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Agonist Response Kinetics of *N*-Methyl-D-aspartate Receptors in Neurons Cultured from Rat Cerebral Cortex and Cerebellum: Evidence for Receptor Heterogeneity

TONY PRIESTLEY and JOHN A. KEMP

Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Harlow, Essex, UK CM20 2QR

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SUMMARY

Accumulating evidence from both radioligand binding and molecular cloning experiments has led to the suggestion that there are heterogeneous populations of *N*-methyl-D-aspartate (NMDA) receptors. In particular, the NMDA receptor associated with cerebellar neurons has been suggested to be different from that in higher brain regions. With these observations in mind, we considered the possibility that the NMDA receptors on cultured neurons from rat cortex and cerebellum may show differences in their affinity for the coagonists, glutamate and glycine, and the ion channel blocker ketamine. A kinetic approach was used to derive the association and dissociation constants for each of the ligands from their respective macroscopic time constants of binding and unbinding. The constants were assessed electrophysiologically by measuring the onset and decay of whole-cell currents in response to drug applications to voltage-clamped neurons. In addition, differences in coagonist affinity were examined using conventional equilibrium concentration-response curve analysis. These experiments revealed that current relaxations after fast applications of either glutamate or glycine decayed more rapidly in cerebellar neurons, compared with cortical neurons. Thus, assuming two independent binding sites per receptor, the microscopic decay time constants (τ_{off}) for glutamate were 341 ± 47 (n = 12) and 934 ± 76 msec (n = 11, $p <$ 0.0001) for granule cells and cortical neurons, respectively. The resulting apparent microscopic dissociation constant (mK_d) for glutamate at cerebellar granule cells, calculated from the forward and reverse rate constants, was >2-fold lower than that for cerebral cortex receptors (496 nM, compared with 251 nM). The difference between the two cell types in the τ_{off} for glycine was more substantial, i.e., 558 ± 53 (n = 15) and 2214 ± 125 msec (n = 19, $p < 0.0001$) for cerebellum and cortex, respectively. Corresponding apparent mK_d values for glycine differed by >4-fold, i.e., 189 nM and 45 nM for cerebellar granule and cortical neurons, respectively. Analysis of data obtained from equilibrium concentration-response curves also revealed differences in coagonist affinity between the two cell populations. The mean mK_d values for glutamate at cerebellum and cortical neurons were 1260 nM and 630 nM, respectively, and those for glycine were 316 nM and 63 nM, respectively. No obvious differences were found between the two cell types with respect to the ion channel-blocking kinetics of the dissociative anesthetic ketamine. In conclusion, these functional data demonstrate differences in coagonist (glutamate and glycine) affinity for NMDA receptors associated with cultured cerebellar granule cells, compared with neurons cultured from the cerebral cortex. In view of the multisubunit structure of these ligand-gated ion channels, it must be considered likely that the observed differences in binding site affinity reflect differences in the subunit composition.

The NMDA receptor is a ligand-gated cation channel that, because of its permeability to calcium, is thought to play a key role in the physiology of synaptic transmission and certain pathological conditions within the central nervous system (1–4). Over the years there have been several indications, particularly from radioligand binding studies, of pharmacologically distinct subtypes or interconvertible affinity states of NMDA receptors. In particular, the NMDA receptor in the cerebellum has often been suggested to be different from that in the forebrain. Thus, the binding of L-[^{3}H]glutamate to membranes of cerebellar neurons is less sensitive to displacement by either NMDA receptor agonists, particularly quinolinic acid (5, 6), or antagonists (7). Cerebellar NMDA receptors have also been suggested to have a different affinity for NMDA ion channel-blocking dissociative anesthetics such as thienylcyclohexylpiperidine (8, 9), ketamine, and SKF 10,047 (10).

The cloning and expression of subunits that comprise functional NMDA receptors have also supported the idea of different cerebellar NMDA receptors. These studies have shown that in adult rodent brain the NMDAR2C (or ϵ3) subunit appears to be almost exclusively expressed in the cerebellum, whereas the NMDAR2B (or ϵ2) subunit is expressed in forebrain structures but is absent from the cerebellum (11, 12).

The mounting evidence in favor of NMDA binding site

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*f*)quinoxaline; EPSP, excitatory postsynaptic potential; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

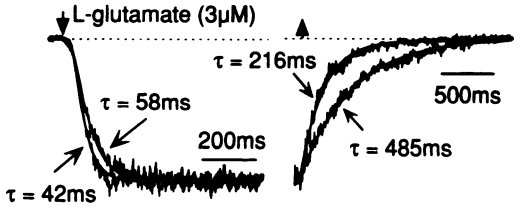

Fig. 1. Comparison of glutamate response kinetics in neurons cultured from cerebral cortex and cerebellum. Averaged inward current responses and their corresponding relaxations after the fast application of glutamate (*down arrowhead*) and its removal (*up arrowhead*), in the continuous presence of glycine, are shown superimposed for a typical cerebrocortical neuron and a cerebellar neuron. Exponential curves (*solid lines*) were fitted to the digitized data (see Methods) and yielded on-rate time constants for glutamate-induced currents of 42 and 58 msec and off-rate time constants of 216 and 485 msec for cerebellar and cortex neurons, respectively. Note that the responses have been scaled to the same amplitude, to facilitate direct visual comparison; actual current amplitudes were ~115 pA (granule cell) and ~220 pA (cortical neuron).

heterogeneity, particularly with respect to the different properties of cerebellar neurons, prompted us to investigate whether there were functional differences in the agonist pharmacology of endogenously expressed NMDA receptors in cultured cerebrocortical neurons and cerebellar granule cells. To do this we investigated the kinetics of NMDA receptor-mediated L-glutamate and glycine responses using concentration-jump applications on whole-cell, voltage-clamped neurons. For comparative purposes, coagonist affinity was also determined using equilibrium concentration-response curve analysis.

Materials and Methods

Preparation of rat cortical cell and cerebellar granule cell cultures. Cultures of rat cortical neurons were prepared from the cerebral hemispheres of rat fetuses (16–18 days of gestation), and essentially pure cultures of cerebellar granule cells were prepared from cerebella of 6–8-day-old rat pups. In both cases, the dissociation procedures and enzyme concentrations were as described previously (13), with the only exception being that the culture medium for the cerebellar granule cells was supplemented with 15 mM KCl.

Whole-cell voltage-clamp recordings. Cell cultures were grown on poly-lysine-coated glass coverslips and were used for electrophysiological experiments after 8–21 days *in vitro*. At the time of the experiment, a single coverslip was transferred to a glass-bottomed, Perspex recording chamber mounted on the stage of a Nikon Diaphot inverted microscope. Cultures were observed using phase-contrast optics and were continuously perfused with a salt solution of the following composition (in mM): NaCl, 149; KCl, 3.25; $CaCl_2$, 2; $MgCl_2$, 2; D-glucose, 11; tetrodotoxin, 0.0003; HEPES buffer, 10. The pH of the perfusate was adjusted to 7.35 using NaOH and the osmolarity was adjusted to 350 mOsm using sucrose. Our cortical cell cultures consisted of a morphologically heterogeneous population of neurons. Many of the larger, pyramidal-type neurons had extensively branched neurites that appeared to project for considerable distances. To minimize any possible artifactual influences due to differences in cell morphology (e.g., efficiency of space clamp and speed of drug equilibration), we restricted our recordings to small, isolated, bipolar cells that possessed only one or two fine neurites and that were of similar dimensions as mature granule cells.

Drugs were diluted from concentrated stock solutions into a modified salt solution that lacked $MgCl_2$ but that was supplemented with NBQX (10 μM), to block non-NMDA receptor-mediated events. Additional measures were taken in the case of experiments involving the measurement of glycine kinetics, where the drug solutions were made up in ultrapure high performance liquid chromatography-grade water and all glassware was rinsed extensively in the same water to remove traces of glycine.

Patch pipettes with a tip diameter of ~2–3 μm (mean resistance, 2.76 ± 0.14 MΩ; $n = 11$) were formed from borosilicate glass using a Mechanex BBCH puller; they were not additionally fire polished or treated to reduce capacitance. The patch pipettes were filled with a solution composed as follows (in mM): CsF, 120; CsCl, 10; HEPES, 10; EGTA, 10; $CaCl_2$, 0.5; pH adjusted to 7.25 with CsOH and osmolarity adjusted to to 330 mOsm with sucrose. Whole-cell currents were recorded from cultured neurons at a holding potential of −60 mV, using a List EPC-7 patch-clamp amplifier, and were averaged (typically >15 but no fewer than 5 responses). Pipette seal resistances were not routinely measured but were generally in excess of 100 GΩ and pipette capacitance transients were cancelled before the membrane was ruptured, but no additional capacitance neutralization or series resistance compensation was applied.

L-Glutamate (high performance liquid chromatography grade; Sigma) and glycine (BDH) were freshly prepared as aqueous stock solutions before every experiment and were diluted in the extracellular medium detailed above but with $MgCl_2$ omitted. They were applied to localized regions of the culture by fast perfusion from a double-barreled pipette assembly (14). The solutions in the wash barrel also lacked $MgCl_2$. The internal diameter of each of the perfusion tubes was 375 μm, and the tubes were positioned close to the bottom of the dish and ~300 μm from the cell. Solution equilibration times around the cells were determined by stepping from a solution of kainate (100 μM) in 10 mM NaCl to one containing 140 mM NaCl. The mean time constant of the exponential increase in membrane current after such a step was 17.2 ± 1.3 msec ($n = 10$).

Exponential curve fitting and measurement of drug on- and off-rates. Cell currents were filtered (cut-off frequency, 10 kHz), digitized (22 kHz) using a CED1401 laboratory interface, and captured on-line to the hard disk of a Compaq Deskpro 486 computer using SCAN software (purchased from J. Dempster, University of Strathclyde, UK). The monoexponential decline of either glutamate- or glycine-evoked current relaxations was measured directly using SCAN. However, the on-rate of either drug response was always better described by a curve-fitting model that assumed that channel gating required the binding of two molecules of agonist to independent recognition sites. For these analyses, curve fitting to digitized data was

TABLE 1

Comparison of L-glutamate kinetics at cortical neuron and granule cell NMDA receptors

Cortical neurons		Cerebellar granule cells	
τ_{on}[a]	τ_{off}	τ_{on}	τ_{off}
msec	msec	msec	msec
72.0 ± 5.8 ($n = 11$)	934 ± 76 ($n = 11$)	48.4 ± 5.5[b] ($n = 12$)	341 ± 47[c] ($n = 12$)

[a] The microscopic τ_{on} was estimated as described in Materials and Methods and the microscopic τ_{off} is twice the measured time constant for the current relaxation. The time constants were used to derive the following forward and reverse rate constants: cortex, $K_+ = 4.3 \times 10^6$ M^{-1} sec^{-1} and $k_- = 1.1$ sec^{-1} ($K_d = 256$ nM); cerebellum, $K_+ = 5.9 \times 10^6$ M^{-1} sec^{-1} and $K_- = 2.9$ sec^{-1} ($K_d = 492$ nM).
[b] Significantly different from cortical neurons, $p < 0.01$ (t test).
[c] Significantly different from cortical neurons, $p < 0.0001$ (t test).

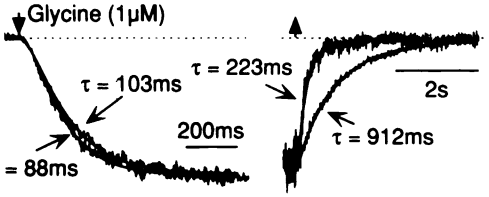

Fig. 2. Comparison of glycine response kinetics in neurons cultured from cerebral cortex and cerebellum. Averaged inward current responses and their corresponding relaxations after the fast application of glycine (*down arrowhead*) and its removal (*up arrowhead*), in the continuous presence of glutamate, are shown superimposed for a typical cerebrocortical neuron and a cerebellar neuron. Exponential curves (*solid lines*) were fitted to the digitized data (see Methods) and yielded on-rate time constants for glycine-induced currents of 88 and 103 msec and off-rate time constants of 223 and 912 msec for cerebellar and cortex neurons, respectively. Responses have been scaled to the same amplitude, to facilitate direct visual comparison; actual current amplitudes were ~90 pA (granule cell) and ~350 pA (cortical neuron).

performed using GraFit software, according to the following mathematical equation:

$$I = B_o - A \times \left(1 - \exp\left(\frac{-t}{\tau}\right)\right)^2 \quad (1)$$

where B_o is the current baseline before the start, A is the current response amplitude, t is time, and τ is the time constant for agonist occupation of each independent site.

Apparent agonist dissociation constants (K_d) were calculated from the aforementioned measures by first deriving the estimated forward (K_+) and reverse (K_-) rate constants according to the scheme:

$$R + 2L \underset{K_-}{\overset{2K_+}{\rightleftharpoons}} RL + L \underset{2K_-}{\overset{K_+}{\rightleftharpoons}} RL_2$$

where R is the receptor, L is the ligand, RL is the monoliganded receptor, and RL_2 is the fully liganded receptor. K_- is twice[1] the measured $1/\tau_{off}$; K_+ was approximated from the following function

$$K_+ = \frac{\frac{1}{\tau_{on}} - K_-}{[\text{agonist}]} \quad (2)$$

and

$$K_d = \frac{K_-}{K_+} \quad (3)$$

Equilibrium concentration-response curves. Equilibrium concentration-response curves for glutamate were constructed by measuring the membrane current response to increasing concentrations in the presence of a constant concentration of glycine (10 μM). Similar experiments involving glycine were compromised by the presence of residual glycine contamination in salt solutions; this problem was exacerbated when glutamate was used as the coagonist. Consequently, equilibrium concentration-response curves for glycine were constructed using NMDA as the coagonist. We previously demonstrated that this would be expected to have only a modest effect on the affinity of glycine for its recognition site (15). Thus, concentration-response curves for glycine were made by using a fixed concentration of NMDA (30 μM) in the presence of different concentrations of glycine. To account for the effects of basal glycine contamination, responses to NMDA (30 μM) were recorded in nominally glycine-free solutions and the response amplitude was used to correct the subsequent concentration-response curves. Best-fit lines were computed for the data using the two-independent site model (see eq. 4). The values for each individual cell were then normalized to the maximum current (I_{max}) predicted by the fitted line and were pooled to generate a mean curve.

$$I = \frac{I_{max}}{\left(1 + \left(\frac{mK_d}{[A]}\right)\right)^2} \quad (4)$$

Affinity estimates are quoted either as the apparent microscopic dissociation constant (mK_d) or as the corresponding negative logarithm (pmK_d), to enable statistical comparisons between data.

Results

Concentration-jump applications of L-glutamate (3 μM) in the continuous presence of glycine (10 μM) and NBQX (10 μM) produced NMDA receptor-mediated inward current responses in whole-cell voltage-clamped (holding potential, −60 mV) rat cortical neurons and cerebellar granule cells. Current relaxations, obtained upon stepping back to L-glutamate-free salt solution, were noticeably faster in granule cells, compared with cortical neurons (Fig. 1). Subsequent analysis of these responses to obtain the microscopic on- and off-rate time constants (τ_{on} and τ_{off}, respectively) revealed significant differences between the two cell types (Table 1). Thus, the estimated τ_{on} and τ_{off} values for L-glutamate in cortical neurons were significantly slower than the corresponding measures in cerebellar granule cells. Estimation of the forward and reverse rate constants (K_+ and K_-, respectively) for agonist binding and unbinding illustrated that these differences were due primarily to the slower dissociation of glutamate from receptors on cortical neurons.

Concentration jumps with glycine (1 μM) in the presence of glutamate (3 μM) revealed that glycine binding and unbinding also proceeded with slower kinetics in cortical neurons than in cerebellar granule cells (Fig. 2). In fact, the difference in affinity, as estimated from the forward and reverse rate constants, was even more pronounced for glycine than for glutamate (Table 2).

The differences in glutamate and glycine affinity between

[1] We have assumed that two agonist molecules binding to two identical independent sites are required to gate the NMDA channel; the measured off-rate reflects the dissociation of just one of these molecules and, therefore, is twice as fast as the true off-rate.

TABLE 2
Comparison of glycine kinetics at cortical neuron and granule cell NMDA receptors

Cortical neurons		Cerebellar granule cells	
τ_{on}[a]	τ_{off}	τ_{on}	τ_{off}
msec	*msec*	*msec*	*msec*
96.5 ± 2.6 (n = 12)	2214 ± 125 (n = 19)	89.0 ± 1.6[b] (n = 12)	558 ± 53[c] (n = 15)

[a] The time constants were used to derive the following forward and reverse rate constants: cortex, $K_+ = 9.9 \times 10^6$ M^{-1} sec^{-1} and $K_- = 0.45$ sec^{-1} (K_d = 45 nM); cerebellum, $K_+ = 9.5 \times 10^6$ M^{-1} sec^{-1} and $K_- = 1.8$ sec^{-1} (K_d = 189 nM).
[b] Significantly different from cortical neurons, $p < 0.05$ (t test).
[c] Significantly different from cortical neurons, $p < 0.0001$ (t test).

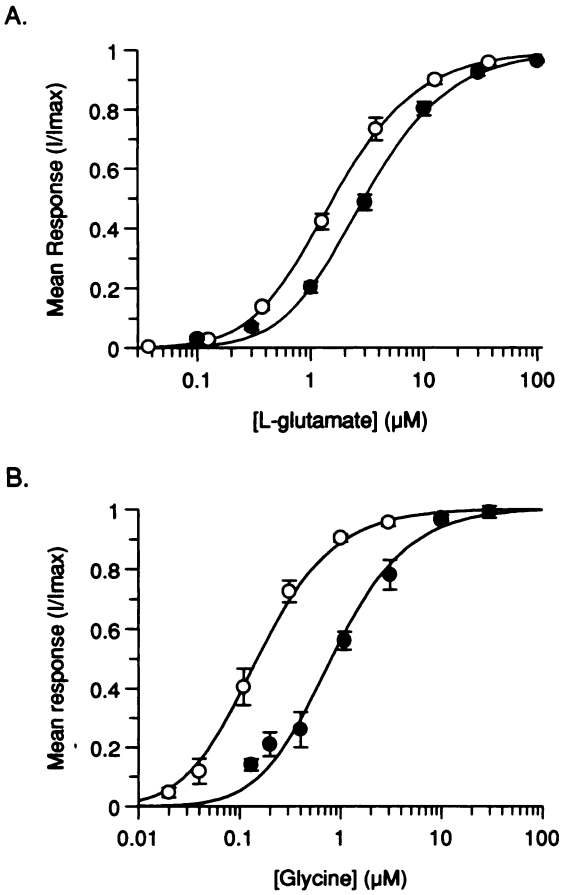

Fig. 3. A, Equilibrium concentration-response curves for L-glutamate at NMDA receptors on cortical and cerebellar granule cells. Concentration-response curves for glutamate-evoked membrane currents were constructed in the continuous presence of glycine (10 μM). The *curves* represent fitted lines through the mean normalized data obtained from cortical neurons (○) or cerebellar granule cells (●) ($n = 5$ in each case). The data reveal glutamate to be approximately 2-fold weaker at the cerebellar receptor than at the cortical receptor, and the corresponding pmK_d values were 5.9 ± 0.04 and 6.2 ± 0.05, respectively (significant difference, $p < 0.001$, unpaired t test). B, Equilibrium concentration-response curves for glycine at NMDA receptors on cortical and cerebellar granule cells. Concentration-response curves for glycine-evoked membrane currents were constructed in the continuous presence of NMDA (30 μM). The *curves* represent fitted lines through the mean normalized data obtained from cortical neurons (○) ($n = 3$) or cerebellar granule cells (●) ($n = 4$), each corrected for basal glycine contamination. The data show glycine to be approximately 5-fold weaker at the cerebellar receptor than at the cortical receptor, and corresponding pmK_d values were 6.5 ± 0.07 and 7.2 ± 0.05, respectively (significant difference, $p < 0.0005$, unpaired t test).

the two cell populations indicated by these kinetic measurements were confirmed using conventional equilibrium concentration-response curve analyses. Although there were minor discrepancies in the absolute dissociation constant estimates generated by these methods, the difference in agonist affinity remained consistent using the two approaches. Thus, equilibrium concentration-response curves for L-glutamate at cerebellar granule cells were to the right of those obtained from cortical neurons (Fig. 3A). The mK_d values derived from these data revealed a roughly 2-fold, but significantly ($p < 0.001$), lower affinity for glutamate at the receptors on granule cells, compared with the receptors on cortical neurons (cerebellum, 1260 nM; cortex, 630 nM). As with the kinetic approach, more substantial differences in affinity were found for glycine. Analysis of the concentration-response curves for glycine confirmed a 5-fold lower affinity for this ligand at the receptors on granule cells, compared with those on cortical cells (mK_d values: cerebellum, 316 nM; cortex, 63 nM; significant difference, $p < 0.0005$; Fig. 3B).

In the case of ligand-gated ion channels, where agonist binding directly evokes a functional response, differences in agonist affinity imply fundamental differences in receptor properties. It is also conceivable that differences in receptor proteins may extend to other features of the NMDA receptor-ionophore complex. Indeed, it was previously suggested that dissociative anesthetics such as ketamine and SKF 10,047, which are open-channel blockers, have a lower affinity for the NMDA receptor ion channel in the cerebellum, compared with that in the cortex (10). We addressed this possibility by examining the kinetics of ion channel block by ketamine in the two cell types. These experiments provided no evidence to suggest that ketamine had a lower affinity for the NMDA receptor-associated ion channels in granule cells, compared with those in cortical neurons (Table 3).

Discussion

The principal finding to emerge from these experiments is that the kinetics of binding and dissociation for both glutamate and glycine at their recognition sites are different for NMDA receptors on cultured cerebellar granule cells and cortical neurons. Although both the measured time constants for the on-rate and decay of the membrane current showed significant differences, calculation of the corresponding forward and reverse rate constants suggested that the differences in agonist affinity at the two receptors were due primarily to a marked difference in the off-rate. Because the fastest of these was at least 1 order of magnitude slower than the drug equilibration time around the cell, it is unlikely that these differences arise from variations in cell size or morphology. Furthermore, these whole-cell kinetic values are similar to those that have been obtained from isolated outside-out membrane patches (16–18). Additionally, the kinetic approach was validated by the fact that similar differences in affinity were obtained using equilibrium concentration-response curve analysis. Thus, the higher affinity of both glutamate and glycine at the NMDA receptor on cortical cells is due to the slower dissociation of the coagonists from their recognition sites.

The kinetic analyses we have used in interpreting these data have assumed that two molecules of both glutamate and glycine are required to bind to the NMDA receptor-ionophore complex to gate the channel (Refs. 16, 19, and 20, but see Ref. 17). Consistent with such a model, the rise time of the membrane current response was always sigmoidal in appearance. Hence, current on-rates obtained in the continuous presence of either glutamate or glycine were always better described by a model that required the binding of two agonist molecules at independent recognition sites. A sigmoidal rise time may be due in part to noninstantaneous drug equilibration at the receptor site, even with the fast agonist application techniques used. However, this alone is unlikely to account for the particularly slow initial phase of the inward current response. Indeed, under conditions where non-NMDA receptors were fully occupied by a saturating concentration of kainate, the delay between initiating a concentration jump into Na^+ and achieving a linear

TABLE 3

Comparison of ketamine kinetics at cortical neuron and granule cell NMDA receptors

The on-rate for ketamine block was best described by the sum of two exponentials, both of which are given [$\tau_{on(fast)}$ and $\tau_{on(slow)}$]. Statistical analyses revealed no significant differences between neuron types for any of the parameters.

Cortical neurons			Cerebellar granule cells		
$\tau_{on(fast)}$	$\tau_{on(slow)}$	τ_{off}	$\tau_{on(fast)}$	$\tau_{on(slow)}$	τ_{off}
msec	msec	msec	msec	msec	msec
46.7 ± 5.8 (n = 6)	530 ± 115 (n = 6)	6027 ± 1404 (n = 6)	55.2 ± 25 (n = 5)	457 ± 159 (n = 5)	5072 ± 1186 (n = 5)

increase in current was in the range of 7–10 msec. This delay, which is presumably due to the speed of solution equilibration around the cell, is appreciably faster than that observed during experiments requiring agonist binding (15–28 msec).

The concept of different NMDA receptor subtypes in rat cortex and cerebellum is supported by evidence from molecular cloning experiments that have demonstrated that these receptors are likely to be composed of different subunits. The NMDAR1 subunit, which is required for the functional expression of heteromeric receptors, has a fairly ubiquitous expression throughout the central nervous system. Expression studies with these subunits have shown that the NMDAR1 subunit in combination with any one of the NMDAR2 subunits results in heteromeric recombinant receptors that have different affinities for glutamate and glycine, depending on the type of NMDAR2 subunit that is present. Thus, the kinetics of glutamate and glycine responses with recombinant receptors composed of NMDAR1 with NMDAR2A are much faster than those evoked from combinations of NMDAR1 and NMDAR2C (11) and, as predicted from this, the affinities of these coagonists are higher with NMDAR1+2C than with NMDAR1+2A recombinant receptors (12). At first sight this may appear paradoxical, with regard to the present data, because *in situ* hybridization studies have revealed that NMDAR2C mRNA is exclusively located in rat cerebellum, with little or no message in cortex (11). However, it has recently been shown that cultured cerebellar granule cells do not express the NMDAR2C subunit but express high levels of NMDAR2A mRNA along with smaller amounts of NMDAR1 and -2B.[2] Thus, unlike their *in vivo* counterparts, cultured granule cells fail to make the developmental switch to the expression of the NMDAR2C subunit. Hence, despite our initial premise, the differences that we have observed in agonist affinity in cultured cerebellar and cortical neurons may not reflect the differences in NMDA receptors in the corresponding brain regions in the adult. In addition, the precise subunit composition of native NMDA receptors is currently unknown and it is possible that such receptors are composed of more than two different subunits. Indeed, recent expression studies in *Xenopus* oocytes indicated that three different subunits, rather than just two, can preferentially coassemble to form functional recombinant NMDA receptors (21). Nevertheless, the present experiments demonstrate that naturally expressed NMDA receptors show different pharmacological characteristics that, in this case, may have important implications for neuronal development.

Additional support for different cortical and cerebellar NMDA receptor subtypes comes from independent studies of the time course of miniature spontaneous and evoked synaptic EPSPs. Silver *et al.* (22) recently reported that the NMDA component of EPSPs recorded in cerebellar granule cells after stimulation of the mossy fiber pathway decays with a time constant of approximately 50 msec. This value is considerably faster than that recorded from cultured hippocampal neurons, where the NMDA component of the synaptically evoked EPSPs typically decayed with a time constant of 250–545 msec (23). Because it is the kinetics of transmitter binding and unbinding that are thought to determine the time course of NMDA receptor-mediated synaptic events (23), these observations suggest that the subsynaptic NMDA receptors located on granule cells and cortical neurons differ in their affinity for endogenously released transmitter.

The time constants for the block of NMDA receptor-mediated responses by ketamine and the recovery of those responses after its removal did not differ between granule cells and cortical neurons. Although this may seem a surprising result, based on the radioligand binding studies (10), it is supported by observations with phencyclidine, which also has a lower affinity for the binding site in cerebellum. However, phencyclidine is as potent in inhibiting NMDA receptor-evoked release of [^{3}H]noradrenaline in cerebellar slices as it is in functional assays other brain regions (24). The kinetics of ketamine found in the present experiments suggest that the ion channel properties of cultured cerebellar granule cell NMDA ionophores are similar, at least with respect to this dissociative anesthetic, to those expressed by cultured cortical neurons.

It has been reported that the stage of development affects NMDA response kinetics in visual cortex neurons (25). To rule out such a possibility in our experiments, responses were obtained from cultures at different times after plating. Essentially the same result was obtained regardless of the stage of maturity of the culture. We consider it unlikely, therefore, that the observed differences between cortical neurons and granule cells could be a result of time in culture.

In conclusion, we propose that the differences in NMDA response kinetics between rat cerebellar granule cells and cortical neurons in culture are due to fundamental differences in recognition site affinity for both of the coagonists, glutamate and glycine. It must be considered highly likely that these differences reflect differences in the subunit structure of the respective receptor-ionophores, the precise details of which remain to be elucidated.

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Send reprint requests to: T. Priestley, Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR, UK.