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The Contribution of NMDA and Non-NMDA Receptors to Fast and Slow Transmission of Sensory Information in the Rat SI Barrel Cortex

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The main objective of this study was to establish the contribution of NMDA receptors to natural processing of somatosensory information within rat SI barrel cortex. Responses of 52 cells in layers I–IV of the rat barrel cortex were analyzed by PSTH (peristimulus histogram) analysis of evoked spikes in reply to brief deflections of the principal whisker in animals anesthetized with urethane. Short and longer peak latency responses within PSTHs were compared in the presence and absence of the specific NMDA and non-NMDA antagonists D(−)-2-amino-5-phosphonovaleric acid and 6,7-dinitroquinoxaline-2,3-dione, which were administered locally to neurons by iontophoresis and additionally tested against their putative specific agonists, NMDA and quisqualate, respectively.

The results suggest the following. (1) The generation of most spikes from cells in layers I–IV is dependent upon activation of NMDA receptors. However, NMDA receptors do not contribute to responses at very short latencies commensurate with monosynaptic thalamocortical relay for layer IV cells. These appear to be entirely mediated through non-NMDA receptors. (2) In the absence of transmission through NMDA receptors, non-NMDA receptors do not generate significant spike activity in later (10–100 msec latency) discharges. (3) NMDA receptor participation in first spike generation is directly dependent upon the latency of response of the cell to principal whisker deflection. (4) Latency of response, non-NMDA receptor-mediated spike generation and laminar location were powerfully covariant. (5) In addition, it was found that cells exhibiting short-duration spikes (<0.7 msec; "fast-spike units") in layer IV responded powerfully at short latencies, first spikes being entirely dependent upon non-NMDA but not NMDA receptor action, later spikes (10–100 msec poststimulus) being >80% dependent upon NMDA receptor action. It is concluded that most sensorially driven spike activity in layers I–IV is dependent upon NMDA receptor action. This appears to be enabled by contingent subthreshold depolarization largely through non-NMDA receptor action, whereas the earliest thalamocortical discharges are evoked solely through non-NMDA receptors.

[Key words: NMDA receptors, non-NMDA receptors, barrel cortex, somatosensory cortex, 2-amino-5-phosphonovaleric acid, thalamocortical, plasticity, latency, iontophoresis]

A generally accepted tenet in recent years has been that the NMDA receptor has a special role in plasticity, but a minor role in normal neural transmission (Collingridge and Bliss, 1987). This idea has its origins in studies on the behavior of the NMDA receptor in hippocampal slices. There, activation of the NMDA receptor is secondary in time and minor in magnitude to non-NMDA receptor activation, and the latter alone is responsible for significant neural transmission (Madison et al., 1991). Similarly most *in vitro* studies on neocortex at best have implied a supplementary role in normal neocortical transmission for the NMDA receptor (Artola and Singer, 1987, 1990; Sutor and Hablitz, 1989), although the functional participation of NMDA receptors in sensory cortex has been known for a number of years (Hicks and Guedes, 1983). Thus, neocortical NMDA receptors are characteristically associated with late EPSPs peaking some 20–60 msec after fast non-NMDA receptor-generated EPSPs. Second, in contrast to "fast" non-NMDA EPSPs, NMDA-mediated EPSPs are only easily shown under conditions designed to minimize IPSPs such as in the presence of GABAergic antagonists (Artola and Singer, 1987, 1990), or by careful choice of stimulus magnitude (Sutor and Hablitz, 1989) or using zero extracellular magnesium concentrations (Thomson et al., 1985; Thomson, 1986; Huettner and Baughman, 1988) to alleviate the voltage-dependent block of the NMDA receptor (Mayer et al., 1984; Nowak et al., 1984).

However, recent evidence from *in vivo* studies suggests a more substantial role for NMDA receptors in sensory transmission in neocortex. In several studies it has been shown that NMDA receptors contribute strongly to spike activity evoked by natural sensory stimulation in both somatosensory and visual neocortex (Tsumoto et al., 1987; Armstrong-James, 1989; Fox et al., 1989; Miller et al., 1989). In addition, within the ventrobasal complex both NMDA and non-NMDA receptors are involved in generating spike discharges in reply to whisker stimulation in the rat (Salt, 1987; Salt and Eaton, 1989). The importance of the role of NMDA receptors in normal sensory transmission in neocortex is highlighted by the current debate on a specific role for NMDA receptors in plasticity of the developing visual cortex (Kleinschmidt et al., 1987), where it has recently been suggested that suppression of NMDA receptor activity suppresses normal sensory transmission, which in turn could be responsible for failure of plasticity (Miller et al., 1989; Constantine-Paton et al., 1990; DeFreitas and Stryker, 1990).

In view of these findings, the present study seeks to disclose

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the contribution of NMDA receptors to normal transmission of sensory information in a different but highly organized area of somatosensory cortex, the barrel cortex of the rat, using iontophoretic application of antagonists to NMDA and non-NMDA receptors. The study capitalizes on recent studies on receptive field organization of the representation of mystacial vibrissae in this area in SI that have allowed a dissection of thalamocortical (T-C) and intracortical origins for responses (Armstrong-James and Fox, 1987; Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991). The fast and secure transmission of sensory information from whiskers to layer IV in this area also affords a distinct advantage over the visual system by allowing separate analysis of components of differing latency within the response and to determine if these are generated through different receptors.

From receptor binding studies (Monaghan and Cotman, 1985), NMDA receptor density is least in layer IV and greatest in the most superficial neocortical layers. Accordingly, two specific hypotheses are tested. The first is that the contribution of NMDA receptors to a cell's response varies according to its layer, and the second is that this is determined by its latency of response to the afferent stimulus. Implicit within the second hypothesis may be a dependence for NMDA receptor expression upon the number of serial intracortical synaptic relays interposing between T-C inputs and the response. To distinguish these variables, an extensive analysis of responses is undertaken of early and later components of peristimulus time histograms (PSTHs) for cells in different layers, and their modification by controlled iontophoresis of NMDA and non-NMDA antagonists.

The results suggest that non-NMDA receptors only appear to generate significant spike activity in reply to whisker deflection at very short latencies in barrel cortex commensurate with monosynaptic relay for layer IV cells. For the generation of nearly all other spikes from cells in layers I–IV transmission through NMDA receptors is required. These include fast responses generated at latencies within 3–5 msec of arrival of the T-C volley in layer IV.

Materials and Methods

Anesthesia and surgery. Male Wistar rats weighing from 220 to 340 gm were anesthetized with urethane (1.5 gm/kg body weight, i.p.; 20% solution in water). Following full anesthesia the scalp was infiltrated with local anesthetic and the posteromedial barrel subfield area of SI neocortex, here referred to as barrel cortex, was exposed 4–7 mm lateral to the midline and 0–4 mm posterior to bregma. Body temperature was maintained at 37°C by a rectal thermistor controlling a heating pad. Since anesthetic level profoundly affects response characteristics (Armstrong-James and George, 1988), anesthesia was maintained by supplementary urethane injections (10% original dose) such that layer V neurons, sampled after completing recordings in layer IV, exhibited bursts characteristic of stage III slow-wave sleep and light anesthesia (2–4 bursts/sec; Armstrong-James et al., 1985; Fox and Armstrong-James, 1986). This condition enabled rats to be maintained in full but light anesthesia whereby no signs of discomfort are expressed by animals. Respiration was regular (80–110 breaths/min in nearly all animals) and spontaneous movement absent. Long-latency and sluggish hindlimb withdrawal was achieved only with severe pinching of the hindfoot, but not always. However, at higher rates of bursting of layer V neurons (4 per second) slow eye blink reflexes could sometimes be achieved, though other movement or change in respiration was absent; in these cases, supplementary anesthetic was given.

Single-cell recording. Single-unit recording, focal EEG activity, and iontophoretic administration of drugs were achieved through low-noise multibarrel carbon-fiber microelectrodes (Armstrong-James and Millar, 1979; Armstrong-James et al., 1980). Single cells were discriminated using a waveform/window discriminator (Millar, 1983). All penetrations were radial, normal to the cortical surface, this being enabled by appropriate angling of the micromanipulator and reference to the atlas of Paxinos and Watson (1982). Tridimensional microdrives with an accuracy of 5 μm in all three dimensions were used for placement. The positions of all penetrations were mapped together with the subpial depths of neurons investigated. Penetration of the subarachnoid space was determined optically by penetration of the black tip of the microelectrode and additionally was heralded with an increase of noise by the electrode (about 50%). No dimpling of the cortical surface occurs with the slender profile of these electrodes, and consequently with egress of microelectrodes cell positions were invariably accurate to within 25 μm.

Data recording and analysis. Spike durations were measured for the majority of cells used for the analysis (44 of 51 cells). Early negative-going and the first positive-going phases were used for this purpose, duration being evaluated to the time when the positive-going component crossed zero potential. The mean of three spike durations was assessed on a 12-bit Nicolet digital storage oscilloscope.

Peristimulus time histograms (PSTHs) were generated by the application of 50 brief (3 msec duration, 0.5 msec rise time) 200 μm deflections of principal vibrissae 10 mm from the face (Armstrong-James and Fox, 1987). The principal whisker was defined as that giving the earliest latency response, or where no discrimination on this basis could be made the highest magnitude of response. For each cell the preferred stimulus orientation was assessed to the nearest quadrant of up, down, backward, and forward excursion of the whisker. PSTHs were constructed from one millisecond bins, where bin 0-1 was registered as the first bin poststimulus (latency, 0–1 msec). Latency histograms (LHs) were constructed for all cells in parallel. Where latencies are given in the text, these refer to modal latencies where the modal latency is defined as the most common first spike poststimulus time generated by the 50 stimuli (Armstrong-James and Fox, 1987).

Response magnitudes were quantified by cumulative counts of spikes generated in PSTHs during periods of 0–100 msec poststimulus. Counts were corrected for spontaneous activity by subtracting the number of spontaneous events arising 50 msec prior to the stimulus, this count being adjusted for the poststimulus period analyzed (Armstrong-James and Fox, 1987). Studies were restricted to cells responding to principal vibrissae in the caudal C–E rows. Stimulation repetition rate was maintained at one stimulus per 2 sec, as apposed to 1 stimulus per second in earlier studies (Armstrong-James and Fox, 1987; Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991). Principally, this increased the magnitude of responses of cells by 1.5–2-fold and in particular allowed an increase in probability of later discharges to single stimuli. Peak latencies were assessed to the nearest millisecond. For cells showing multimodal PSTHs, earlier responses achieved higher magnitude than later responses, so peak latencies refer to the early and not late responses. Data collection and analysis was enabled by a Dell 386 computer with a 1401plus CED interface (Cambridge Electronic Design Ltd., Cambridge, UK). Histogram data were stored on hard disk for later output to a Hewlett Packard digital plotter. Statistics and graphical analysis were carried out on exported data using a Macintosh IIsi computer and STATWORKS and CRICKET graph software (Apple).

Analysis of PSTHs. For this form of analysis PSTHs were broken down into inclusive poststimulus time epochs of 5–8, 8–10, 10–20, 20–45, and 50–100 msec, and spike counts for each epoch were registered. These were corrected for spontaneous activity registered for the 50 msec period prior to stimulation. The mean count for this period was subtracted from each epoch after adjustment *pro rata* for the epoch period. Epoch analysis ignores the position of any major component of the response but allows blocks of the PSTH to be analyzed at constant poststimulus intervals. The earliest epoch (5–8 msec) should encompass the very earliest latency spikes evoked within cortex by the stimuli used here (Armstrong-James and Fox, 1987; Armstrong-James et al., 1991) although not necessarily encompassing the major transient response, which was later for most cells. To assess the effect of the drug, for each epoch the number of spikes generated in the presence of the drug was expressed as a percentage of the number generated in the control response.

Cell classification. Cells were grouped by latency class, cell layer, and spike shape for the various forms of PSTH analysis given in Results. Usable results were achieved from 2 to 10 cells for each animal. The location of cells was established histologically by marking every penetration with two to four microlesions using tip negative currents of 1.5–

2.5 μA passed for 10 sec. For at least two cells in each penetration (nominally one in layer IV and one in layers II–III) lesion sites were placed at the positions of recorded cells following their examination for response. When placed within a barrel, such lesions have been found in the past to destroy a zone occupying <2% of the barrel volume (Armstrong-James and Fox, 1987). Following the termination of the experiment, animals were perfused with 200 ml of phosphate-buffered saline (PBS) at pH 7.3 with added 2% lignocaine and heparin (1.0 ml and 0.5 ml) and then with 4% paraformaldehyde and 2% glutaraldehyde in PBS. Brains were then removed and coronal 30 μm serial sections were stained with cresyl violet for identification of lesion sites. All layer IV cells were found to be located between 450 and 780 μm below the cortical surface as given by microelectrode drive readings. Since we were unable to establish a clear division between layer II and layer III, layer II/III subdivisions were nominally allocated by registering cells in the top half of layers II–III as layer II neurons and those in the lower half as layer III neurons. Cells ascribed in this way are labeled as layer II and layer III cells within the text.

Administration of drugs. Unless otherwise stated in the text, in standard experiments four iontophoretic barrels were available for drug applications, where agonists and antagonists to excitatory amino acid (EAA) receptors were employed. All drugs were made up on the day of the experiment. 6,7-Dinitroquinoxaline-2,3-dione (DNQX; Tocris Ltd.) was made up in 150 mM NaCl at a concentration of 4 mM (pH 9.0) and placed in one barrel, and D(−)-2-amino-5-phosphonovaleric acid (AP5; Sigma Inc.), *N*-methyl-D-aspartate (NMDA; Tocris Ltd.), and quisqualic acid (QUIS; Tocris Ltd.) each at a concentration of 100 mM (pH 7.8) were placed in other barrels. The pH of solutions was adjusted with 0.1 M HCl or 0.1 M NaOH. Negative current ejection was used for all drugs.

Protocol for iontophoresis. Newly filled electrodes were allowed to stabilize in saline for 30 min. Effective retaining (positive) currents were tested for microelectrodes in exploratory penetrations and ranged from 2 to 5 nA for NMDA, QUIS, and AP5 and from 5 to 13 nA for DNQX.

All cells were challenged with NMDA and QUIS using cyclical ejection currents applied to the appropriate barrels. Routinely, agonists were ejected in alternate cycles for 15 sec followed by 35 sec of retaining current application for each drug. Ejection currents for agonists were adjusted to elevate firing rates above control levels (0–1 Hz for most cells) by a minimum of 5 Hz and maximum of 25 Hz for different cells. Maximal firing rates to NMDA and QUIS administration were made similar. To establish rapid action, AP5 and DNQX were administered at an ejection current of 20–30 nA for 30 sec to achieve rapid efflux (Armstrong-James and Fox, 1983), followed by ejection at zero current while cyclical agonist trials were carried out. Following two trials with each agonist (NMDA and QUIS alternately), current for the antagonist barrel was progressively raised until response to the appropriate agonist was suppressed to a level of >90% but <100%. Specificity was then registered in a manner modified devised by Hicks et al. (1991): specificity $A' = 1 -$ (% decrement of control response A /% decrement of control response B), where A' is the nominal specific antagonist to agonist A and B is the other agonist. "Response" was assessed by automatic counting of numbers of spikes evoked over a 25 sec period commencing at the beginning of application of ejecting current for the agonist. In all cases background spontaneous firing rate was measured in the absence of agonist administration and the background count was subtracted to allow measurement of increase in firing rate above background. The mean count for two trials was used, these trials being those established when the effect of the antagonist reached the optimal value given above. Although agonists were only administered for 15 sec, the 25 sec period for assessment of response was required since responses typically outlasted the administration period (see Fig. 1).

When DNQX, a putative non-NMDA antagonist, was tested *alone* against responses to cyclical applications of QUIS and NMDA (12 cells), specificity for QUIS receptors was found to be poor. DNQX typically had the effect of suppressing both QUIS- and NMDA-induced discharges in parallel. In six cases virtually no specificity could be achieved (specificity index < 60%, where 50% = equal suppression of response to both agonists). The remaining six cases achieved specificity indices of 83%, 72%, 65%, 62%, 62%, and 61%, respectively. In view of this difficulty, DNQX was administered *in conjunction with* AP5 after cyclical QUIS and NMDA trials and response trials with AP5 alone were completed. DNQX administration was adjusted to reduced existing responses to QUIS agonist administrations by 90–100% (mean ± SEM, 94 ± 0.8%). Responses to whisker stimulation then were tested to evaluate remaining responses attributable to QUIS (non-NMDA) receptors. Few difficulties were experienced using AP5 as a specific antagonist of NMDA receptors. Further details are given in Results.

Stimulation trials were conducted immediately after the correct antagonist level had been established over a subsequent period of 100 sec. The ejection current for the antagonist was held at the same level while retaining currents were applied to agonist barrels. For any particular microelectrode, antagonist ejection currents required for this procedure typically varied by less than 3 nA. This allowed a rapid establishment of the correct ejection level for antagonists once the first cell had been characterized (this cell was not used for analysis).

Following stimulation trials in the presence of AP5 and DNQX, recovery to within 80% of the response magnitudes achieved for control trials typically took less than 20 min. No data were accepted if recovery to within 80% levels of the overall magnitude (0–100 msec count) of control responses failed to occur within 30 min.

Results

Cell populations studied

The studies presented here are confined to neurons within layers I–IV. Investigations were not carried out on cells in deeper layers largely due to the uncertainties of being able to administer drugs effectively over the substantial dendritic radii of those cells. Apical dendrites from cells in these layers (layer V–VI) penetrate most if not all superficial layers that render much of the dendritic membrane inaccessible to drugs administered iontophoretically in layers V and VI.

In preliminary studies we found that cells responded more profoundly when low stimulus repetition rates were used. In this study, stimuli were delivered at a rate of one every 2 sec and responses from single cells were analyzed for a period of 100 msec poststimulus from PSTH data. These responses were of greater magnitude and longer in duration than those generated by the higher-frequency (1/sec) stimulation rates used in previous investigations on rat barrel cortex neurons (e.g., Armstrong-James, 1989; Armstrong-James et al., 1991). Only responses to the principal (center-receptive) vibrissa are described here.

Useful data were achieved from 51 cells. For all 51 cells sensitivity to AP5 was tested. Of these, 30 cells were tested both with AP5 alone and AP5 and DNQX (administered together). The remaining 21 cells were tested only for sensitivity to AP5 since the latter had their response magnitudes reduced by >95% in the presence of AP5 ejected at a level adequate to suppress responses to NMDA by >92%, <100%. Consequently, they were not tested further with DNQX and AP5 together on the supposition that all responses were virtually all generated through NMDA receptors.

Specificity of AP5 and DNQX for NMDA and QUIS receptors

All 51 cells were subjected to cyclical administrations of NMDA and QUIS in the absence and then in the presence of AP5 to establish the relative specificity of the latter for NMDA receptors (see Materials and Methods). An example is shown in Figure 1. One condition for a cell to be acceptable for study was that the ratio of response to QUIS and AP5 administered together to QUIS alone had to exceed 0.7. If this was not the case, this meant than AP5 had depressed QUIS-evoked responses by more than 30%. Two cells failed to meet this criterion (0.55 and 0.33, respectively) and were rejected from the analysis. Their values are not included for construction of Figure 2.

For the acceptable cells, specificity indices for the suppression by AP5 of NMDA-induced discharges vis à vis QUIS discharges are shown in Figure 2. The results suggest that AP5 was highly

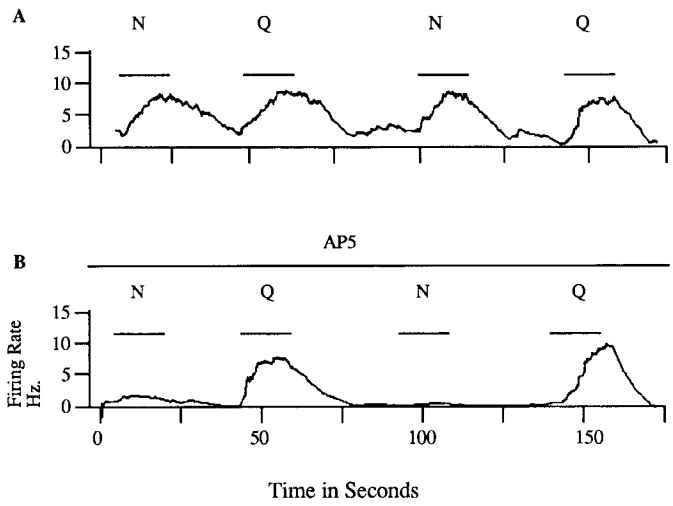

Figure 1. A typical example of results from a single neuron used to assess the specificity of AP5 for NMDA receptor-mediated discharge. *A*, NMDA (*N*) and QUIS (*Q*) are administered by iontophoretic ejection cyclically in alternate cycles to generate their agonist-induced discharges from the cell; 5 nA of ejection current for NMDA and 7 nA of ejection current for QUIS were used. *B*, As for the *A* but in the presence of AP5 (4.5 nA ejecting current). Full details of the methodology are given in Materials and Methods.

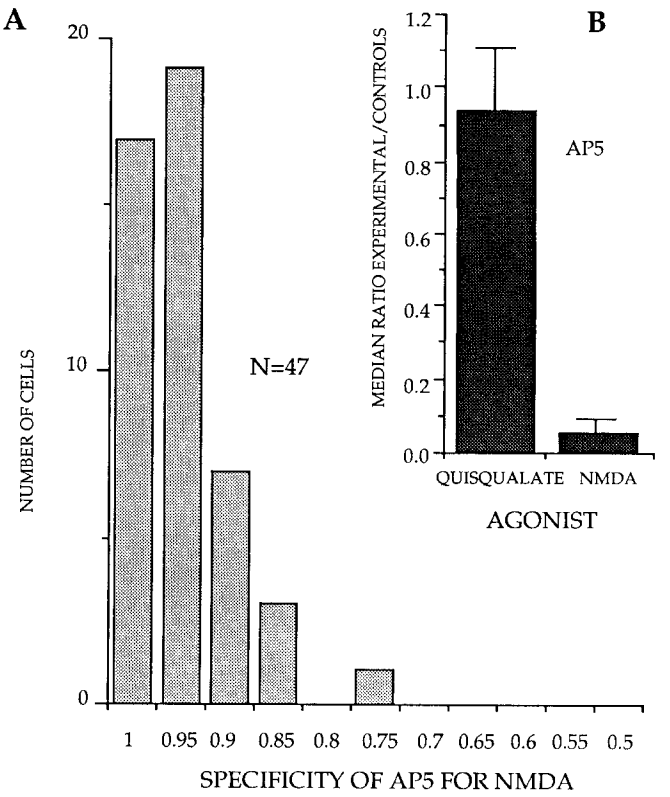

Figure 2. *A*, Specificity of AP5 for NMDA receptors evaluated for 47 of the 49 cells used for final analysis in Results. The two cells omitted had specificities of <0.7. Two additional cells failed to respond physiological to administrations of QUIS. These were used for further analysis and are described in Results. The method for evaluating specificity is given in Materials and Methods, and follows that devised by Hicks et al. (1991) for iontophoretic experiments. *B* shows the median values for the magnitude of responses to iontophoretically administered QUIS and NMDA divided by the magnitude of responses to the same agonists in the presence of AP5. *Bars* are interquartile ranges.

specific for NMDA receptors. For 2 of the 49 cells (not included in Fig. 2) we were unable to evoke satisfactory responses to QUIS during agonist trials. These cells were both superficial (layer II cells). Both required abnormally high ejecting currents for QUIS (50 and 65 nA) to evoke any discharge, this being highly irregular and accompanied by massive discharge of surrounding cells. Both cells responded adequately to NMDA at low currents (3 and 4.5 nA), these responses being highly efficiently blocked by AP5. These cells were included for analysis with regard to AP5 sensitivity, on the supposition that they possessed few, if any, QUIS receptors but efficient NMDA receptors.

PSTH and LH analysis

PSTHs and LHs were constructed for all response trials and printed out for visual inspection. From these it was evident that cells located in layer IV commonly exhibited bimodal PSTHs whereas those located in layer II most commonly exhibited unimodal or diffuse PSTHs. Layer III cells tended to be intermediate, some exhibiting bimodal, others unimodal histograms. Examples of PSTHs for layer II and IV cells are shown in Figure 3. This figure additionally shows the effect of AP5 and AP5 and DNQX together on the responses of these cells to principal whisker stimulation. Typically the earliest latency evoked spikes (5–8 msec poststimulus) were insensitive to AP5, but sensitive to DNQX, whereas longer-latency evoked spikes were sensitive to AP5.

In previous studies using PSTH analysis, spikes evoked at latencies of between 5 and 8 msec to principal whisker stimulation have been identified as arising by monosynaptic relay T-C relays via the ventral posterior medial nucleus (VPM) of the thalamus (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991). In view of this, for all forms of analysis given below, PSTHs were divided into sequential epochs of 5–8, 8–10, 10–20, 20–50, and 50–100 msec poststimulus, and these epochs used for analysis of sensitivity to AP5 and AP5 and DNQX together. This type of analysis is defined here as "epoch analysis."

Epoch analysis of sensitivity to AP5 and DNQX—laminar organization

Following classification of cells by laminar location, the data from PSTHs were grouped for each poststimulus epoch for cells classified into layers II ($N = 11$), III ($N = 10$), and IV ($N = 28$) categories, numbers referring to cells successfully studied in full. The averaged magnitudes of responses for each epoch were then derived by summing all responses by epoch for cells in each layer. The averaged responses are shown in Figure 4*A*. Several features are evident for cells in different layers that could commonly be detected also in individual responses. First, a progressive increase in response magnitude for response in the earliest epoch (5–8 msec poststimulus) is apparent on moving from layer II to III to IV. No responses were detected earlier than 5 msec poststimulus. Second, cells in layer IV exhibited bimodal PSTHs with modes at 5–8 msec and 20–50 msec poststimulus. Third, the second mode (20–50 msec) is common for cells in all three layers. Finally, overall response magnitude (sum of all epochs) is considerably greater for layer IV cells on the average than for cells in the supragranular layers.

Figure 4*B* shows the statistical effect of iontophoresis of AP5 at levels specific for NMDA receptors on cells grouped for layers

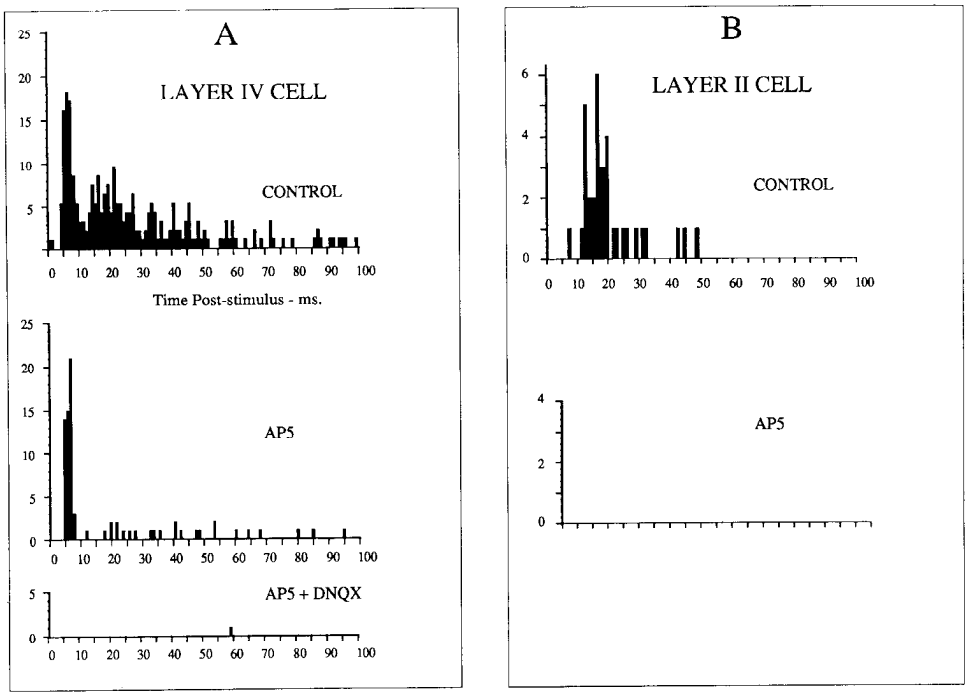

Figure 3. Examples of PSTH data achieved for neurons in layers IV (*A*) and II (*B*) in response to 50 deflections of the principal (center-receptive) whisker for the column. See Materials and Methods for definition of principal whisker and details for PSTH construction. In the *upper histograms* responses were generated in the absence of drugs (control responses); *center histograms* are in the presence of iontophoresis of AP5 alone; *lower histogram* in *A* is in the presence of iontophoresis of AP5 and DNQX. Note the characteristic bimodal response of layer IV cells and unimodal response of layer II cells. Note also the resistance to AP5 at a dose specific for NMDA receptors of early responses (5–8 msec) for the layer IV cell and the elimination of this response by co-iontophoresis of DNQX. Most responses and virtually all in layer II cells were removed by AP5 alone.

II–III and for layer IV cells, illustrated by epoch analysis. AP5 had virtually no overall effect on responses generated in the first epoch (5–8 msec) poststimulus, but a substantial effect on responses in subsequent epochs. Statistical analysis for layer II–III cells indicated a significant increase in sensitivity to AP5 for responses in the 20–50 msec epoch compared with those in the 10–20 msec epoch ($p = 0.037$, paired t test). For other sequential epoch pairs, no significant differences were apparent ($p < 0.05$). For layer II–III cells responses in the first epoch were too small for statistical analysis. For layer IV cells differences in sensitivity to AP5 were highly significant between the 5–8 and 8–10 msec epochs ($p < 0.002$), and between the 8–10 and 10–20 epochs ($p < 0.005$). The very slight fall in sensitivity to AP5 for the 20–50 msec epoch compared with the previous epoch (10–20 msec) was significant at the 0.02 level. Comparing the 20–50 msec epoch with the last epoch (50–100 msec), no significant difference arose ($p = 0.24$). Remaining responses were deemed solely as generated through non-NMDA receptors on the finding that virtually all remaining responses for all cells in the presence of AP5 were eliminated by DNQX and AP5 at doses adequate to just suppress QUIS-generated responses (see Materials and Methods). Responses in the presence of DNQX were considered null if less than three spikes in the first epoch remained, or less than two spikes in any other epoch. For cells in layer IV, 26 of 28 cells matched this criterion. For the remaining two cells, three spikes remained in the first epoch. For all layer II/III cells the above criterion was matched where DNQX was applied (for cells where AP5 did not remove all spikes generated). In summary, the results suggest a progressive and rapid involvement of NMDA receptors in responses from 8–10 msec poststimulus, particularly for all neurons with an initial solitary involvement of non-NMDA receptors for earliest discharges of layer IV cells.

Cells classified by latency

One objective was to find if NMDA receptor participation in responses differed for cells exhibiting different modal latencies. Cells successfully studied were divided into short-latency (5–8 msec; $n = 17$) and long-latency (8 msec and greater; $n = 32$) categories according to the peak modal latencies registered from LH analysis. Cells were categorized regardless of layer. Similar analyses to those presented for cells classified by layer (Fig. 4) are shown in Figure 5. Figure 5*A* illustrates the averaged shapes of PSTHs arranged by epochs for cells classified as short-latency cells (class A cells), which exhibited modal latencies of 5–8 msec, and longer-latency cells (class B cells), exhibiting modal latencies of 8 msec or greater. Short-latency cells predominated in layer IV and were not found in layer II. In this respect, 14 of 28 cells in layer IV, 3 of 10 cells in layer III, and 0 of 11 cells in layer II were categorized as short-latency class A cells. Class A cells clearly exhibited bimodal PSTHs, as is apparent from the epoch analysis shown in Figure 5*A*, whereas class B cells overwhelmingly exhibited unimodal PSTHs (upper right, Fig. 5*A*). Average response magnitudes for class A cells were greater. The greatest disparity arose for the earliest epoch (5–8 msec poststimulus), where class A cells derived their additional early modal response. Unlike class A cells, class B cells exhibited a broad late mode within the range of 10–50 msec poststimulus, whereas the second mode for class A cells was clearly at 20–50 msec poststimulus.

Statistical differences in sensitivity to AP5 by epoch analysis are shown for the two cell classes in Figure 5*B*. In general, a pattern similar to the differentiation evident for layer II/III cells and layer IV cells is apparent, which largely but not totally arises from the preponderance of short-latency cells located in layer IV, 50% of which were class A.

For class B cells, a substantial difference in sensitivity to AP5 is apparent between the first and second epochs (5–8 and 8–10 msec). However, this difference was not significant due to the very small number of cells in this class (5 of 32, all layer III) yielding any significant response at 5–8 msec poststimulus. In comparing the second and third epochs (8–10 and 10–20 msec), paired t test analysis indicated a highly significant increase in sensitivity from the earlier to the later epoch ($p = 0.005$). No further statistically significant increases in sensitivity were ap-

CELLS BY LAYER

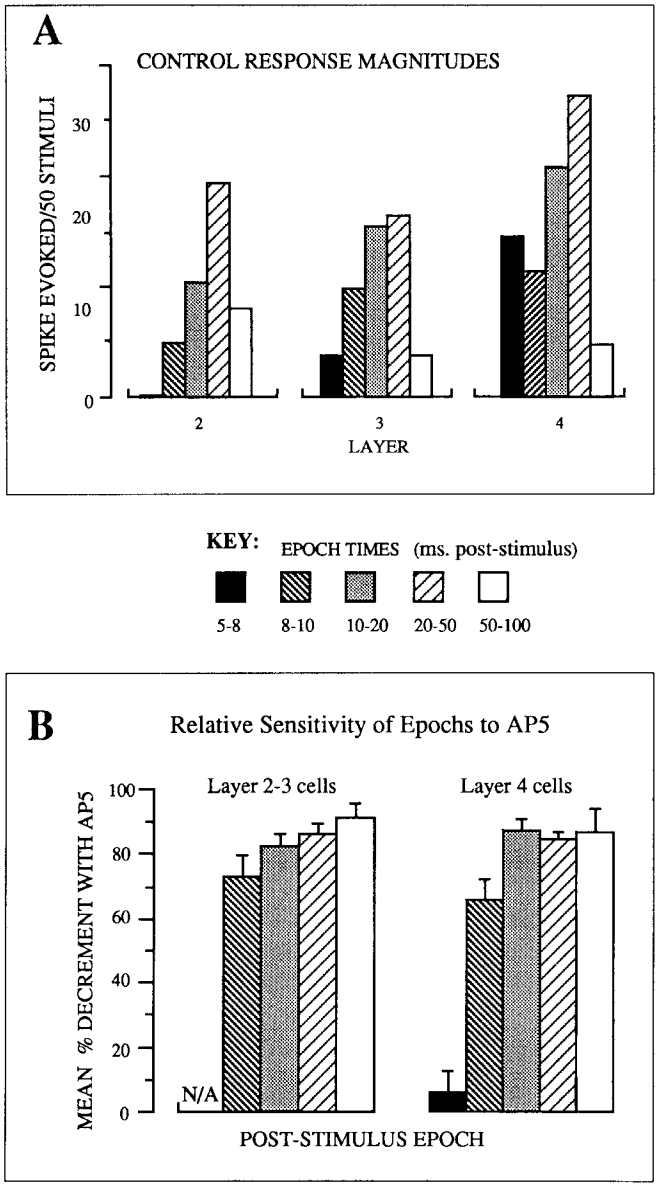

Figure 4. *A*, This set of histograms shows the pooled PSTHs for all cells according to layer, illustrated in terms of response magnitude (RM) for each epoch, where RM = number of spikes generated by 50 deflections of the principal whisker delivered at a rate of one stimulus per 2 sec (see Materials and Methods for further details on stimuli). Each PSTH was divided into sequential epochs of 5–8, 8–10, 10–20, 20–50, and 50–100 msec poststimulus. Note that latencies of 5–8 msec indicated responses with modal latencies arising in the fifth, sixth, and seventh bin poststimulus, where each bin is 1 msec in duration. Other epochs follow the same format. *B*, These histograms illustrate by sequential epoch analysis the mean percentage attenuation of control responses caused by iontophoretically administered AP5 at a dose specific for NMDA receptors (see Fig. 2) . Neurons for layers II and III were grouped since results were very similar for the two layers. An identical form of analysis was used for subsequent figures. Counts in epochs for experimental PSTHs were divided by counts for controls and multiplied by 100 to give percentage deficit in the presence of the drug. Histogram amplitudes are means of the data, and error bars represent SEMs. Where control responses of <5 spikes per epoch occurred, data were rejected for analysis for this and all subsequent figures. For this and all subsequent figures, pooled results for cells in individual neuron classes were statistically analyzed for each epoch using the paired *t* test for comparison between epochs (see Results). Note the resistance of the earliest responses (5–8 msec poststimulus) to AP5.

LONG AND SHORT LATENCY CELLS

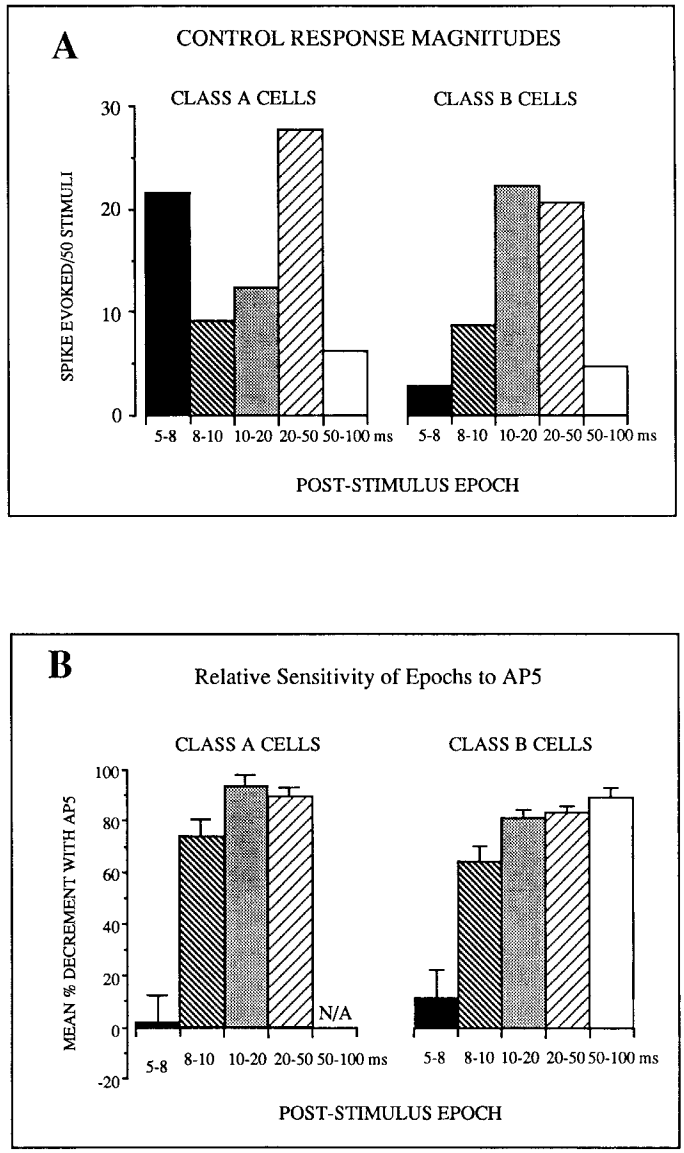

Figure 5. *A*, This set of histograms shows the pooled PSTHs for all cells according to modal latency of the first response, illustrated in terms of response magnitude for each epoch. *Class A cells* were defined as those responding at modal latencies of within 5–7 msec of the stimulus. *Class B cells* are those responding at all greater latencies. For details of epoch analysis, see Figure 4*A*. Note that class A cells differ principally from class B cells in exhibiting a bimodal response with a prevalent early response lacking in class B cells. *B*, These histograms illustrate by sequential epoch analysis the mean percentage attenuation of control responses caused by iontophoretically administered AP5 at a dose specific for NMDA receptors (see Figs. 2, 4*B*). Neurons are grouped according to modal latency class. Histogram amplitudes are means of the data, and error bars represent SEMs. For further details on statistical analysis, see Figure 4*B*. Note the resistance of the earliest responses (5–8 msec poststimulus) to AP5, particularly for class A cells.

parent between sequential pairs of later epochs (10–20, 20–50, and 50–100 msec; $p < 0.05$).

For class A cells, differences in sensitivity to AP5 were highly significant between the 5–8 and 8–10 msec epochs ($p < 0.005$), but not between the 8–10 and 10–20 msec epochs or between the 10–20 and 20–50 msec epochs ($p > 0.05$). The number of cells producing responses at 50–100 msec were too few for statistical analysis.

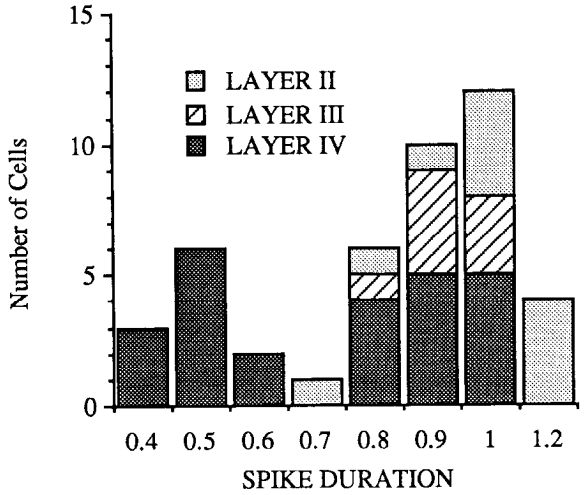

Figure 6. The distribution by spike duration of all cells for which spike duration data was gathered ($N = 44$). The laminar locations of cells is also illustrated. Cells with durations of <0.7 msec were classified as FSUs following the nomenclature of Simons (1978). For the technique of measuring spike durations, see Materials and Methods.

With coapplication of DNQX with AP5, all responses were lost for class B cells, with less than two spikes in any epoch after application of AP5. Two class A cells (found in layer IV) had three spikes, each in epoch 1 (5–8 msec poststimulus), resistant to DNQX at a level adequate for suppression of QUIS-evoked responses. All other class A cells (15) had their remaining responses in the presence of AP5 eliminated by DNQX (<2 spikes/epoch). The results suggest that earliest discharges of short-latency cells (5–8 msec poststimulus) are entirely resistant to AP5. Statistically, discharges of these cells at 8–10 msec poststimulus also show a modicum of resistance to AP5, although the greatest proportion of the response in this epoch is suppressed by the drug. Later responses, from 10 msec poststimulus, are maximally suppressed by AP5 (means, 89.2–93.5%).

Cells classified by spike duration

For 44 cells, spike durations were measured as described in Materials and Methods. The numerical distribution of cells classified by spike duration is illustrated in Figure 6. In addition, the laminar location of cells is shown. The distribution is bimodal, and accordingly, cells with durations of <0.7 msec were categorized as "fast-spike units" (FSUs). Those with durations of 0.8 msec or more were classified as "regular-spike units" (RSUs). The classification scheme follows that of Simons (1978). From Figure 6, all cells with spike durations of 0.6 msec or less were located in layer IV. A substantial proportion of layer IV cells had long-duration spikes, as did all cells in layer III. Apart from the intermediate unit in layer II (0.7 msec duration), layer II cells generally possessed the longest spike durations, with spikes of 1.2 msec in duration all being located in this layer. In summary, the results suggest a decrease in spike duration from layer II to layer III to layer IV.

FSUs and RSUs in layer IV

The responses of these two cell classes found in layer IV were evaluated by PSTH analysis (1) in the absence of drugs and then (2) in the presence of AP5 and finally (3) in the presence of AP5 and DNQX administered together. The findings are presented in Figure 7. From Figure 7*A* it is clear that FSUs generated much larger response magnitudes to principal whisker stimu-

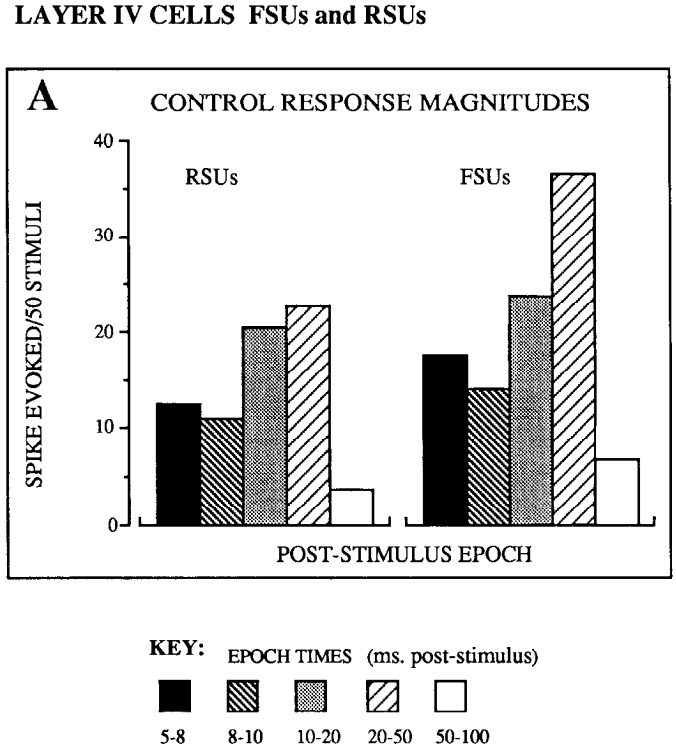

Figure 7. *A*, This set of histograms shows the pooled PSTHs cells in layer IV subdivided according to the duration of their action potentials (see Fig. 6). FSUs ("fast-spike units") were classified as those cells having action potentials of less than 0.7 msec in duration. Other cells were classified as RSUs ("regular-spike units"). For details of epoch analysis, see Figure 4*A*. Note that FSUs differ principally from RSUs in the high magnitude of their response in any epoch compared with RSUs. Both classes of layer IV cells exhibit bimodal PSTHs as shown by epoch analysis. *B*, These histograms illustrate by sequential epoch analysis the mean percentage attenuation of control responses caused by iontophoretically administered AP5 at a dose specific for NMDA receptors (see Figs. 2, 4*B*). Neurons are separately grouped into RSU and FSU classes. Histogram amplitudes are means of the data, and error bars represent SEMs. For further details on statistical analysis, see Figure 4*B*. Note the resistance of the earliest responses (5–8 msec poststimulus) to AP5, particularly for FSUs.

lation than did RSUs. This was the case for each poststimulus epoch and reflected a tendency for FSUs to respond with high-frequency burst discharges. Characteristically, these discharges terminated abruptly after some 40–50 msec. It was also notable that FSUs commonly exhibited a higher rate of spontaneous discharge than RSUs. The maximum number of spikes was generated at 20–50 msec poststimulus, and this was associated with a secondary mode in the PSTHs of these cells. Nearly all had bimodal PSTHs, with a first mode at 5–10 msec poststimulus. RSUs exhibited a very similar form of response to FSUs on the average, but generally response magnitudes were much smaller (mean, 1.14 spikes/stimulus compared with 2.0 spikes/stimulus), particularly later on during the PSTH.

Figure 7*B* compares the statistical findings for sensitivity to AP5 of RSUs ($N = 14$) and FSUs ($N = 11$) where results were available using epoch analysis. With further coadministration of DNQX in the presence of AP5, responses of all cells save two (both RSUs) were eliminated (to <3 spikes in the 5–8 msec epoch, <2 spikes in other epochs).

For both groups of cells, sensitivity to AP5 was maximal for discharges from 10–100 msec poststimulus ($p \ll 0.05$ for difference between epoch pairs, paired t test), suggesting an overwhelming involvement of NMDA receptors in discharges during these poststimulus periods. For FSUs, discharges in the first epoch (5–8 msec poststimulus) were not affected significantly by AP5; in the presence of AP5 these responses did not differ significantly from control responses ($p = 0.36$, paired t test). For RSUs, first responses (5–8 msec poststimulus) in the presence of AP5 were also not significantly different from control responses ($p = 0.09$). When comparing sensitivity for sequential pairs of epochs, differences between the first two epochs for FSUs (5–8 and 8–10 msec) were significant ($p < 0.05$), as were differences between the second and third epochs (10–20 and 20–50 msec; $p < 0.02$). The findings for FSUs suggested that NMDA receptors did not participate in responses during the first epoch, but contributed significantly during the second epoch, some 50% of responses here being suppressed by AP5. By 10 msec poststimulus virtually all responses generated in these cells were realized through NMDA receptors. For RSUs, differences in sensitivity between the first and second, and second and third epochs (respectively, 5–8 and 8–10, and 8–10 and 10–20 msec) were highly significant ($p < 0.001$ and $p < 0.003$, respectively). For RSUs there is a rapid rise in participation of NMDA receptors in responses from low values at 5–8 msec poststimulus (mean, 17%) to saturation (90%) levels at 10 msec poststimulus. A small but significant generation of responses solely through non-NMDA receptors is implied at 8–10 msec poststimulus by the substantial significant difference between values for this and the subsequent epoch in suppression by AP5.

Discussion

Specificity of antagonists

DNQX has been claimed to be specific for non-NMDA receptors in a number of preparations (Collins and Buckley, 1989; Yamada et al., 1989; Hartveit and Heggelund, 1990), whereas in our study administration of DNQX alone substantially reduced spike discharges evoked by iontophoretically applied NMDA at current levels just adequate to eliminate discharges evoked by iontophoretically applied QUIS. However, the conductance of the NMDA receptor channel is highly voltage dependent, blockade of the channel at membrane potentials of −30 to −80 only being relieved by contingent depolarization of the postsynaptic cell (Mayer and Westbrook, 1985). Thus, the most likely explanation for the actions of DNQX alone on NMDA-evoked discharge in our study is that prevailing subthreshold EPSPs generated through non-NMDA receptors are antagonized, which afforded adequate depolarization of cells to allow NMDA receptors to overcome their voltage dependence blockade and evoke discharges. Under these conditions DNQX would remain "specific" for non-NMDA receptors but would also prevent discharge through NMDA receptors by removing contingent non-NMDA-mediated EPSPs. This is supported by *in vivo* studies on neurons in the rat VPM responding to air jet stimuli applied to the whiskers (Salt and Eaton, 1989). The authors suggested that the NMDA-mediated component of their responses was dependent upon a contingent depolarization through non-NMDA receptors, since DNQX when administered iontophoretically alone suppressed responses previously blocked by AP5 alone.

The actions of DNQX alone strongly contrasted with equivalent tests for AP5, which was found to be highly specific in eliminating NMDA-evoked spikes while leaving QUIS-evoked spikes essentially intact. Thus, dependency for generation of spike responses on NMDA receptor action could therefore be ascertained directly by their sensitivity to AP5. In our protocol for iontophoresis, any discharges that remained in the presence of AP5 which subsequently were eliminated by co-iontophoresis of DNQX could be assumed to be entirely dependent upon non-NMDA receptor action. This can be ascertained with some certainty since (1) here DNQX would only be acting on non-NMDA receptors, (2) no effect of DNQX on receptors other than EAA receptors has been reported, (3) AP5 did not affect responses to QUIS, and (4) in contrast to NMDA receptors, non-NMDA receptor channels are not voltage sensitive (Mayer and Westbrook, 1984).

Participation of NMDA and non-NMDA receptors in early and later responses

A principal finding was that spikes generated in barrel cortex by principal whisker stimulation at very short latencies (5–8 msec poststimulus) were almost entirely mediated through non-NMDA receptors. These responses were essentially unaffected by AP5 but eliminated by DNQX. Second, NMDA receptor action was required for nearly all spikes generated from 10 to 100 msec poststimulus, although contingent coactivation of non-NMDA receptors at levels subthreshold for spike generation is also presumably required to bring NMDA receptors close to threshold. The possibility that subthreshold depolarizing influences through nonspecific inputs to cortex also contribute is discussed below. Suppression by AP5 of late discharges (20–100 msec poststimulus) attained values ranging from 82% to 100% (mean values). At early latencies of some 3–5 msec following the very earliest discharge (8–10 msec), NMDA receptor action was required for generating most spikes from layer II–III cells where >70% of the response is dependent upon their action. For this epoch, NMDA receptors contributed least to the generation of spikes for the FSU cell class (49% suppression by AP5), with the remaining spike responses in the epoch being mediated solely through non-NMDA receptors.

Previous findings strongly suggest that virtually all spikes at 5–8 msec latencies are generated directly by T-C afferents (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991).

In reply to principal whisker deflection, these spikes evoked at these latencies are virtually restricted to cells in layer IV, where T-C afferents overwhelmingly terminate (Killackey, 1973; Jensen and Killackey, 1987). Spikes at 5–8 msec latencies were deduced to represent the bulk of T-C–evoked first spikes, since >90% of cells in the thalamic relay, the VPM, responded to the same stimuli applied to principal whiskers with latencies of 4–6 msec (Armstrong-James and Callahan, 1991). From these and the present findings we conclude that first discharges of layer IV cells to the monosynaptic relay from VPM are almost exclusively mediated through non-NMDA receptors.

From the analysis of sensitivity to AP5 of responses within the second epoch (8–10 msec poststimulus), we conclude that NMDA receptor action is required for some 50–70% of the discharges evoked within some 3–5 msec of the arrival of the T-C input to layer IV cells, which in turn implies that fast EPSPs are evoked through NMDA receptors in response to natural sensory stimulation. Whether some of these discharges are evoked directly by T-C inputs remains to be established. A mono- or disynaptic intracortical relay may also intervene, although if this were so the rise time of EPSPs through NMDA receptors would be exceptionally fast.

The finding that spikes generated solely through non-NMDA receptors are largely confined to layer IV cells is in accordance with findings from receptor binding studies that non-NMDA receptors are most densely concentrated in layer IV in rat parietal neocortex (Monaghan and Cotman, 1985). Our findings also compliment those of Fox et al. (1989), who in iontophoretic studies on cat visual cortex *in vivo* found that visual responses of layer II and layer III cells were greatly suppressed by AP5, although their layer IV cells were more resistant to AP5 (9–22% response reduction for four cells, 0% for six cells). No evidence for differentiation by latency of spikes according to dominance by NMDA or non-NMDA receptors was found in that study, however, since moving bars of light were used to evoke discharges, precluding latency measurements.

In cat visual cortex *in vivo* (DeFreitas and Stryker, 1990), cat lateral geniculate *in vivo* (Hartveit and Heggelund, 1990), and cat somatosensory cortex (Hicks et al., 1991), DNQX has been shown to antagonize non-NMDA receptor-mediated responses effectively. Although laminar locations or latencies were not measured, the careful study by Hicks et al. (1991) indicates a more powerful involvement of non-NMDA receptors in *directly* evoking spikes than in our study, their contribution being about double that of NMDA receptors. One reason for the difference from our findings may lie in the fact that barbiturate anesthesia was used in their study and urethane anesthesia in ours. T. P. Hicks (personal communication) has expressed agreement with this possibility but also suggests that a species difference may well be involved.

Bimodal and unimodal responses

Cells responding with a significant early (5–8 msec) discharge exhibited bimodal histograms, the second mode nearly always occurring at 20–50 msec poststimulus. This "secondary" mode was apparent in cells of all types, whether they were classified in terms of layer, latency class, or spike duration. A variable-amplitude first mode at 5–8 msec poststimulus occurred for cells classified in these different ways. For layer II cells the early mode was absent, suggesting a lack of T-C input to these cells. For these cells the second mode time coincided on the average with the second mode time for layer IV cells. For layer III cells the early mode was very much smaller in magnitude compared to any type of layer IV cell, suggesting a very limited involvement of direct T-C inputs. The observations for the first-mode responses are entirely in accordance with the known pattern of termination of T-C inputs originating from VPM that innervate layer IV massively and to a small extent the bottom of layer III (Killackey, 1973; Jensen and Killackey, 1987).

FSUs and RSUs

The cells that exhibited the greatest magnitude of response were FSUs. These were distinguished by the high amplitude of both the first response (5–8 msec) and the second mode at 20–50 msec poststimulus. These were exclusively found in layer IV in the present study. Similar neurons have been described in other layers of somatosensory cortex in the rat (Simons, 1978) and visual and somatosensory cortex of the rabbit (Swadlow, 1988, 1989). In common with our findings, these authors found they had short action potential durations, responded with high response magnitudes, had high spontaneous firing rates, and were most commonly found in layer IV. Average response magnitudes for these cells in our study were some twofold those of layer IV RSUs. Studies *in vitro* in rodent SI neocortex have suggested that cells exhibiting short action potentials are inhibitory interneurons (McCormick et al., 1985; Agmon and Connors, 1992), which also have been found to be glutamic acid decarboxylase immunopositive (McCormick et al., 1985). Of interest to the present study is the finding that these cells fired at the very shortest latencies to principal whisker inputs, and possessed the largest earliest spike responses attributable solely to non-NMDA receptor action. This suggests that the first response in layer IV in reply to stimulation of the principal input is compounded of both inhibition and excitation of layer IV neurons.

Comparison with in vitro *studies on neocortex*

The fast generation of spikes through NMDA receptor action found in our study is not apparent in most *in vitro* neocortical studies (Thomson, 1986; Artola and Singer, 1987, 1990; Sutor and Hablitz, 1989; Thomson et al., 1989b). Peak latencies for AP5-sensitive components of EPSPs in layer II/III cells have been estimated typically at 25–60 msec to white matter stimulation (Sutor and Hablitz, 1989; Artola and Singer 1990), whereas in the present experiments the earliest spike activity dependent upon NMDA receptor action in layers III and IV dominated responses within 5 msec of the arrival of the T-C input to layer IV. The latencies given for the above studies are more akin to the latencies for our second-mode responses, including the only mode for layer II cells, and may well arise from secondary intracortical relay. Similarly, spikes generated solely through non-NMDA receptor action generally peaked within 3 msec of the T-C volley reaching layer IV in our study, compared with mean latencies of 20 msec or so for non-NMDA EPSPs generated by white matter stimulation *in vitro* (Thomson et al., 1989b; Artola and Singer, 1990), the latencies in these studies being equivalent to second-mode responses in ours. We could find little evidence that non-NMDA receptors reached threshold for generating spikes at such latencies, since virtually all spikes at these latencies were removed by AP5. The long latency for AP5-sensitive EPSPs *in vitro* is usually taken to suggest a polysynaptic origin (Thomson, 1986; Sutor and Hablitz, 1989). Sup-

port for this comes from the finding that about 80% of all axodendritic (excitatory) synapses originate from intrinsic neurons in layer IV of barrel cortex (White, 1979), and second, the common second mode (20–50 msec poststimulus) for the late discharge for cells in all layers cannot be attributed to a direct T-C origin in layer II. Third, surround receptive field responses, which have an intracortical origin arising from column-to-column relay (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991), are entirely eliminated by AP5 (Armstrong-James, 1989).

However, in recent studies on slices of visual cortex of the rat and cat, NMDA receptor components of EPSPs generated at short (4–6 msec) latency from layer II/III and V pyramidal cells in reply to intracortical stimulation have been demonstrated (Jones and Baughman, 1988; Shirokawa et al., 1989; Nishigori et al., 1990). Short-rise-time (about 3 msec) NMDA components of mixed non-NMDA/NMDA EPSPs also have been revealed by spike-triggered averaging for coupled layer II/III cells in the rat *in vitro* (Thomson et al., 1989a). Thomson (1986) found also that about one-half of the EPSPs elicited by threshold callosal stimulation in rat neocortical slices in normal physiological ringer were suppressed by AP5.

Origins of short-latency NMDA receptor activity in sensory responses

For short-latency cells, our findings suggest that significant proportions of NMDA receptor-gated ion channels are within 3–5 msec of generating full conductance in reply to natural T-C volleys. However, this speed of activation is unexpected for the activation of the NMDA receptor-gated ion channel based on the observed slow dynamics of the receptor–ligand interaction *in vitro* (Mayer et al., 1984; Nowak et al., 1984). The short latency of the fast NMDA-mediated EPSPs precludes fatigue of inhibitory circuitry by the afferent volley. High-frequency summation of afferent volleys as a precondition for summation at NMDA receptors would also seem unlikely in view of an independence in contribution to magnitude of response (Fox et al., 1991) and the finding that weak inputs from surround receptive fields preferentially activate NMDA receptors (Armstrong-James, 1989).

One explanation for fast NMDA-mediated excitation is that enough EAA neurotransmitter liberated by spontaneous T-C activity remains bound to the NMDA receptor at the time of the input volley to allow rapid opening of the ion channel, if EAA neurotransmitter binding is the main rate-limiting factor for NMDA receptor activity. Although this is possible, the spontaneous discharge of ventrobasal thalamic cells under identical conditions to those used here is low (<2 Hz; Armstrong-James and Callahan, 1991). However, a subset of synapses possessing bound glutamate may be activated by the afferent volley, this subset varying according to which group of VPM cells has recently spontaneously discharged.

A further condition should be that the ongoing average depolarization of the cell induced by other inputs, perhaps through non-NMDA receptor action, is adequate *in vivo* to relieve the voltage-dependent magnesium block of the NMDA receptor ion channel (Mayer et al., 1984; Nowak et al., 1984; Mayer and Westbrook, 1985), but not *in vitro*. This would also explain the independence of expression of NMDA-mediated components from magnitude of response (Fox et al., 1991). In common with unanesthetized animals (Armstrong-James and Fox, 1988a), most cells exhibited spontaneous activity. With *in vitro* studies little spontaneous activity is evident, and this is presumably due to a total lack of active inputs to neocortex. Under these conditions cells will be expected to exhibit little or no ongoing depolarization or bound transmitter. *In vivo*, however, a plethora of potentially depolarizing influences on cells occur from a multitude of afferent inputs that are absent *in vitro*, including serotoninergic, cholinergic (Sato et al., 1987), and noradrenergic inputs (Armstrong-James and Fox, 1983) and glutamatergic intralaminar inputs (Fox and Armstrong-James, 1986; Armstrong-James and Fox, 1988a), which together establish the pattern of spontaneous activity. Significantly, spikes evoked through intralaminar inputs are largely dependent upon NMDA receptor action (Armstrong-James et al., 1985; Fox and Armstrong-James, 1986). Statistically, there would seem to be a high chance of adequate depolarization, and binding of EAA neurotransmitter through specific and nonspecific thalamic inputs, to allow any remaining voltage dependence of the NMDA receptor to be overcome most times that the somatic input is activated. A logical outcome of this hypothesis would be that cortical cells *in vivo* exhibit average membrane potentials closer to threshold than *in vitro*, although this has yet to be established due to the damaging effects of penetrating the membrane (Ferster and Lindstrom, 1983).

In conclusion, it seems likely that several factors contribute to the prevalent activity of NMDA over non-NMDA receptors in generating spikes for normal transmission in rat barrel neocortex *in vivo*. Enough NMDA receptors on neurons in layer IV appear to be depolarized beyond their voltage-dependent level and perhaps have sufficient EAA transmitter bound on arrival of the T-C volley to produce a fast NMDA receptor-mediated EPSP, which in turn evokes spike output from the cell. Coactivation of non-NMDA receptors by afferent and/or other inputs appears to be necessary for all cells to enable NMDA receptors to generate copious spike discharges in reply to sensory stimulation, although non-NMDA receptors alone do not evoke significant spike discharge in cells other than to T-C volleys for fast layer IV cells. Active corticopetal pathways *in vivo*, which are inactive or destroyed *in vitro*, are assumed to cooperate in generating the primary depolarizing conditions whereby release of NMDA receptors from magnesium block (Mayer and Westbrook, 1985) enables their activation for virtually all neocortical cells in reply to the somatosensory input.

Consistency with current models of NMDA receptor activation

The model for activation of NMDA receptors most commonly quoted largely arises from studies *in vitro* on the CA1 field of the hippocampus (Herron et al., 1986; Cotman et al., 1988; Collingridge and Lester, 1989). From these studies the notion arose that any significant activation of NMDA receptors is conditional upon powerful postsynaptic depolarization, such as occurs under conditions of high-frequency stimulation (Collingridge and Lester, 1989), and that significant activation of NMDA receptors leads to long-term potentiation (LTP). The conditions of their model also predict that low-magnitude responses are solely generated through non-NMDA receptors whereas larger responses may or may not contain additional (but small) NMDA-driven components as the voltage-dependent threshold for NMDA receptor activation is exceeded. This is in contrast to *in vivo* studies where no dependence of the NMDA component of the discharge upon magnitude of response has been found (Fox et al., 1991) and to the present study where lowest-magnitude responses (layer II cells) involving intracortical relay were

dominated by mediation through NMDA receptors. Apart from an absence of intact modulatory inputs, another possibility for low NMDA receptor-mediated activity *in vitro* is that inhibitory conductances of cells in neocortical slices far exceed (by about sevenfold) those found for the same neocortical cells *in vivo* (Berman et al., 1989), which might suggest a high level of GABAergic suppression of NMDA receptor function in most slice preparations.

Relevance to sensory cortical plasticity

Our findings support the view outlined in the introductory remarks that NMDA receptors do not solely hold a special role in neuronal plasticity. That NMDA receptors overwhelmingly dominate normal sensory responses in barrel cortex suggests that factors additional to those that generate large responses through NMDA receptors must be sought where their activation is required for plasticity or use-dependent modification of EAA transmission. Thus, activation of NMDA receptors in neocortex may be considered necessary but not sufficient for synaptic plasticity. NMDA receptor activity engaged in somatosensory processing in neocortex is also unlikely to be directly linked to LTP in any simple manner. If this were the case, then the predominant role of NMDA receptors in normal sensory transmission uncovered in the present study, and a substantial role in other studies *in vivo* (Fox et al., 1989; Miller et al., 1989; Nishigori et al., 1990), would imply a condition of continuous potentiation, leading to continuous expansion of receptive fields with use. However, apposed to this is the finding that receptive field size in rat somatosensory and cat visual cortex decreases with increasing age (Armstrong-James, 1975; for review, see Armstrong-James and Fox, 1988b), despite an intracortical origin for surround excitatory receptive fields (Armstrong-James and Callahan, 1991; Armstrong-James et al., 1991).

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