Scheme 2 incorporated two explicit binding steps for glutamate or homoquinolinate as well as two desensitization steps given the dual exponential time course for desensitization; binding and desensitization parameters were free to vary in these fits. Scheme 2 produced excellent fits to the macroscopic peak open probability and time course, suggesting that our single-channel measurements accurately describe the independently recorded peak macroscopic response (Fig. 8C). Table 2 summarizes the results from fitting scheme 2 to macroscopic data for both glutamate and homoquinolinate and confirms that an independent subunit gating model with two pregating steps can accurately predict the magnitude and time course of macroscopic currents over a range of agonist concentrations and application durations, as well as generate the experimentally determined peak open probability. Furthermore, simulations show that the time constant describing deactivation is virtually identical (33.3–34 ms) for 1, 5, 10, 15, and 20 ms pulses of glutamate, confirming that the application duration we chose did not confound determination of rate constants. Fitted data in Table 2 also show a reduced association rate for homoquinolinate, an increased $K_{D}$, and an accelerated dissociation rate, consistent with predictions from MD simulations.

**Discussion**

There are three main findings of this study. First, homoquinolinate is a low-affinity partial agonist at NR1/NR2A receptors with predicted increased intrapocket motion relative to glutamate. Second, NR1/NR2A receptors activated by glutamate open faster and have a higher open probability than those activated by the
Mechanism of partial agonism at glutamate receptors

Although the concept of partial agonism was developed more than 50 years ago (Ariens, 1954; Stephenson, 1956; Del Castillo and Katz, 1957), only recently has an understanding of the structural and functional basis of partial agonism been elucidated for at least one receptor, the AMPA subtype of glutamate receptors. It has been shown for AMPA receptors that partial agonists induce only partial closure of the agonist-binding S1S2 domain purified in isolation from the receptor (Armstrong and Gouaux, 2000), suggesting that partial agonism in some ways reflects the ability of different structural agonists to induce distinct receptor domain conformations. Functionally, activation of AMPA receptors by agonist induces multiple conductance levels, and evidence exists that the permeation properties of each conductance level may be independent of the structure of the agonist. It has been proposed that each AMPA receptor subunit can gate independently and make an incremental contribution to ion permeation (Rosenmund et al., 1998; Smith et al., 2000). This important result drove the compelling convergence of structure and function achieved by Jin et al. (2003), who demonstrated that the degree of domain closure of the S1S2 domain in GluR2 induced by partial agonists that differ by only a single atom correlates directly with the functional efficacy for coupling agonist binding to channel gating. However, there is evidence that the glycine binding domain of NR1 does not exhibit a similar correlation between domain closure and agonist efficacy (Furukawa and Gouaux 2003; Inanobe et al., 2005).

Unlike AMPA receptors, NMDA receptors must be fully liganded to open. There is evidence that multiple conformational changes are required to transduce agonist binding into channel gating (Banke and Traynelis, 2003; Popescu et al., 2004). Although NMDA receptor activation is thus distinct from that of AMPA receptors (Erreger et al., 2004), there are still important precedents operating for AMPA receptors that hold implications for NMDA receptors. For example, both AMPA and NMDA receptor subunits can contribute kinetically distinct features to channel activation, suggesting that individual NMDA receptor subunits can have independent effects on gating. In addition, Armstrong and Gouaux (2000) first showed that partial agonism at AMPA receptors involves at least some degree of altered protein conformation. Whereas direct empirical struc-
tural data on agonist binding to NR2A are unavailable, our MD docking simulations intimate that homo-quinolinate and glutamate induce different conformations within the binding cleft. In particular, MD analysis of agonist docking suggests that homo-quinolinate, a constrained analog of glutamate, engages in a signifi- cantly different set of interactions within the binding domain and thereby stimulates a qualitatively variable response from the protein by comparison with glutamate. Thus, the different con- formations induced by the glutamate- and homo-quinolinate-bound agonist binding domains may control efficacy. The divergent efficacies could reflect global perturbation in the structure of the agonist binding domain when homo-quinolinate is docked (Fig. 2, helix F). Alternatively, the partial agonism of homo-quinolinate may imply increased intraprotein motion and decreased stability of the binding pocket (Fig. 5), which might not spend as much time in the fully closed conformation hypothe- sized to activate the ion channel. Although we cannot yet distin- guish between these two hypotheses, the combined structural and functional approach described here helps define more clearly the molecular basis of partial agonism for NMDA receptors.

References