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Principles of *N*-Methyl-D-aspartate Receptor Allosteric Modulation

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ABSTRACT

N-Methyl-D-aspartate (NMDA) receptors are glutamate-gated ion channels with complex participation in synaptic transmission, integration, and plasticity. They are highly permeable to Ca^{2+}, activate with characteristic kinetics, and generate currents with distinct amplitudes according to stimulation frequency. Multiple endogenous and pharmacological agents bind at distinct locations throughout the protein and modulate NMDA receptor responses with allosteric mechanisms. The NMDA receptor activation pathway consists of a series of consecutive, stepwise structural rearrangements rather than a binary, closed-open reaction. This high-resolution multistate gating reaction is used here to investigate the effects of ideal, state-specific modulators on physiologically relevant parameters of the macroscopic responses to single-pulse and high-frequency repetitive stimulation. The simulations suggest three significant aspects of NMDA receptor modulation: 1) modest, 1 kcal/mol bidirectional perturbations in receptor free energy cause up to a 50-fold change in the total charge transferred; 2) activators modulate primarily the response time course, whereas inhibitors are more effectively modulating current peak amplitude; and 3) state-specific modulators have opposite effects on charge transfer and current potentiation by high-frequency stimulation. The results imply that the magnitude of the NMDA receptor-mediated Ca^{2+} influx and the receptor's ability to discriminate stimulation frequency can be controlled separately. Thus, a detailed mechanistic characterization of NMDA receptor allosteric effectors may identify function-specific modulators and provides a road map for the development of combinatorial strategies for local, transient tuning of specific receptor functions.

Glutamate-triggered ionic fluxes through NMDA receptors (NRs) contribute to synaptic transmission, synaptic integration, and bidirectional long-term synaptic plasticity in the central nervous system (McBain and Mayer, 1994; Dingledine et al., 1999). Multiple mechanisms control NR signals; gene expression, differential splicing, and protein targeting and turn-over determine the number, molecular composition, and cellular location of NRs. According to brain region, developmental stage, and synaptic versus nonsynaptic location, NRs generate currents with signature waveforms (Cull-Candy et al., 2001). In addition, the kinetics of the NR response can be effectively modulated by ligands binding at multiple allosteric sites (Yamakura and Shimoji, 1999).

NRs are under tight allosteric control in vivo and represent important pharmacological targets. Endogenous extracellular ions including Zn^{2+}, Mg^{2+}, and H^{+} maintain NRs under physiological tonic inhibition (Mayer et al., 1984; Westbrook and Mayer, 1987; Traynelis and Cull-Candy, 1990). Polyamines, arachidonic acid, neurosteroids, ethanol, and nitric oxide, as well as redox and phosphorylation reactions, also modify channel activity (Yamakura and Shimoji, 1999; Loftis and Janowsky, 2003). Inappropriate NR activity contributes to severe neuropathologies, including acute and chronic neurodegeneration, chronic pain, and addiction and mental disorders (Hardingham and Bading, 2003). Still, the promise of NR-targeted therapies remains largely unfulfilled because of unacceptable side effects of indiscriminate NR inhibition (Kemp and McKernan, 2002). The development of pharmacological agents that can suppress harmful NR activities while preserving this receptor's vital functions remains an elusive objective and requires a more in-depth understanding of the principles that govern the allosteric modulation of NR activation.

Allosteric theory postulates that allotropic ligands alter a protein's activity by binding with distinct affinities to resting versus active receptor conformations, in effect forcing a dynamic redistribution of receptors among the two functionally distinct states (Monod et al., 1965; Changeux and Edelstein,

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ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; NR, *N*-methyl-D-aspartate receptor; HEPBS, *N*-(2-hydroxyethyl)piperazine-*N*′-(4-butanesulfonic acid).

1998). This conceptual framework describes adequately the allosteric modulation of proteins whose activation follows a two-state isomerization reaction. However, most receptors visit a larger repertoire of functionally discrete states during activation, and state-specific ligands are likely to have correspondingly distinct sets of modulatory effects (Kenakin, 2003). Multistate kinetic models have been used successfully to describe the activation reactions of two NR isoforms (Banke and Traynelis, 2003; Popescu and Auerbach, 2003). Both schemes account well for the complex patterns of currents observed in single-channel records and for the characteristic waveforms of the ensemble responses. In addition, the detailed description of the NR1/2A activation demonstrated that NR current amplitudes vary according to stimulation frequency (Popescu et al., 2004).

The roles played by NRs at central synapses are complex and incompletely understood. At least three characteristics of the NR ensemble response are of particular interest: 1) the total charge transferred; 2) the current wave form; and 3) its sensitivity to stimulus frequency. Because of NRs' substantial Ca^{2+} permeability, the total charge transferred scales directly with the amount of Ca^{2+} injected into the postsynaptic cell (Burnashev et al., 1995; Schneggenburger, 1996). Postsynaptic Ca^{2+} transients initiate in a concentration-dependent manner bidirectional plastic changes or may trigger neurodegeneration (Tymianski et al., 1993; Cormier et al., 2001; Ismailov et al., 2004). In turn, the shape of the NR current, which is strongly influenced by its relaxation kinetics, sets a temporal window for the effective summation of responses across multiple synapses and contributes to temporal and spatial integration of synaptic inputs (Carmignoto and Vicini, 1992; Watanabe et al., 1992). Last, the ability of NRs to double their response amplitudes when stimulated with high-frequency pulse trains makes them into veritable frequency discriminators (Popescu et al., 2004). This ability to translate frequency-encoded information into intracellular Ca^{2+} amplitudes may be crucial to the NR involvement in long-term plasticity. Explicit correlations between state occupancies, physiologically relevant macroscopic behaviors, and biological functions have not yet been described.

To investigate the principles governing the allosteric control of NR functions, I used the multistate activation scheme to examine correlations between fluctuations in specific rate constants, as elicited by state-specific modulator types, and the physiologically relevant properties of the ensemble current. The results illustrate that distinct response phenotypes are elicited according to the specific kinetic transitions modified by a particular heterotropic effector, suggest that charge transfer is highly sensitive to allosteric modulation and can be regulated without affecting the receptor's sensitivity to stimulus frequency, and predict that fluctuations in a distinct set of rate constants control the receptor's sensitivity to high-frequency stimulation with opposite effects on charge transfer. These novel predictions encourage further characterization of the kinetic mechanisms used by particular endogenous and pharmacological NR allosteric modulators with the promise of identifying function-specific agents.

Materials and Methods

All simulations were done in the Simu interface of the QUB kinetic analysis software (http://www.qub.buffalo.edu). Starting with a user-specified Markov model (number and conductance properties of states, state connectivities, and associated rates for each postulated transition), the program calculates for each channel the most probable sequence of transitions with associated state-occupancy durations. Macroscopic currents are calculated from time-dependent probabilities of occupying conducting states and each state's specified conductance. Control currents from 10,000 channels were simulated with the model and rates in Fig. 1a, in which the unitary conductance of C states was set at 0 and that of O states at 10 pA. This single-channel current amplitude corresponds to NR1/2A channels in cell-attached patches exposed to 150 mM NaCl, 2.5 mM KCl, 10 mM HEPBS buffered at pH 8, and driven by +100 mV in the recording pipette. At time 0, all channels populated the resting, unliganded, and nonconducting state C^U. After the stimulation protocols described below, currents developed in a time-dependent manner mirroring the combined dynamic occupancies of the O_1 and O_2 states. These currents were sampled at 20 kHz.

In this study, state-specific allosteric modulators were defined as ligands that bind preferentially to distinct kinetic states and that alter only one rate constant. The effect of a positive/negative state-specific modulator on channel kinetics was modeled as a 5-fold increase (5× that of control) or decrease (0.2× that of control) in a specific rate constant, each reflecting a change in receptor free energy of ~1 kcal/mol. Activators were considered to increase forward rate constants (toward O_2) or decrease backward rate constants (from O_2). Conversely, inhibitors were considered to decrease forward rate constants or increase backward rate constants.

Stimulation protocols consisted of either a single pulse or a θ-like burst, which consisted of a train of five consecutive pulses with a 10-ms interpulse interval (100 Hz), as indicated in the text. Each

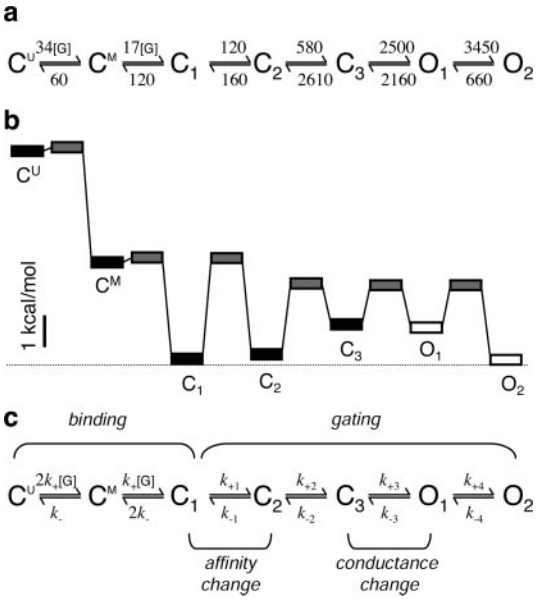

Fig. 1. NR reaction mechanism. a, minimal kinetic scheme proposed for the activation of NR1/2A receptors. All rates are given in s^{-1}, except for k_+, which is given in millimolar^{-1} second^{-1}. [Reprinted from Popescu G, Robert A, Howe JR, and Auerbach A (2004) Reaction mechanism determines NMDA receptor response to repetitive stimulation. *Nature (Lond)* **430:**790–793. Copyright © 2004 Nature Publishing Group. Used with permission.] b, free energy diagram calculated from the rates shown in a, and a glutamate concentration, [G] = 1 mM. Conformational transitions associated with gating ($C_1 \leftrightarrow O_2$) proceed along a relatively low-barrier, flat free-energy landscape. c, functional correlates of kinetically defined transitions. Distinct kinetic states also differ with respect to glutamate occupancy (C^U, unliganded; C^M, mono-liganded; C_{1-3} and O_{1-2}, fully liganded); glutamate affinity (C^U, C^M, and C_1 have measurable affinity: $K_d = k_-/k_+$, whereas glutamate dissociation is statistically negligible from $C_{2,3}$ and $O_{1,2}$ states); and ionic permeability (C, nonconducting; O, conducting).

pulse was approximated as a square step into 1 mM glutamate lasting 1 ms. For each state-specific effector (i.e., set of rate constants) and a given stimulation protocol (single-pulse or θ-burst), 10 current traces (1 s each) were simulated and averaged for analysis. Time constants for the rise and the relaxation phases of the averaged traces (RT, τ_{decay}) and the maximal current amplitudes (I_{peak}) were determined by fitting each current phase to a single exponential function directly in the QUB program. Open probabilities (P_o) were calculated from $I = nP_o\gamma$, in which n represents the number of channels, and γ represents the unitary conductance. Charge transfer was calculated in Origin (OriginLab Corp., Northampton, MA) as the integrated area under the curve of stimulus-evoked current from time 0 to 1 s. The simulations described here were done with a large number of channels to reduce fluctuations caused by the probabilistic nature of gating transitions; however, at most synapses, only few (<10) receptors are present. Therefore, charge transfer was normalized to the number of channels to allow easy comparison with results in the literature.

The activation energy diagram (Fig. 1b) was constructed relative to the ground state C_1. The free energies of all other states were calculated from the rate constants in Fig. 1a using the relationship $\Delta G_0 = -RT(\ln K_{eq})$, where R is the molar gas constant, T is the absolute temperature, and K_{eq} is the equilibrium constant of the transition considered, calculated as the ratio of the forward to reverse rate constants. Barrier heights were represented as $E^{\ddagger}_n = \Delta G^0_n + (10 - \ln k_{+n})$.

Results

Effects on the Magnitude and Shape of the NR Response to Single-Pulse Stimulation. NRs activate with uniquely slow kinetics that reflect receptor oscillations between several nonconducting conformations before reaching active, ion-permeable states. Because the intermediate states leading to open conformations are sufficiently long-lived to be detected with suitable resolution in recordings of single-channel currents, the main kinetic events associated with NR gating have been successfully described with statistical methods (Banke and Traynelis, 2003; Popescu and Auerbach, 2003; Erreger et al., 2004). This approach has demonstrated that the NR activation can be minimally conceptualized as the receptor's serial occupancy of seven energetically and functionally distinct states (Fig. 1). The six receptor transitions describing agonist-binding and receptor gating are characterized by associated pairs of forward/backward rate constants that have been estimated for NR1/2A receptors by fitting this scheme directly to entire sequences of single-channel intervals (Fig. 1a) (Popescu et al., 2004). Using standard thermodynamic relationships, this kinetic description of the NR activation pathway can be translated into relative free-energy fluctuations, thus illustrating the free energy landscape experienced by NRs during activation (Fig. 1b). In functional terms, the NR activation sequence is initiated by glutamate binding to resting or unliganded receptor conformations and continues with three successive, stepwise receptor isomerizations. The first conformational change ($C_1 \rightarrow C_2$), which is rate-limiting for both the activation (toward O states) and the deactivation (away from O states) pathways, most probably coincides with a structural rearrangement that results in a change in receptor affinity for glutamate, as indicated by a negligible probability of glutamate dissociating from subsequent receptor states ($C_{2,3}$ and $O_{1,2}$) (Popescu et al., 2004). The conformational change represented by the $C_3 \rightarrow O_1$ transition coincides with a switch in the conductance properties of the receptor, such that a receptor's observable response (ionic flux) will be determined by the combined fractional occupancies of the two ion-permeable states, O_1 and O_2 (Fig. 1c).

To determine whether general rules can be inferred that relate changes in individual rate constants with macroscopic receptor behaviors, control currents were simulated from 10,000 channels (10 pA each) with the model and rates illustrated in Fig. 1a. To estimate receptor responses in the presence of active concentrations of state-specific positive and negative allosteric modulators, currents elicited by a single glutamate pulse were simulated with models in which each rate constant, as identified with the notations in Fig. 1c, was in turn increased or reduced 5-fold. The simulated currents were subsequently analyzed in terms of maximal amplitudes and time constants for the rise and decay phases and of the total charge transferred per channel. The results are illustrated in Fig. 2a. The values in Table 1 (single-pulse stimulation) reflect the modulation by a state-specific positive effector ligand, one that increases a forward reaction rate or reduces a reverse one according to the kinetic mechanism used. Table 2 summarizes results for a negative state-specific effector.

The simulations clearly indicate that the total charge transferred through NRs after single-pulse stimulation was effectively controlled at all kinetic transitions investigated, except for the association rate constant k_+, for which a 5-fold change (increase or decrease) had no detectable effect on charge transfer. This latter observation was anticipated because the glutamate concentration used (1 mM = ~300 K_d) strongly dominated the kinetics of the glutamate association reaction even after a 5-fold change in k_+. At all other transitions, a 5-fold change in any single rate constant resulted in ~5-fold changes in the amount of charge transferred. Therefore, charge transfer through NRs was uniformly and effectively controlled at all of the kinetic steps investigated. In contrast, the shape of the response varied with the kinetic mechanism used by the modulator.

The rise times for currents driven with a single pulse were fitted poorly by single exponentials, were always considerably faster than the decay times, and varied only modestly (~2-fold) with a 5-fold change in any rate constant in the model (Tables 1 and 2). Thus, the simulations indicate that the major contributors to the large changes in charge transfer observed were caused by changes in peak open probability (peak P_o) and decay kinetics (τ_{decay}).

Overall, activators increased charge transfer by inducing responses with longer relaxation times, whereas decreases in charge transfer prompted by inhibitors reflected primarily the lower amplitudes of such responses (Fig. 2, a and b). Thus, activators and inhibitors, even when equally effective in controlling charge transfer, had distinct effects on the shape of the NR current. In particular, changes in the kinetics of the first receptor isomerization reaction ($C_1 \leftrightarrow C_2$) affected differentially the current's decay and amplitude. For a similar change in charge transfer, a decrease in the forward rate k_{+1}, modeling the action of a negative modulator, resulted in currents with 4-fold lower amplitudes but only 2-fold faster decay kinetics. In contrast, a decrease in k_{-1}, which simulated the action of a positive effector, resulted in currents with >4-fold longer τ_{decay} and only 2-fold higher amplitudes (Fig. 2b). The relative resistance of rise and decay

times to being accelerated suggests that the NR reaction mechanism has a built-in limit to how much a single state-specific modulator can shorten the activation time.

To investigate the sensitivity of NR deactivation time course to modulation, currents were simulated with models in which the rate-limiting step in the deactivation pathway, the dissociation rate constant k_{-}, was varied over 4 orders of magnitude. Results show that although decay time constants

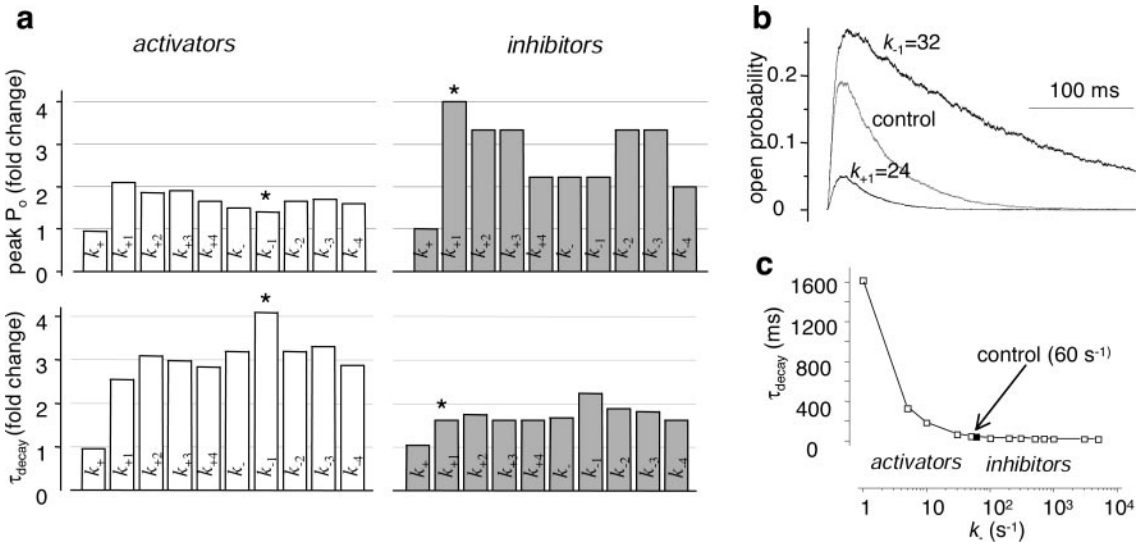

Fig. 2. Modulation of NR responses to single-pulse stimulation. a, for a similar ~5-fold change in charge transfer, activators have more pronounced effects on decay kinetics, whereas inhibitors more readily change current amplitudes; ⋆, these effects are illustrated in b. b, current amplitude is most effectively controlled by decreasing k_{+1}; decay time is most markedly modified by decreasing k_{-1}. c, decay time constants vary steeply with k_{-} until they reach a lower limit at ~20 s^{-1}.

TABLE 1

Effects of positive modulators on macroscopic properties of NR responses according to the kinetic mechanism used

See Fig. 1a for control values for all rate constants.

	Rate	Single-Pulse Stimulation				θ-Burst Stimulation			Charge Ratio θB/SP
		Rise Time	Peak P_o	τ_{decay}	Charge	Peak P_o	τ_{decay}	Charge	
	s^{-1}	*ms*		*ms*	*fC*		*ms*	*fC*	
Control		8.8	0.2	47	120	0.35	47	275	2.3
k_{+}	85	10.1	0.19	45	110	0.35	45	272	2.5
k_{+1}	600	5.1	0.42	120	550	0.52	111	800	1.5
k_{+2}	2900	6.3	0.37	145	590	0.66	140	1114	1.9
k_{+3}	12,500	8.3	0.38	140	590	0.7	139	1185	2.0
k_{+4}	17,250	11.4	0.33	133	490	0.68	132	1053	2.1
k_{-}	12	10.9	0.3	150	510	0.38	147	683	1.3
k_{-1}	32	10.5	0.28	192	510	0.49	186	1019	2.0
k_{-2}	522	8.5	0.33	150	530	0.64	148	1133	2.1
k_{-3}	432	11.7	0.34	155	570	0.71	171	1393	2.4
k_{-4}	132	11	0.32	135	500	0.67	167	1270	2.5

θB, θ-burst; SP, single pulse.

TABLE 2

Effects of negative modulators on kinetic parameters of NR macroscopic response according to the kinetic mechanism used

See Fig. 1a for control values for all rate constants.

	Rate	Single-Pulse Stimulation				θ-Burst Stimulation			Charge Ratio θB/SP
		Rise Time	Peak P_o	τ_{decay}	Charge	Peak P_o	τ_{decay}	Charge	
	s^{-1}	*ms*		*ms*	*fC*		*ms*	*fC*	
Control		8.8	0.2	47	118	0.35	47	278	2.4
k_{+}	3.4	6.6	0.2	45	121	0.35	48	286	2.4
k_{+1}	24	10.9	0.05	29	21	0.12	33	75	3.6
k_{+2}	116	12.5	0.06	27	23	0.11	30	62	2.7
k_{+3}	500	14.5	0.06	29	23	0.09	28	61	2.7
k_{+4}	690	8.9	0.09	29	36	0.14	30	99	2.8
k_{-}	300	8.9	0.09	28	33	0.21	28	181	5.5
k_{-1}	800	8.1	0.09	21	21	0.13	19	70	3.3
k_{-2}	13,050	9.7	0.06	25	22	0.11	25	62	2.8
k_{-3}	10,800	9.7	0.06	26	21	0.09	25	61	2.9
k_{-4}	3300	9.3	0.1	29	37	0.13	28	98	2.6

θB, θ-burst; SP, single pulse.

vary steeply for k_- values that are slower than controls (i.e., activator modulation), the effect of a single state-specific negative modulator on NR1/2A current decay is limited by the reaction mechanism (Fig. 2c). The limitation stems from a deactivation pathway in which two separate transitions have comparably slow, potentially rate-limiting kinetics: the glutamate dissociation step, represented by k_-, and the receptor structural rearrangement, represented by k_{-1}. In effect, this intrinsic kinetic arrangement ensures that a perturbation that increases only one of these two rate constants will have minimal effect on τ_{decay}. In contrast, there was no apparent limit to how much the current decay phase of NR1/2A receptors could be extended (Fig. 2c). A similar mechanism seems to limit the effects of a single rate-specific activator on receptor rise time, perhaps acting to preserve the characteristic slow activation of these receptors. In summary, these simulations suggest that the NR gating machinery contains intrinsic rate-stabilizing components.

Effects on Responses to Pulse Trains. NR1/2A receptors generate currents with distinct amplitudes according to the frequency of the glutamate pulses with which they are stimulated. In response to a single pulse, NRs generate currents with half-maximal amplitudes; a second pulse arriving at a 10-ms interval (100 Hz) increases the response amplitude (Popescu et al., 2004). It has been suggested that at central excitatory synapses, this so-far-unique receptor behavior serves to translate the information encoded in the frequency of the incoming electrical pulses into distinct postsynaptic Ca^{2+} transients. Because both the frequency of synaptic discharge and the postsynaptic Ca^{2+} concentration carry pertinent information to synaptic physiology, the ability of NRs to, in effect, act as frequency-to-amplitude converters is likely to be the target of physiologically critical regulatory mechanisms.

To determine how many pulses in a high-frequency train are necessary to elicit currents with maximal amplitudes, activity was simulated (10,000 channels, 10 pA) in response to trains of 2 to 10 pulses (100 Hz) with the model and rates in Fig. 1a. Irrespective of the model used, a θ-like burst consisting of four to six high-frequency pulses was sufficient to elicit responses of maximal amplitude (data not shown). This high-frequency stimulation protocol was used further in this study. θ-Burst–elicited currents had similar rise and decay kinetics as those elicited with a single-pulse protocol. This observation suggests that single pulse- and θ-burst–elicited NR responses differ mainly in the amount of charge transferred as dictated by current amplitudes.

To examine possible effects of allosteric modulators on the ability of NRs to vary the amount of charge transferred according to the stimulation protocol, θ-burst–induced currents were simulated with the model in Fig. 1a and with models in which rates were modified, in turn, 5-fold to mimic the action of individual state-specific modulators. Responses were then compared with the corresponding currents elicited by single-pulse stimulation in terms of peak open probabilities and amount of charge transferred per channel (Tables 1 and 2, θ-burst stimulation). The results were counterintuitive: inhibitors increased the receptor's ability to respond differentially to θ-burst stimulation, whereas activators strongly reduced this ability. These behaviors are illustrated in Fig. 3a. Fluctuations in glutamate dissociation kinetics (k_-) and the kinetics of the $C_1 \leftrightarrow C_2$ transitions (k_{+1}) emerged as the most effective in controlling the NRs' ability to discriminate stimulus frequency (Fig. 3b). Even for the modest, single rate constant changes used in this study, negative modulators affecting these particular transitions more than doubled the ratio of θ-burst to single-pulse charge transfer per channel (from 2.4 for control to 5.5 for changes in k_{-1}); on the other hand, positive effectors virtually eliminated the ability of NRs to respond differentially to θ-burst stimulation (ratio of θ-burst to single-pulse charge transfer per channel is 1.3 and 1.5 for changes in k_- and k_{+1}, respectively). These results suggest that the NR ability to discriminate stimulation frequency is sensitive to allosteric modulation and that this property is controlled by a subset of NR effectors only. Thus, the relative insensitivity of the frequency-dependent differential charge transfer to variations in the k_{+2}, k_{+3}, and k_{+4} rate constants suggests that allosteric effectors that modify these NR transitions will effectively influence charge

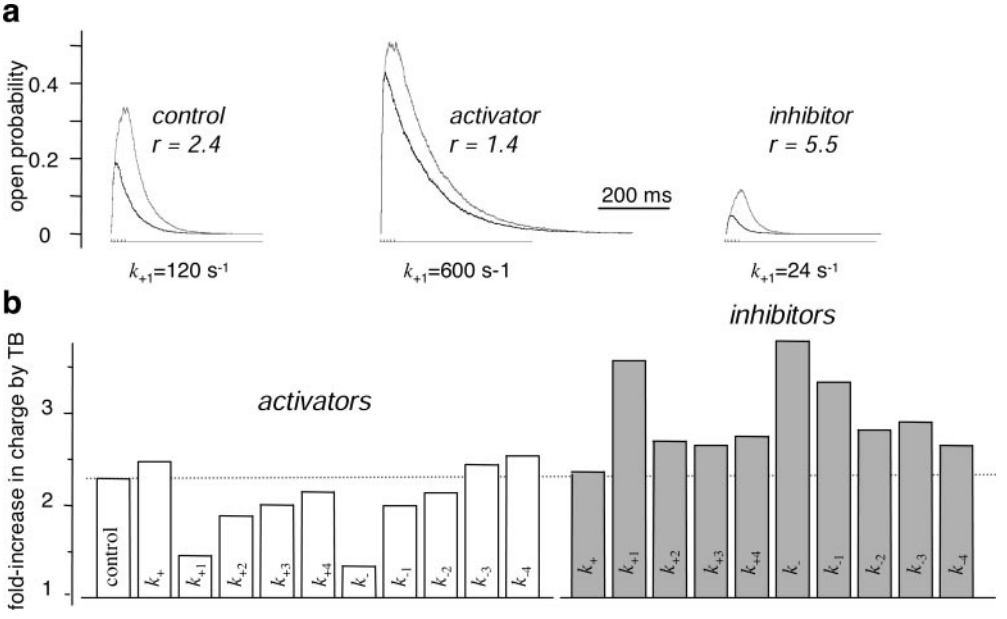

Fig. 3. Modulation of NR current potentiation by θ-burst stimulation. a, responses to a single pulse (black line) and to a θ-like burst (gray line). Increasing k_{+1} increases total charge transfer and eliminates θ-burst–elicited potentiation. In contrast, reducing k_{+1} amplifies θ-burst–induced potentiation while reducing charge transfer; r, ratio of single pulse- to θ-burst–elicited charge transfer per channel. b, activators (□) reduce and inhibitors (▨) increase θ-burst–dependent potentiation. The magnitude of the effect is mechanism-specific.

transfer but will leave intact the receptor's frequency discrimination properties.

Discussion

The principles that govern the allosteric regulation of NR activity were systematically investigated in silico by examining the effects of modest bidirectional fluctuations in individual microscopic rate constants on the observable parameters of the ensemble responses. Results show that response amplitude, decay time course, and frequency discrimination are differentially impacted by changes in individual rate constants according to the identity of the kinetic transition affected.

NR-mediated charge transfer was highly sensitive to allosteric modulation. Charge transfer in response to a single pulse was efficiently and uniformly modulated by fluctuations in any one rate constant, except for the glutamate association rate constant. A modest 1-kcal variation in receptor state free energy elicited up to 50-fold change in the amount of charge transferred, with ~5-fold bidirectional change after single-pulse stimulation and an additional ~2-fold increase after a high-frequency train. Because NRs have high unitary conductance (Stern et al., 1992) and high Ca^{2+} permeability (Jahr and Stevens, 1993; Burnashev et al., 1995; Schneggenburger, 1996), modest fluctuations in receptor gating translate into substantial fluctuations in Ca^{2+} influx.

NR-mediated Ca^{2+} influx is a salient biochemical signal for the post synaptic cell. In particular, at excitatory synapses onto spiny neurons in which free Ca^{2+} diffusion is restricted to the minute volumes of dendritic spines, allosteric NR modulation may result in substantial changes in intracellular Ca^{2+} concentration even when only a few synaptic receptors are expressed (Nimchinsky et al., 2004). This observation may have important implications for neurophysiology and neuropathology alike. Distinct physiological levels of intracellular Ca^{2+} concentrations determine whether a synapse will be strengthened or weakened in response to synaptic activity, whereas sustained, pathologically high levels of Ca^{2+} trigger apoptotic cascades responsible for excitotoxic neurodegeneration (Mody and MacDonald, 1995; Sattler et al., 1998; Cormier et al., 2001; Ismailov et al., 2004). The striking sensitivity to allosteric modulation exhibited by NRs suggested by these simulations raises the possibility that NR allosteric effectors may wield powerful control over NR-dependent postsynaptic physiological and pathological phenomena. Agents that modify charge transfer but preserve the receptor's ability to discriminate stimulus frequency may represent better-tolerated pharmacological interventions.

Activators and inhibitors had distinct effects on the shape of the NR current. For changes in charge transfer of similar magnitude, activators extended the deactivation phase of the response up to 4-fold, whereas inhibitors had a lesser (~2-fold) effect. This result correlated with the observation that there was a limit to how fast currents decayed after changes in a single rate constant. NR current decay time is a parameter of particular relevance to several important neuronal functions. It sets the time frame within which activity at neighboring synapses will be integrated, participates in the generation of dendritic spikes, contributes to supralinear summation of excitatory postsynaptic currents, and sets the temporal window within which synaptic discharge and depolarization in the postsynaptic dendrite are detected as coincidental (Magee, 2000; Schiller et al., 2000; Schiller and Schiller, 2001). NR current decay time is developmentally and regionally regulated by differential expression and dynamic subcellular targeting of NRs with distinct subunit compositions (Carmignoto and Vicini, 1992; Watanabe et al., 1992). Allosteric modulation may represent an additional, fast, reversible, and perhaps local mechanism to adjust the NR current decay time.

The results presented here suggest that, at least for NR1/2A isoforms, current decay time is resistant to further decrease by allosteric inhibitors but can be easily increased by positive modulators. It is interesting that the NR1/2A receptor is the isoform with the fastest decay kinetics (Vicini et al., 1998). Its apparent resistance to further acceleration suggests that these receptors may serve to set the shortest time frame within which synaptic activity will be integrated.

A single θ-like burst stimulus was sufficient to trigger maximal current amplitude (and thus maximal charge transfer) from NR1/2A receptors. The implication that NRs recognize and respond distinctly to a physiologically occurring electrical pattern may be directly relevant to the roles played by NRs in higher brain functions. Many neurons in the central nervous system have the ability to generate short bursts of action potentials consisting of four to six pulses at 100 to 200 Hz, which repeat with a 200-ms periodicity. This rhythmic activity pattern known as θ rhythm is prevalent in the hippocampus, in which entire populations of cells fire synchronously with this frequency. In this brain region, θ oscillations have been ascribed a role in cellular plasticity and behavioral learning processes (Berry and Seager, 2001). More specifically, it has been demonstrated in vitro that cholinergic induced θ-like activity increases NR-dependent plasticity phenomena (Huerta and Lisman, 1996). In this context, NR inhibition may represent means to enhance NR sensitivity to θ-burst stimulation while tuning out low-frequency signals.

It is noteworthy that current potentiation by high-frequency stimulation was differentially affected by fluctuations in individual rate constants. The most effective control points were the k_- and k_{+1} rate constants, and these had opposite effects on frequency discrimination and charge transfer (Fig. 4). It is noteworthy that in most systems, stimulation frequency determines the direction of the ensuing activity-dependent synaptic modification, with high-frequency stimulation (100 Hz) resulting in long-term potentiation, and tonic, low-frequency stimulation (1–10 Hz) initiating long-term depression (Herron et al., 1986; Dudek and Bear, 1993). Thus, it is highly plausible that NRs are the molecular transducers that translate patterns of neurotrans-

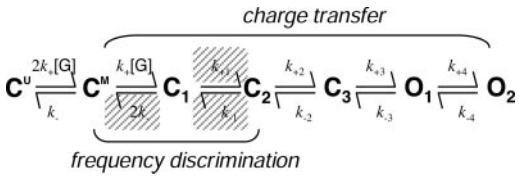

Fig. 4. Principles of NR allosteric modulation. Charge transfer is uniformly and effectively modulated by changing any rate constant in the activation reaction. The ability of NRs to respond with increased amplitude to θ-burst stimulation is modulated in opposite direction by fluctuations in a specific subset of rate constants (▨).

mitter release into distinct levels of intracellular Ca^{2+} to trigger differential plasticity. The simulations presented in this study suggest that allosteric modulators can intervene to increase or annihilate a frequency-dependent differential Ca^{2+} influx and thus control how the cell will respond to a particular stimulation frequency. Thus, NR allosteric modulators may contribute to physiological mechanisms for differential synaptic transmission (Markram et al., 1998).

Several limitations inherent to this study need to be stated. This first systematic examination of correlations between state-specific modulators and characteristic behaviors of ensemble NR responses was made possible by the recent high-resolution characterization of the NR1/2A activation reaction; necessarily, the results are limited to this receptor isoform. In addition, although the model captures most recognizable NR physiological behaviors, it is still incomplete because it lacks explicit association/dissociation transitions for the coagonist glycine and desensitization steps. NR1/2A receptors have remarkably low desensitization, and thus it is likely that excluding this latter kinetic component will not greatly affect the results presented here. Still, both the glycine binding and the desensitization reactions may represent physiological targets for NR modulation and should be addressed in the future. Last, only the effects of "ideal" modulators (i.e., those that affect the rate constant for a single conformational transition) were investigated here. In reality, the action of a particular modulator may result in changes in a set of rate constants (see below).

The inhibitory effects demonstrated for calmodulin on NRs native to hippocampal granule cells illustrate several aspects of NR modulation highlighted by the present study. It was estimated that calmodulin decreases the amount of charge transferred by a single receptor ~3-fold (from 69 to 23 fC) (Rycroft and Gibb, 2002). Despite differences in the preparation investigated (native versus recombinant) and experimental conditions (i.e., ionic concentrations, pH, and membrane potential), these values are remarkably similar to the ones estimated here for ideal inhibitors, ~5-fold decrease (from 118 fC for control to 36–21 fC in the presence of modulator; see Table 2). First, this comparison between experiment and simulation indicates that the small free-energy fluctuations assumed in this study are within the range of receptor free-energy fluctuations induced by a physiological modulator. Second, when the effects of calmodulin on NR activity were modeled with a two-state gating scheme, it was found that calmodulin changed the NR opening rate ~7-fold, the channel closing rate ~2-fold, and the desensitization rate ~2-fold (Rycroft and Gibb, 2002). Although these estimated opening and closing "rates" amalgamate several microscopic rate constants, it is clear that calmodulin binding to NRs changes the kinetics of several NR transitions whose integrated effects result in a unique macroscopic phenotype: decreased open probability, and increased current decay rate. Last, the effects of calmodulin or of any other known NR modulator on NR's frequency discrimination properties have not been yet investigated. The simulations presented here demonstrate that it is critical to determine specifically which microscopic rate constants are affected by a modulator to evaluate the modulator's impact on NR's sensitivity to stimulus frequency. The predictions afforded by a known kinetic mechanism would help design targeted experimental paradigms to further investigate such effects.

The systematic correlations delineated here between fluctuations in specific microscopic rate constants and physiologically relevant kinetic properties of the macroscopic response provide a theoretical foundation to the search for function-specific modulators, make a strong case for undertaking a detailed characterization of the kinetic mechanisms used by endogenous and pharmacological allosteric modulators, and offer a clear road map for combinatorial approaches to the rational control of NR functions. The goal is to be able to predict NR responses in the simultaneous presence of several modulators, as is the case in vivo, and the effect of pharmacological agents on NRs operating in particular physiological or pathological conditions.

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