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Effect of changing extracellular levels of magnesium on spontaneous activity and glutamate release in the mouse neocortical slice

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1 The mouse neocortical slice preparation, maintained in a two compartment, grease gap bath, exhibits spontaneous depolarizing activity (with or without rhythmic after potentials) after perfusion with magnesium-free artificial cerebrospinal fluid.

2 If the magnesium concentration is decrementally lowered over an extended time period, then incrementally raised following a similar time course, the spontaneous depolarizing shift activity shows a hysteresis (with regard to both frequency and amplitude), the depolarizing shifts being more resistant to magnesium during the the incremental period.

3 The amino acid content of the perfusing fluid was analysed by high performance liquid chromatography (h.p.l.c.). Although a basal efflux of 6 amino acids was quantifiable, only glutamate levels increased following superfusion of the preparation with magnesium-free, artificial cerebrospinal fluid.

4 Glutamate release increased to 266% of the resting release in the presence of magnesium within the first 12 min of the change into magnesium-free artificial cerebrospinal fluid. This increase in release preceded the onset of spontaneous depolarising activity. The release of glutamate remained elevated at 182% of control up to 60 min after perfusion with magnesium-free buffer, when depolarizing activity was well established.

5 A model is presented and discussed for the genesis and maintenance of the spontaneous depolarizing shifts. It is suggested that the maintenance of this spontaneous activity reflects a long term enhancement of neocortical neurone excitability which may be related to long term potentiation in the hippocampus.

Introduction

The receptors for the excitatory amino acids have been classified into at least 3 subtypes (N-methyl-D-aspartate (NMDA), kainate and quisqualate) as a result of pharmacological investigations (for reviews see Watkins & Evans 1981; McLennan 1983; Stone & Connick 1985; Mayer & Westbrook 1987; Stone & Burton, 1988). Responses involving the activation of the N-methyl-D-aspartate (NMDA) receptor are very sensitive to changes in the extracellular concentration of magnesium (Evans *et al.*, 1977). This antagonism is thought to result from a voltage-dependent blockade of the ionic channels activated by the NMDA receptor (Nowak *et al.*, 1984; Mayer *et al.*, 1984). In the rat neocortex the relief of this blockade upon removal of magnesium from the artificial cerebrospinal fluid (ASCF) also gives rise to potentiation of responses due to NMDA receptor-mediated processes (Thomson & West, 1986). This implies that when magnesium is at physiological levels (approximately 1–2 mM) (Davson *et al.*, 1987) there will be substantial, but not necessarily total, antagonism at the NMDA receptor.

Several groups of investigators have recently observed that slices of rat or mouse neocortex maintained in a twin compartment, grease gap bath (Harrison & Simmonds, 1985) produce spontaneous depolarizing shifts often with rhythmic after potentials when the magnesium content of the superfusing medium is lowered from a normal content of 1 mM to 0 mM (Horne *et al.*, 1986, Burton *et al.*, 1987). This phenomenon is also seen in human cortex (Avoli *et al.*, 1987). These depolarizing shifts usually appear within 30–60 min of superfusion with magnesium-free ACSF and gradually increase in both size and

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frequency over the following 1 to 2 h (Burton et al., 1987). Not all slices exhibit this activity spontaneously although most can be induced into producing depolarizing shifts following depolarization with an NMDA receptor agonist. Such depolarizing shifts are sensitive to NMDA antagonists such as 2-amino-5-phosphonopentanoate (APV), 2-amino-7-phosphonoheptanoate (APH), kynurenic acid, and ketamine, and are abolished by tetrodotoxin (Horne et al., 1986; Burton et al., 1987).

Similar paroxysmal depolarizations also appear after 15–20 min perfusion with 50 μM bicuculline in normal (1 mM magnesium) Krebs medium (Horne et al., 1986). These depolarizing shifts were also abolished by NMDA antagonists. It is therefore possible that changes in the concentration of GABA in a cortical perfusate could also contribute to the epileptiform activity seen in zero magnesium medium (Heyer et al., 1982).

Since other forms of self perpetuating depolarizing activity such as long term potentiation (LTP) have been associated with an increase in glutamate release (Dolphin et al., 1982) we have endeavoured to examine the changes in endogenous amino acid release concomitant with the onset of spontaneous depolarizing activity initiated by a lowering of extracellular magnesium. To date there have been no reports on the effect of removal of magnesium ions on the release of the endogenous excitatory amino acids, and only few reports on its effect on γ-aminobutyric acid (GABA) release (e.g. Drejer et al., 1987). Some of the present results have been given previously in a preliminary form (Smith & Stone, 1988; Connick et al., 1988).

Methods

Full details of the preparation and a qualitative description of its properties appear elsewhere (Burton et al., 1987).

Tissue preparation

Male MF1 mice (4–8 weeks old) were killed by cervical dislocation followed by decapitation and the whole brain removed quickly into ice-cold artificial cerebrospinal fluid (ACSF). Coronal sections about 500 μm thick were cut with a Vibroslice (Campden Instruments). Acceptable sections had as rostral and caudal limits the genu of the corpus callosum and the rostral region of the cerebral ventricles respectively. These were transferred to an incubation chamber containing a humidified 95% O_2, 5% CO_2 atmosphere at room temperature (20–24°C). A suitable slice was subsequently divided in the midline and further cuts were made to produce several wedge shaped pieces of tissue about 1.5 mm wide at the pial surface and about 1 mm wide at the corpus callosum. One such wedge was transferred to a two chamber bath, so that most of the cortical tissue was contained in one chamber, and the corpus callosum and a little cortical tissue contained in the other. A high resistance seal between the two compartments was achieved by use of high vacuum silicone grease (BDH). Each chamber was continuously perfused at 2.5 ml min^{-1} (or 2.0 ml min^{-1} for release studies) via droppers with ACSF gassed with a mixture of 95% O_2 and 5% CO_2. The callosal compartment of the bath was perfused with magnesium containing ACSF at all times. Where reference is made to a change from magnesium-containing to magnesium-free ACSF this only applies to the cortical (pial) compartment.

ACSF composition

ACSF normally had the following composition (in mM): NaCl 123.8, KCl 3.3, $MgSO_4 \cdot 7H_2O$ 1.0 (or as stated in the text), KH_2PO_4 1.22, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, D-glucose 10.0, and was continuously bubbled with a 95% O_2, 5% CO_2 mixture to bring the pH to about 7.4. The ACSF was approximately 325 mOsm. The magnesium content of the nominally Mg-free ACSF, prepared by omission of $MgSO_4$, was not greater than 11.3 μM (estimated from manufacturer's data) and typically was 2–3 μM (estimated by Atomic Absorption spectroscopy).

Experimental protocol

The d.c. potential between the two compartments was continuously monitored via Ag/AgCl electrodes and a high input impedance amplifier. It was displayed on a storage oscilloscope and for permanent records a chart recorder. Unless otherwise stated, after an incubation of at least 1 h in ACSF the cortical chamber was perfused with nominally Mg-free ACSF.

Amino acid release After 1 h in ACSF (1 mM magnesium) the cortical side of the bath was perfused with Mg-free ACSF. Samples of perfusate (2 min) were collected and 500 pmol of D-α-aminoadipic acid was added as an internal standard prior to concentration in a vacuum centrifuge. Fractions were resuspended in 100 μl of water and the endogenous amino acids analysed by high performance liquid chromatography (h.p.l.c.) using a method based upon that of Turnell & Cooper (1982).

o-Phthalaldehyde (OPT)/2-mercaptoethanol derivatives were produced by taking 100 μl of OPT reagent solution (Sigma, UK) and mixing with 100 μl of amino acid mixture (standards or sample). The mixture was immediately vortexed to produce the 1-

alkyl-thio-2-alkyl-substituted isoindole derivative (Roth, 1971). After 30 s, 20 μl of the mixture was injected onto the chromatographic column for analysis.

A Gilson gradient system was used, and detection performed by a Gilson Spectra Glo fluorimeter with an excitation wavelength of 390 nm and emission cut off filter at 475 nm, at maximum sensitivity. Separation of the derivatised amino acids was performed on a reverse phase 'Resolve' C_{18} analytical column (Waters, UK) fitted with a C_{18} 'guard pac' pre-column (Waters, UK).

Changes in magnesium concentration After 1 h in normal ACSF the concentration of magnesium in the ACSF superfused over some slices was lowered in a stepwise fashion, while with other slices the magnesium concentration was reduced to 0 in one step. Upon achieving a nominally magnesium-free state, slices were equilibrated for approximately 1 h before increasing the magnesium content of the ACSF. Slices were then returned to zero magnesium ACSF as a recovery control. Each magnesium concentration change was allowed to equilibrate for 30 min.

Atomic absorption spectroscopy Samples of perfusate (200 μl) were collected throughout the normal washout of magnesium from the bath (2 mM to 0 mM magnesium in one step). These samples were then analysed for magnesium content in a Pye Unicam SP9 atomic absorption spectrophotometer with a magnesium vapour lamp (285.2 nm) against a calibration curve of $MgSO_4$ in reagent grade water.

Materials

All chemicals were of 'Analar' grade and were obtained from BDH Ltd. Amino acids were from the Sigma Chemical Co. Reagent grade water was prepared by running tap water through Milli RO4 and Milli Q water purification systems (Millipore, UK).

Statistics

Throughout this study, statistical significance has been assessed relative to control conditions by use of either a paired or unpaired Student's t test as appropriate.

Results

In none of the experiments in which tissues were bathed continuously in magnesium-containing ACSF were depolarizing shifts obtained (Burton *et al.*, 1987). Spontaneous depolarizing shifts appeared within about 1 h of superfusion with magnesium-free ACSF in about 60% of the viable slices prepared (mean $\pm$ s.e.mean; 67.6 $\pm$ 7.1 min to approx 50% final amplitude; $n = 13$). The depolarizing shifts occurred with or without rhythmic after potentials (see Figure 1). Each depolarizing shift consisted of an initial brief (50–100 ms) negative going potential in the pial compartment followed by a slow repolarization over the next 2–10 s. In most wedges a series of repetitive negative going potentials were superimposed on the slow repolarising phase.

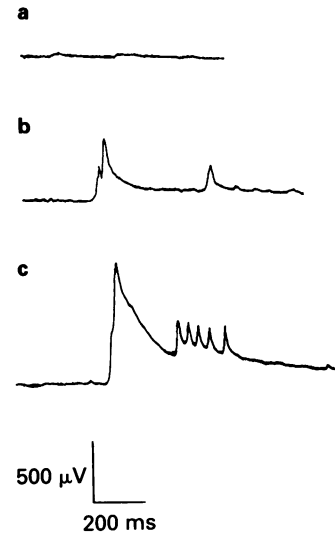

Figure 1 Traces of spontaneous activity in mouse neocortical slice; (a) before introduction of magnesium-free ACSF; (b) during the development of spontaneous depolarizing activity; (c) fully developed depolarizing shift showing rhythmic after potentials.

Effect of incremental rise in magnesium concentration

In seven slices the magnesium concentration was reduced in one single step to zero, when depolarizing shifts occurred. The magnesium concentration was then raised to assess the sensitivity of the spontaneous activity to magnesium concentration. When incubated with ACSF containing 200 μM magnesium (slices were allowed to equilibrate for 30 min following the change in magnesium concentration) 3 slices showed increased depolarizing shift amplitude (109.8 $\pm$ 3.9% of controls; mean $\pm$ s.e.mean) although at all magnesium concentrations the frequencies of the shifts were reduced. It is interesting to note that of the original seven slices, five were still spontaneously active when the magnesium concentration was raised above control (1 mM) levels; in

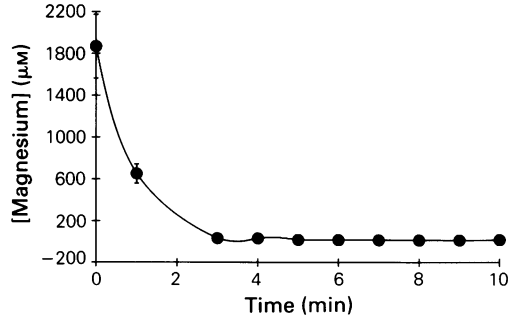

Figure 2 Graph of washout of magnesium from the cortical chamber of the two compartment grease gap bath. The initial magnesium concentration is 2 mM and the magnesium concentration was measured by atomic absorption spectroscopy (see text). Each point is the mean from three separate experiments with s.e.mean shown by error bars except where smaller than symbol.

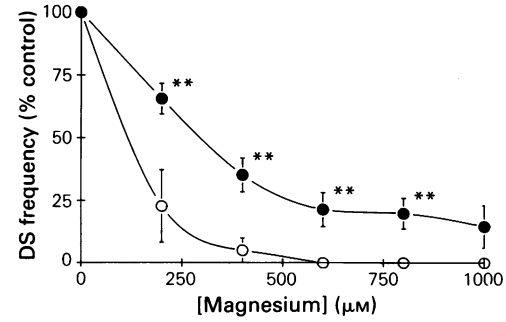

Figure 4 Graph to show hysteresis of spontaneous depolarizing shift (DS) frequency in mouse neocortical slices to decreasing (open circles) and increasing (filled circles) magnesium concentrations. Each point represents the mean number of depolarizations per minute over the last 5 min of each 30 min step at each individual magnesium concentration; s.e.mean shown by vertical bars. $^{**}P < 0.01$. Magnesium concentrations given in μM.

addition, the spontaneous activity in two slices was not blocked at 2.6 mM magnesium. In the two slices that were still active at 2.6 mM, the washout of magnesium was rapid (see Figure 2) and the depolarizing shifts increased in frequency and amplitude in a similarly rapid manner following the final removal of magnesium from the ACSF (see Figure 3).

Effect of a stepwise decrease and increase of magnesium concentration

The effect of reducing the magnesium content of the ACSF in steps of 200 μM, each lasting 30 min, on the frequency and amplitude of the depolarizing shifts can be seen in Figures 4 and 5. The onset of depolarizing shifts was only apparent at concentrations of magnesium of 400 μM or less in the decremental part of the experiment. In one slice, depolarizing shifts began at 400 μM (frequency 40% of value obtained in magnesium-free solution) but were of very small amplitude (19% of control value obtained in magnesium-free solution). Two other slices also began to show spontaneous activity at 200 μM magnesium, the amplitudes now being greater (28% and 63% of magnesium-free values) and the frequencies more variable (5% and 71% respectively). When the slices were subsequently perfused with nominally magnesium-free ACSF, they developed their depolarizing shifts as previously reported (Horne *et al.*, 1986; Burton *et al.*, 1987). After incubation in nominally magnesium-free ACSF, the reintroduction

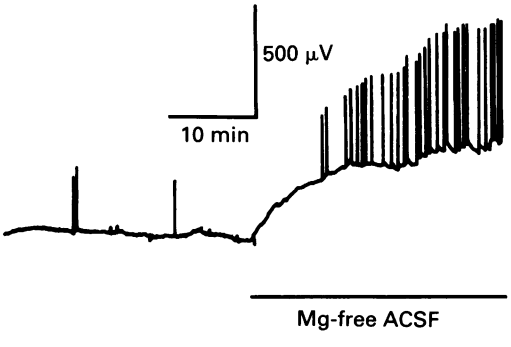

Figure 3 Reappearance of spontaneous depolarizing shifts in mouse neocortical slice following washout of 2.6 mM magnesium. Note rapid return of both frequency and amplitude to near control levels. (Retouched)

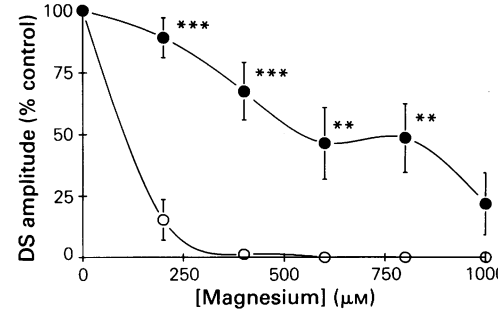

Figure 5 Graph to show hysteresis of spontaneous depolarizing shift amplitude to decreasing (open circles) and increasing (filled circles) magnesium concentrations in mouse neocortical slices. $^{**}P < 0.01$; $^{***}P < 0.001$. Concentrations of magnesium given in μM.

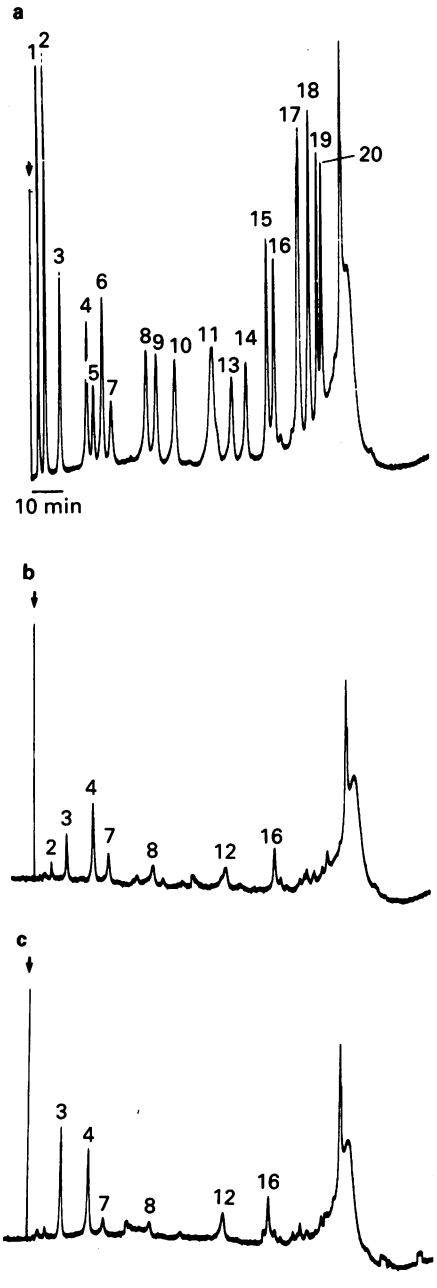

Figure 6 High performance liquid chromatography (h.p.l.c.) chromatograms of (a) a standard injection of 20 μl of a mixture of 25 pmol each of (1) phosphoserine, (2) aspartic acid, (3) glutamic acid, (4) α-aminoadipic acid, (5) asparagine, (6) serine, (7) glutamine, (8) glycine, (9) threonine, (10) citrulline, (11) alanine, (13) taurine, (14) aserine, (15) β-aminoisobutyric acid, (16) tyrosine, (17) methionine, (18) valine, (19) tryptophan and (20) phenylalanine. (b) A typical trace of amino acids released from a slice at t_{-28}; peak 12 is that of GABA. (c) A typical trace of amino acids released from a slice at t_{60}. The traces in (b) and (c) are efflux from the same slice. The broad peak at the end of each chromatogram represents the elution of a number of unresolved compounds corresponding to the rapid increase in the elution gradient. Chromatographic conditions were as follows: Solvent (a): 0.05 M sodium acetate, 0.05 M disodium hydrogen phosphate, pH 7.4: methanol: tetrahydrofuran, 96:2:2. This mixture was then filtered through a 0.45 μm Durapore filter (Millipore, UK) under vacuum. Solvent (b) consisted of 65% methanol in water. This was premixed and filtered under vacuum. Chromatographic conditions: The gradient programme, expressed as time in minutes from injection (% solvent b) was; 0(0), 4.5 (3), 5.4 (6), 6.4 (7), 7.3 (8.6), 9.1 (10), 10 (10.7), 10.9 (11.4), 11.8 (12.5), 12.7 (13), 13.6 (14.3), 27.3 (23), 47 (100). The flow rate was 1.5 ml min^{-1} at room temperature.

of ACSF containing magnesium led to an overall loss of frequency and amplitude, although in 2 of 8 slices the amplitude was greater than that of control (105% and 103% of controls). All the slices showed reduced depolarizing shift frequencies. At all higher magnesium concentrations both frequency and amplitude were depressed below the values in zero magnesium although at all levels of magnesium below 1 mM, the frequency and amplitude were significantly higher than observed during the progressive reduction of magnesium concentration. Graphs of the amplitude and frequency of depolarizing shifts therefore exhibit a hysteresis phenomenon in which their values at any given magnesium concentration depend on the direction of magnesium concentration change (Figures 4 and 5).

Amino acid release

Although the detection system used was routinely capable of resolving 10 to 50 pmol of up to 23 amino acids, analysis of the ACSF superfusing the pial area of the slice showed a detectable efflux of only aspartate, glutamate, glutamine, glycine, GABA and tyrosine. These amino acids were identified on a basis of their retention times and their concentration assessed by measurement of their peak height relative to the internal standard as previously described (Connick & Stone, 1988). In addition, the identities of individual peaks were confirmed by 'spiking' the sample with specific amino acids with the exception of tyrosine which was identified by its retention time alone.

Typical chromatograms of amino acid standards (Figure 6a), and typical samples from perfusates taken 28 min before and 60 min after removal of magnesium are shown in Figure 6b and c respectively. The only amino acid to change in the profile

detected was glutamic acid. The efflux of the compound was typically between 100 to 150 pmol ml^{-1} in the superfusing ACSF. A more exact measurement of the release of glutamate in terms of tissue weight was not possible because of the nature of the preparation. Typically the total weight of the cortical slice was between 2 and 3 mg with a variable proportion of the slice lying on the callosal side of the bath. The efflux of glutamate therefore represents that from approximately 1–2 mg of tissue.

During the course of the experiment the amounts of aspartic acid, glutamine and glycine usually declined however, this was not the result of the removal of magnesium from the medium. The efflux of GABA and tyrosine remained at a constant low level throughout the experiment.

In the presence of magnesium no spontaneous activity was detectable (Figure 1a) and glutamate release was stable at 99.7 ± 5.4% of the mean release ($n = 3$ to 5) at time 0 (t_0) (Figure 7). Upon removal of magnesium at t_0, there was an immediate increase in glutamate release (Figure 7) corresponding to the washout of magnesium from the bath (Figure 2). Glutamate release was increased to 352.5 ± 23.8% between t_0 and t_{40} ($n = 3$ to 5), the large s.e.mean representing the very large increase in release found in some slices and the difficulty in normalizing data from slices with varying frequency, amplitude and time of onset of their depolarizing shifts. Comparing the six fractions immediately either side of t_0, there was an increase in release of 266% ($P < 0.05$), that is within 12 min of the change to magnesium-free solution.

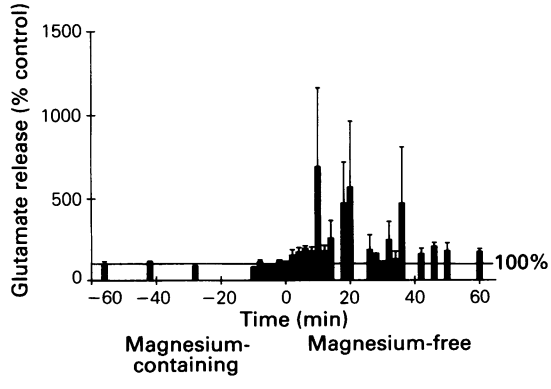

Figure 7 Histogram of glutamate release as a % of release at time zero (t_0). Each column represents mean (s.e.mean shown by vertical bars) from between 3 and 5 experiments. Magnesium-containing ACSF (1 μM) was exchanged for magnesium-free ACSF at t_0. Spontaneous depolarizing activity appeared 56.4 ± 8.6 min (mean ± s.e.mean) after the change to zero magnesium ACSF.

After the initial dramatic increase in glutamate release up to 40 min after t_0 (354% of the resting efflux; $P < 0.05$) the efflux stablized between 40 and 60 min corresponding to the maturation of the depolarizing shifts and the development of numerous afterpotentials superimposed upon each depolarizing shift (Figure 1c). The average release of glutamate between 40 and 60 min was 181.4 ± 9.5% and although less than that occurring between 0 and 40 min, it was still substantially higher (182%; $P < 0.001$) than the release into magnesium containing ACSF immediately before the change to zero magnesium buffer.

Discussion

The spontaneous depolarizing shifts described here are similar to those seen in the neocortical slice preparation of the rat (Harrison & Simmonds, 1985; Horne *et al.*, 1986). The fast rise time of the initial peak (around 50 ms) and the appearance of rhythmic afterpotentials are characteristic of both preparations. In this paper we are primarily concerned with the initial event as the appearance and frequency of afterpotentials per depolarizing shift was found to be inconsistent between preparations.

It is interesting to speculate as to the origin and mechanism of these depolarizing shifts in the light of the results presented in this paper. We are unable to give any information as to the anatomical site of origin of the spontaneous depolarizing shifts owing to the non-focal nature of the grease gap recording technique. However, a recent paper (Aram & Lodge, 1988) in which the whole neocortical slice preparation of the rat and microelectrode recording techniques were used, has shown that in magnesium-free conditions the greatest amplitude of these spontaneous epileptiform events was found in layers II/III. This of course does not necessarily reflect origin but probably reflects the greatest concentration of cells involved in the spontaneous activity. In fact these workers note that there are multiple foci from which these epileptiform bursts spread to both contra- and ipsilateral cortices. One would expect the mouse neocortex to behave similarly owing to the close phylogenetic relationship between the two species.

The depolarizing shifts seen on lowering extracellular magnesium levels, may be attributable to 3 mechanisms. A general increase in the level of cell excitability due to the removal of magnesium could be the cause although Horne *et al.* (1986) found that if magnesium, after removal, was subsequently replaced by an equivalent concentration of calcium, then the frequency of the depolarizing shifts did not change. This may indicate that the general level of cell excitability is not the major mechanism of depolarizing shift genesis.

Another mechanism by which the initiation and maintenance of spontaneous depolarizing shifts may be explained could be by regional anoxia in the area around the grease gap (Rothman & Olney, 1986). However this too would seem unlikely as Aram & Lodge (1988) have demonstrated that the spontaneous depolarizing shifts can be initiated by low magnesium levels in a standard slice (i.e. not grease gap) bath and with orthodox methods when the neurones showed no signs of deterioration.

An alternative cause of the spontaneous activity could be the reduction of the magnesium blockade of the ion channel associated with the NMDA receptor, an effect which has been suggested by results obtained by Coan & Collingridge (1987) in the hippocampus. This unmasking of the NMDA receptor would lead to an increased level of excitatory tone in all neurones receiving an NMDA receptor-mediated excitatory input.

Since an increase of extracellular magnesium levels has been shown to induce a reduction of neurotransmitter release (del Castillo & Engbaek, 1954), the removal of magnesium could also lead to an increase in neurotransmitter release acting at those receptors (Jenkinson, 1957; Richards & Sercombe, 1970). Although one would expect this to apply to the release of inhibitory neurotransmitters (Drejer *et al.*, 1987) as well as excitatory ones, the present results indicate that the only increase in putative neurotransmitter efflux is that of glutamate. This result would suggest that depolarizing shifts are due to an increase in glutamate release but would not necessarily exclude the unmasking of the NMDA receptor channel also having an important role. Indeed the fact that the increase in glutamate release does not follow the same time course as the development of spontaneous activity may even suggest a model which includes the involvement of the NMDA receptor channel in the genesis and maintenance of depolarizing shifts. It is important to note that the initial increase in glutamate release peaks before the full development of the depolarizing shifts and then tails off whilst remaining significantly higher than the resting release which occurs during the control period. Furthermore the effects of magnesium on the depolarizing shifts are different when investigated before or after the full development of the spontaneous activity. It is feasible that the removal of magnesium initially causes an increase in transmitter release (in this case presumably glutamate) as indicated by the results. This increased level of glutamate in turn increases the excitatory tone of neurones which should be self perpetuating as raised levels of glutamate would in turn cause increased glutamate release (Connick & Stone, 1988). The increase in excitatory tone would also tend to overcome the voltage-dependent magnesium blockade of the NMDA receptor-associated ion channel. This in turn would lead to recruitment of a suitable proportion of neurones that presumably fire synchronously via synaptic interconnections. Alternatively a discrete population of sensitive 'initiator' neurones may experience enhanced excitatory tone and presumably recruit other neurones eventually leading to fully developed depolarizing shifts.

The potentiated involvement of NMDA receptors could explain the observed hysteresis in the susceptibility of the depolarizing shifts to blockade by reintroduction of magnesium into the bath. Whilst it is possible that the hysteresis could simply reflect a lag between the change of magnesium concentration and equilibrium in the 30 min steps used, this is unlikely since depolarizing shifts typically appear after about 30 min, reaching half their maximum amplitude after 67 min. In addition, the fact that some slices still showed spontaneous activity at up to 2.6 mM magnesium following the full development of the depolarizing shifts argues against this explanation. The observed hysteresis is similar to an effect noted previously by Anderson *et al.* (1986).

NMDA receptor antagonists are able to prevent the initiation of long term potentiation (LTP) in both the cortex and hippocampus (Bliss & Lomo, 1973; Artola & Singer, 1987), but once established these antagonists have no effect on the perpetuation of LTP. Huang *et al.* (1987) have shown that the induction of LTP is facilitated by lowering magnesium concentrations. Skrede & Malthe-Sorenssen (1981) found that high frequency stimulation of afferent fibres to CA1 neurones in the hippocampus (conditions similar to that needed for the induction of LTP) caused a long lasting increase in the resting release of [^{3}H]-D-aspartate from pre-loaded cells. Furthermore Dolphin *et al.* (1982) using a push-pull perfusion technique, found that LTP induced in the perforant path/dentate gyrus granule cell pathway of the hippocampus produced a long lasting increase in the release of newly synthesized glutamate. The present finding of an increased release of glutamate is therefore consistent with the proposal that the maintenance of depolarizing shifts is partly due to a long term change of excitability akin to LTP.

The role of GABA in the genesis of the depolarizing shifts is unclear. Removal of magnesium has been reported to enhance NMDA-induced but not glutamate-induced release of GABA from cultured cortical tissue (Drejer *et al.*, 1987). The present failure to detect any change of GABA release is therefore entirely consistent with our demonstration of the selective release of glutamate by the slices.

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