To our knowledge there are no reports showing biochemical evidence of somatostatin-induced increases in PLA2 or lipoxygenase activity. It is possible that a GTP-binding protein may be involved in activation of PLA2 (ref. 22). Leukotrienes are synthesized in several regions of the brain, including the hippocampus23. Specific LTC4 binding sites are also found in several brain regions and are among the most numerous in the hippocampus; binding sites for LTB4 and LTE4 are weak and those for LTA4 undetectable24. The exact subcellar location of receptors for the lipids however, is still unknown: given the great membrane permeability of the lipids, they could act on the M-channel by either an intracellular or extracellular route, as suggested by others2,25,26. Thus, presynaptic or feedback effects cannot be excluded. Although the exact physiological role and site of action of the relevant lipid(s) cannot be stated at this time, it seems likely that, as in some invertebrate neurons2,20, a single ion channel type in mammalian central neurons can be regulated in opposing directions by two different ligands (somatostatin and acetylcholine) acting through different second messenger systems. Such bidirectional regulating mechanisms should provide sensitive biasing of ion channel activity, and therefore neuronal excitability.

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Dual-component NMDA receptor currents at a single central synapse

E. D’Angelo*, P. Rossi* & J. Garthwaite†

Department of Physiology, University of Liverpool, Brownlow Hill, PO Box 147, Liverpool L69 3BX, UK

* Present thinking about the way that the NMDA (N-methyl-D-aspartate) class of glutamate receptor operates at central synapses relies mainly on information obtained from single-channel and whole-cell recordings from cultured neurons stimulated by exogenous NMDA receptor agonists1,2. The mechanisms that operate in the postsynaptic membrane of a normal neuron following release of the natural transmitter are far less clear. An important problem is that most normal neurons receive many excitatory synapses (105–106 per cell) and these synapses are located on slender dendritic elements far away from the somatic recording site, making the study of discrete synaptic events difficult. Typically, when populations of synapses are activated, NMDA receptor-mediated synaptic potentials appear as slowly rising, long-lasting waves superimposed on faster, non-NMDA-receptor potentials2,4,6. Although believed to be critical for NMDA receptor function9,10, this slow time-course would not be predicted from single-channel kinetics and its origin remains puzzling. We have now analysed the events occurring at the level of a single excitatory synapse using a simple, small, neuron—the cerebellar granule cell—which has an unusually simple glutamatergic5 input. By applying high-resolution whole-cell recording techniques to these cells in situ11, we were able to study the nature of elementary NMDA receptor-mediated synaptic currents. Contrary to expectations, the prominent currents are fast but are followed by slow ones. Both types of current are strongly voltage-dependent but differ subtly in this respect. Furthermore, the currents are absent unless glycine is provided.

Cerebellar granule cells are only about 6 μm in diameter and receive a simple monosynaptic excitatory input from mossy fibres, which originate mainly in nuclei in the brain stem and spinal cord. The total number of excitatory synaptic boutons supplied to each cell amounts to only 3 or 4, one on each short dendrite12,13. Granule cells near the surface of parasagittally cut rat cerebellar slices were selected for study. One or two of the dendrites of these more superficial cells would have been cut off, leaving one or two synapses buried within the slice (Fig. 1a). The patch electrodes contained Nystatin to obtain ‘perforated’ whole-cell recordings, which minimize loss of cytoplasmic components14, and CsCl as the main electrolyte. All bathing solutions contained tetradoxin and bicuculline to block action potentials and inhibitory currents. The membrane capacitance was 3.3 ± 0.37 pF (mean ± s.d. of 87 cells in 11 slices) which, assuming a specific capacitance of 1 μF cm−2, gives an equivalent mean spherical cell diameter of just 10 μm.

The most obvious currents were fast and of a shape suggesting them to be synaptic currents produced by spontaneous release of quanta of transmitter from presynaptic terminals15,16. Consistent with this interpretation, their frequency was increased by perfusion of K+ to depolarize the nerve terminals (Fig. 1c) and their amplitude distribution was bell-shaped (data not shown). The currents were identified as NMDA receptor-mediated on the basis of the following observations.

First, the currents were absent unless glycine was supplied (Fig. 1b). Low concentrations of glycine augment responses to NMDA by binding to a high-affinity site on the NMDA receptor17. In cultured neurons, fast perfusion techniques are needed to prevent activation of this site by glycine continuously released from nearby cells. In our initial tests, the incubation bath (1.5 ml capacity containing 3–5 slices) was left static for 0.5–3 h before recording. Nevertheless, all 17 cells studied (4 slices) were initially silent. But 14 of them displayed spontaneous currents on local addition of glycine. With 10 μM glycine in a continuously perfused medium (subsequently adopted), 70% of recorded cells displayed spontaneous currents.

Second, the currents were reversibly abolished by the NMDA antagonist APV (D-2-amino-5-phosphonovalerate; Fig. 1d), but

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FIG. 1 Spontaneous synaptic activity is revealed by addition of glycine (c) and is enhanced by K"+ (c) and abolished by APV (d). Whole-cell recordings were made from granule cells in thin slices of rat cerebellum (shown schematically in a) but no activity could be observed, in this case recording over 10 min with the slice bathed for 2 h in nominally Mg"+-free medium, until glycine was delivered from a pipette (containing the amino acid at a concentration of 10 μM positioned 50–100 μm from the recorded cell. In a different experiment, K"+ and APV were also perfused locally (30 nM and 100 μM in the pipette, respectively) with 10 μM glycine and 1.2 mM Mg"++ in the bath. The effect of APV was observed all five cells tested and, with K"+, quantitation of current frequency (currents s−1) showed a significant increase (2- to 16-fold three cells) within the first 30 s of application. The holding potential was −40 mV in all cases and the currents were filtered at 2 kHz. The currents were unaffected when 10 μM strychnine, an antagonist acting at inhibitory glycine receptors, was perfused together with glycine (six cells; two slices; see also Fig. 2 legend). a. Left: Mol. molecular layer; WM, white matter; gr, granule cell layer. b. Right: Mf, mossy fibre; gr, granule cell. Go ax. Goji cell axon.

METHODS. Thin (70-120 μm) parasagittal slices of cerebellar vermis from young (12-15-day-old) rats were prepared using a Vibroslicer. A microknife cut was made across the upper part of the granule cell layer (see Fig. 1a) to improve visibility (Olympus C22 inverted microscope equipped with phase contrast optics) and sever the long granule cell axons. The slices were transferred to a polystyrene-coated Petri dish containing 1.5 ml of a solution maintained at 30 °C and containing NaCl (130 mM), KCl (3 mM), NaH2PO4 (1.2 mM), CaCl2 (2 mM), MgSO4 (1.2 mM), glucose (11 mM), Tris–HCl (15 mM) (pH 7.4), tetrodotoxin (1 μM) and bicuculline (10 μM). In some experiments, MgSO4 was omitted. The slices were immobilized by nylon threads fixed to a silver ring. A stream of prewarmed solution was gently superfluenced from a local pipette over a selected folium for 10–30 min. Except in the earlier experiments (see text) the bathing medium was continuously exchanged (1 ml min−1). The recording microelectrodes (10–12 mΩ) were filled with a solution containing Nystatin (100 μg ml−1) and CsCl (140 mM), NaCl (4 mM), CaCl2 (0.5 mM), EGTA (5 mM), HEPEPS buffer (5 mM), pH 7.2 (CsOH). The Nystatin method was adopted both for theoretical reasons and because the success rate in gaining access to the cell interior was considerably higher than with conventional whole-cell recording. Whole-cell currents were derived using a List EPC7 amplifier. The access resistance averaged 70 MΩ and the steady-state input resistance was typically 10 GΩ.

were unaffected by CNQX (6-cyano-2,3-dihydroxy-7-nitroquinoxaline), which is a relatively selective non-NMDA antagonist in the presence of 10 μM glycine (Fig. 2b). Third, with a physiological Mg"++ concentration (1.2 mM) in the bathing solution, current–voltage relationships (Fig. 3a) were linear (R = 0.99) from positive holdings to −20 mV, reversed at −6.5 mV (uncorrected value; see Fig. 2 legend) and, as is characteristic of NMDA currents, displayed a negative slope conductance between −30 and −70 mV. In the absence of added Mg"++, the curve was linear from positive potentials down to −40 mV and the currents thereafter were larger than with Mg"++ (Fig. 3a) and were consistent with a block of the NMDA channels by this ion, and the presence of residual Mg"++ in the nominally Mg"+-free extracellular solution. Over the linear range, the slope was 255 pS (1.2 mM Mg"++) / 246 pS (0.2 mM Mg"++) / 246 pS (1.2 mM Mg"++ / 10 μM CNQX). Assuming a single channel conductance of 50 pS, the fast currents are composed, on average, of the synchronous opening of five NMDA channels.

Kinetic analysis of these currents resulted in mean rise times (measured between 20–80% of the peak; means ± s.d.) of 0.57 ± 0.65 ms at −60 mV and 0.77 ± 0.55 ms at +50 mV (n = 10). The corresponding times to peak were 0.77 ± 0.65 ms and 1.13 ± 0.55 ms. These rapid kinetics are reminiscent of those of spontaneous endplate currents recorded at the neuromuscular junction. The decay of the individual currents, however, was complex, as expected from the random closure of so few NMDA channels. Digital averaging (Fig. 2b) suggested a decay time constant (τd) at +50 mV of 7.1 ± 0.9 ms (mean from best exponential fits of the averaged current decay in three cells ±s.d.). This value is in good agreement with the reported mean lifetime of unblocked NMDA channels and the decay became faster at negative potentials (Fig. 2b); at −50 mV, τd was 3.1 ± 1.1 ms (P < 0.02). This could reflect a reduced channel-burst duration at negative membrane potentials.

Beyond the main peak, the kinetics of single currents were not easily resolved. This was because the rapid events were often followed by a noisy tail, highly variable in duration, but the events lasting for tens of milliseconds (Fig. 2a). In averaged records, these tails generated a 'hump', peaking 20–25 ms after the start of the fast component (Fig. 2b). The current–voltage plot of the hump over the range +50 to −70 mV was like that of the fast current (Fig. 3c), but between −70 and −80, the fast current increased whereas the hump decreased. The ratios of the currents at these two potentials were 1.93 ± 0.31 for the fast component and 0.84 ± 0.29 for the hump (means ± s.d.; data from Fig. 3a and c; P < 0.002).

It can be concluded that quanta of excitatory transmitter are released spontaneously at this central synapse and that they elicit postsynaptic currents exclusively through NMDA receptors. It is likely that other synapses are similar in this respect.
as ‘tonic’ NMDA receptor activation has been detected as a background current in hippocampal neurons in the absence of synaptic stimulation. Such spontaneous activity could have an important influence on neuronal excitability. Non-NMDA receptors are present on granule cells and can be activated following electrical stimulation of mossy fibres, but they may need a higher concentration of transmitter to become effective.

The primary (fast) synaptic current elicited through NMDA receptors has largely predictable kinetics, assuming quanta of transmitter bind to receptors located close to the release site and so cause the synchronous opening of about five NMDA channels. The subsequent slow current, however, is likely to be the one responsible for the slow synaptic NMDA potentials previously detected in this and several other pathways following electrical stimulation of afferent fibres. We can now exclude several hypotheses that have attempted to explain the slow time-course of this component by invoking network-related factors, such as polysynaptic pathways. Instead, an attractive possibility is that after the channels mediating the fast component have closed, the transmitter dissociates and then rebinds to receptors asynchronously. Given the delay to the peak of the slow wave, the operative receptors could be located some distance away, perhaps extrasynaptically. One prediction of this hypothesis is that the time to the peak of the slow wave should shorten as the decay rate of the fast currents increases, as indeed the averaged records (Fig. 2b) suggest.

Finally, the precise action of glycine and whether the ambient levels of glycine are limiting for NMDA receptor function or not, are both controversial issues. Our results strongly suggest that glycine is an obligatory co-agonist at the synaptic NMDA receptor, as no currents could be detected in the absence of added glycine. This, in turn, implies that the ambient levels in the synaptic cleft are very low, probably ≤30 mM (from glycine dose–response curves). Although it cannot be excluded that diffusion of glycine out of the slice is responsible, our findings with preparations bathed for long periods in unstirred solutions would not favour this possibility. Moreover, the availability of glycine is limiting for NMDA receptor function in the cerebellum in vivo. Thus, it is more likely that there is a mechanism maintaining glycine at a low concentration in this synapse. The axon terminals of Golgi cells are equipped with a high-affinity glycine carrier and are in an anatomically appropriate position to undertake this function. Being rich in glycine the same terminals could supply the amino acid to the synapse under appropriate conditions and so regulate granule cell NMDA currents.

FIG. 3 Mean current–voltage (I–V) relationships for the fast current (a, b) and the hump (c). In a, the bathing solution contained either 1.2 mM Mg2+ (filled circles) or no added Mg2+ (open circles). Standard deviations are omitted for clarity but averaged 24% of the means. The data in b (±s.d.) were from cells incubated with 1.2 mM Mg2+ and 10 μM CNXQ. Each point (a and b) represents the mean peak amplitude of individual currents in five cells in two different slices. All currents of significant size greater than or equal to twice background noise were included in the analysis; the mean current at ~70 mV in Mg2+-containing solution may thus be an overestimate (noise level, 1.15 pA). The current amplitudes were not significantly affected by strychnine; for example, at +40 mV, with no added Mg2+ and 10 μM strychnine in the perfusing solution, the mean current in three cells (±s.d.) was 8.4 ± 2.2 pA. The inset (b) shows the I–V curve for a single cell in the presence (solid symbols) and absence (open symbols) of 10 μM CNXQ. The I–V curve for the hump (c) was obtained from measurements of its peak amplitude in averaged currents (Fig. 2b) from four different cells (means ±s.d.). The solid line (a and b) fits data points in 1.2 mM Mg2+, zero CNXQ (by linear regression at potentials above −20 mV, the remainder by eye). The apparent reversal potential for these currents is ~−6.5 mV. In the perforated patch whole-cell recording configuration, a Donnan equilibrium will develop between the pipette and the cytoplasm, which should make the pipette 8.9 mV positive and thus shift I–V relationships to the left by this value. As the reversal potential of the NMDA current (considering only the Nystatin-permeant ions in the pipette and in the bath) can be calculated to be −4.3 mV (ref. 29), the synaptic currents should theoretically reverse at −4.6 mV, which is in reasonable agreement with the experimental value.

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Linkage of a nasopharyngeal carcinoma susceptibility locus to the HLA region


* People's Regional Hospital, Nanning, Guangxi Autonomous Region, People's Republic of China
† Medical Research Council Biostatistics Unit, 5 Shaftesbury Road, Cambridge CB2 8WX, UK
§ INSERM U 93, Hayem Research Center, Saint Louis Hospital, Paris, France
‖ Microbiology Department, National University, Singapore
¶ Immunogen Type Laboratories, Immunoresearch, Melbourne, Australia
** Department of Biostatistics, University of Washington, Seattle, Washington 98195, USA.
†† Section of Epidemiology, Institute for Cancer Research, Sutton, Surrey SM2 5NG, UK
†‡ CNRS Laboratory of Epidemiology and Immunovirology of Tumors, Faculty of Medicine Alexis Carrel, 69372 Lyon Cedex 8, France
†‖ Institute of Virology, Chinese Academy of Preventive Medicine, People's Republic of China
†* To whom correspondence should be addressed.

The frequency of nasopharyngeal carcinoma is nearly 100-fold higher in southern Chinese than in most European populations. Earlier studies have suggested that an increased risk of nasopharyngeal carcinoma is associated with specific haplotypes in the HLA region: relative risks slightly over twofold were found for haplotypes A2, Bw46 and the antigen B17 (refs 2-4). We now report a linkage study based on affected sib pairs which suggests that a gene closely linked to the HLA locus confers a greatly increased risk of nasopharyngeal carcinoma. The maximum likelihood estimate is of a relative risk of approximately 21. The relationship between this suspected disease susceptibility gene (or genes) and known viral and environmental aetiological factors remains to be elucidated.

Our approach was to identify sibships that had more than one individual affected with nasopharyngeal carcinoma (NPC) and for which sufficient individuals could be typed to permit unambiguous assignment of haplotypes. The study began in Singapore and Hong Kong in 1976 and extended in 1983 to Nanning in the Guangxi Autonomous region of the People's Republic of China, where more sib pairs were available (see Table I legend). In each area, the members of each sibship, their parents and children were visited and blood samples taken. Thirty-four sibships with more than one case of NPC were identified, 31 with two cases and the remainder with three cases (Table I). Of the sib-pair families, four had to be excluded, one because the pair were twins of unknown zyosity (but HLA identical) and three because an excess of haplotypes was seen in the family and parental age was ambiguous.

HLA typing on the samples from Hong Kong and Singapore was performed in Singapore and restricted to locus A and B antigens, using a panel of over 200 antisera of Chinese, Malay, Japanese, Filipinos and Caucasian origin. HLA typing in Nanning used a panel of 143 allelic typing antisera (from Saint Louis Hospital, Paris) corresponding to HLA A, B, C and in most cases DR loci. Of the 38 NPC cases tested, the most common DR antigens were DR2 (18/38) and DR4 (16/38).

Table 1: Sib-pair analysis

<table>
<thead>
<tr>
<th>2 Expected under</th>
<th>Number of shared haplotypes</th>
<th>0 Expected under</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>No linkage</td>
</tr>
<tr>
<td>Sib pairs*</td>
<td>16</td>
<td>6.75</td>
</tr>
<tr>
<td>Triples†</td>
<td>2</td>
<td>2.25</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>8.00</td>
</tr>
</tbody>
</table>

Number of sib pairs sharing two, one or zero HLA haplotypes by descent, together with the expected numbers under the null hypothesis of no linkage, and under the best fitting dominant and recessive models. For the sib trios, all possible sib pairs are considered.

Goodness-of-fit of the hypothesis of no linkage, $\chi^2$ (2 df) for sib pairs only $=17.00 < 0.001$, for all pairs (both sib pairs and triples) $=3.20 < 0.005$. Goodness-of-fit tests of the recessive and dominant models are each given by a 1 df likelihood ratio test against a three-parameter model, with relative risk $r_2$ for the dominant model $=2.95 (P=0.08)$.

* 18 from China, 3 from Hong Kong, 5 from Singapore and 1 from Malaysia.
† 2 from China and 1 from Hong Kong.

Figure 1: For the model fitting, inference was based on the log-likelihood difference conditional on the disease phenotypes, or lod score. The use of this conditional likelihood allows inferences to be made free from ascertainment bias. Likelihood calculations were performed using the program LINKAGE for single-gene dominant and recessive models, varying the gene frequency relative risk associated with the disease susceptibility gene, and the recombination fraction $\theta$ between the susceptibility gene and the HLA region. In particular, the lower 95% confidence limit for the relative risk associated with the susceptibility gene, $r_2$, is given by:

$$\frac{2 \max_i \frac{\log (1+r_2 f_i, \theta) - \max_i \frac{\log (r_0, f_i, \theta)}{\theta}}{\theta}}{\theta} = 3.84$$

where $\log (1+r_2 f_i, \theta)$ denotes the log-likelihood function and 3.84 is the upper 95% point for a $\chi^2$ distribution on 1 degree of freedom. The contours shown define confidence regions for $r$ and $f$ at the 95%, 90%, 85% and 50% level with $\theta=0$. The absolute maximum of the likelihood occurs when $r=0$, with values of $f=0.29, r=20.9$ and is marked by a cross (+).