Sensitivity of hippocampal neurones to kainic acid, and antagonism by kynurenate

T.W. Stone

Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland

- 1 The sensitivity to kainic acid of neurones in the CA1 and CA3 regions of rat hippocampal slices has been examined by microiontophoresis and by superfusion methods.
- 2 When the iontophoretic currents needed to produce comparable plateaux of firing were compared, neurones in the pyramidal cell layer of the CA3 region were approximately 5 times more sensitive than cells in the CA1 region. No difference was noted in sensitivity to N-methyl-D-aspartate (NMDA) or quisqualate.
- 3 When kainate was superfused at known concentrations, the threshold for eliciting excitation in CA1 was $2.1 \mu\text{M}$. The threshold concentration in CA3 was $0.24 \mu\text{M}$.
- 4 Two weeks after the stereotaxic intrahippocampal injection of colchicine, the granule cells of the dentate gyrus and thus the mossy fibre projections to CA3 were destroyed. In slices prepared from animals thus treated the threshold concentration of kainate for eliciting excitation had risen to $1.64 \mu\text{M}$.
- 5 Kainate was less effective in promoting the development of epileptiform bursts of neuronal firing in colchicine-treated slices than in controls.
- 6 Kynurenic acid antagonized the excitation of CA1 neurones elicited by kainate, NMDA or quisqualate. In the CA3 region kynurenate antagonized selectively responses to microiontophoretic NMDA, with little effect on responses to kainate or quisqualate.
- 7 In slices taken from colchicine-treated rats kynurenate was able to block responses to kainate in the CA3 area in parallel with responses to NMDA.
- 8 Taken together the results suggest that the excitatory responses to kainate in the CA3 region may be partly due to a presynaptic action on mossy fibre terminals to release endogenous amino acids. The differential action of kynurenate in normal and lesioned slices may, therefore, indicate that the postsynaptic kainate receptors are sensitive to antagonism by this compound whereas the presynaptic receptors are resistant to kynurenate.

Introduction

High affinity binding sites for kainic acid are located in many regions of the central nervous system but occur with highest density in the CA3 region of the hippocampus (Foster *et al.*, 1981; Unnerstall & Wamsley, 1983; Greenamyre *et al.*, 1985; Monaghan *et al.*, 1985). This location corresponds to a high sensitivity of neurones to locally applied kainic acid (de Montigny & Tardif, 1981; Robinson & Deadwyler, 1981). De Montigny & Tardif (1981) demonstrated that pyramidal neurones in the CA3 region were approximately 80 fold more sensitive to iontophoretically applied kainate than pyramidal neurones in the CA1 region.

However, the kainate binding sites in CA3 appear to be localized, at least partly, on the presynaptic terminals of mossy fibre afferents arriving from dentate gyrus granule cells, since destruction of this pathway causes a profound loss in the number of binding sites (Represa *et al.*, 1987). This change is accompanied by a reduced excitatory potency of iontophoretically applied kainate (de Montigny *et al.*, 1987). It is possible therefore that the greater sensitivity of CA3 neurones to kainate is partly the result of its acting presynaptically to promote the release of endogenous compounds, which contribute to the excitatory response. Consistent with this idea is the demonstration that kainic acid can promote the release of both glutamate and aspartate from nerve terminals (Collins *et al.*, 1983; Ferkany & Coyle, 1983; Potashner & Gerard, 1983; Connick & Stone, 1988). In the present study we have therefore compared the potency of kainic acid on CA3 pyramidal cells in the normal hippocampal slice and slices prepared from animals pretreated with colchicine to remove the mossy fibre projection.

Kynurenic acid was described as a selective amino acid antagonist in the cerebral cortex by Perkins & Stone (1982)

and has since been used widely to block the three major classes of excitatory amino acid receptors (N-methyl-D-aspartate, NMDA, kainate and quisqualate) in many regions of the nervous system and a variety of synaptic pathways (reviewed in Stone *et al.*, 1989). However, on some neurones in the CNS kynurenic acid will distinguish, to a variable extent, between NMDA receptors and those for kainate and quisqualate, usually producing a greater degree of blockade of the former (Ganong *et al.*, 1983; Herrling, 1985; Elmslie & Yoshikami, 1985). As a second objective in the present study therefore we have attempted to examine the effects of kynurenic acid in both CA1 and CA3 regions, in order to determine whether the removal of the mossy fibre projection changes the sensitivity of amino acids to blockade by kynurenate.

Methods

Slices were prepared at a thickness of $500 \mu\text{m}$ from male Wistar rats killed by stunning and cervical dislocation. The slices were preincubated in an artificial CSF medium (composition in mM: NaCl 115, KCl 5, KH_2PO_4 1.5, NaHCO_3 25, CaCl_2 2.5, MgSO_4 1.2, glucose 10) saturated with 5% CO_2 in O_2 . The slices were maintained in this medium at room temperature ($21\text{--}24^\circ\text{C}$) for at least one hour before experimentation. Individual slices were then transferred onto a nylon net submerged in a 0.5 ml perfusion chamber, where they were superfused with medium of the same composition at a rate of approximately 3 ml min^{-1} and a temperature of 30°C .

In order to obtain quantitative estimates of the sensitivity of neurones to kainate, two methods were used. Firstly the compound was applied by microiontophoresis (Stone, 1985) in a regular sequence with quisqualate and NMDA, so as to

produce a plateau of cell firing rate of around 30 Hz. The ejecting currents of each agonist required to produce these plateaux were then taken as an indication of the relative sensitivity of the tested neurones. Compounds were applied from 5 or 7 barrelled micropipettes (Clarke Electromedical) broken back to an overall tip diameter of about $2\ \mu\text{m}$. The barrels were filled with a selection of the following solutions in water: kainate 5 mM, pH 7; N-methyl-D-aspartate (NMDA) 20 mM, pH 7; quisqualate 5 mM, pH 7.5. One barrel contained 3 M NaCl for current balancing and ejection was performed by use of a regular time cycle, so as to prevent changes in the amount of compound released for a given current.

In order to obtain more accurate estimates of the absolute sensitivity of neurones to kainate, the agonist was applied directly into the superfusing medium at known concentrations while single cell firing was recorded by a microelectrode filled with 1 M NaCl. This arrangement also served to detect epileptiform bursts of localised groups of cells which sometimes developed following the initial excitation of units by kainate. In all such superfusion experiments 2 min kainate applications were made at least 15 min apart, so as to minimize any tendency to overdepolarization, desensitization or excitotoxicity. Since virtually all the cells tested in this way were initially silent, it was possible to quantify the degree of excitation by counting the total number of spikes produced during the response using a digital counter (Neurolog).

For the subsequent examination of the antagonism of amino acid excitation by kynurenate, a comparison was made of the sensitivity to several agonists applied by micro-iontophoresis. The details of solutions used are noted above. Recordings of cell firing rate were made through a separate single electrode filled with 1 M NaCl and glued alongside the multibarrel assembly (Stone, 1985). The position of the recording electrode was adjusted so that the tip protruded 40–50 μm beyond the multibarrelled iontophoretic assembly.

Action potentials were amplified, discriminated and counted to produce instantaneous ratemeter recordings and histogram records of firing rate. In order to minimize the recognised variability which can exist in the ejecting characteristics of different iontophoretic electrodes, cell sensitivity was always compared with the same electrode in the CA1 and CA3 areas on any one slice.

Mossy fibre lesions

Small amounts of colchicine can be used to destroy selectively the mossy fibre projection from the granule cells to CA3 pyramidal cells (Goldschmidt & Steward, 1980; Represa *et al.*, 1987; de Montigny *et al.*, 1987). Rats were anaesthetized with pentobarbitone, $60\ \text{mg kg}^{-1}$ i.p., and a small burr hole made in the skull to allow the stereotaxic insertion of a microsyringe into the hippocampal formation. Colchicine $2\ \mu\text{mol}$ was injected into the hippocampus in $0.25\ \mu\text{l}$ of saline at coordinates AP 3 mm (caudal to bregma), lateral 2.8 mm, vertical 3.0 mm according to the atlas of Pellegrino *et al.* (1981). The scalp was sutured and the animal allowed to recover for two weeks, at which time the animal was killed for the preparation of brain slices. One slice from each animal was preserved in 10% formaldehyde in saline for subsequent histology to confirm the extent of lesioning.

Quantitative data are presented as mean $\pm$ s.e.mean. Statistical comparisons were made by use of Student's two-tailed *t* test for superfusion data (between slices) and paired *t* test for iontophoretic data (in which comparisons were made between subregions of each slice).

Results

The sensitivity of CA1 and CA3 pyramidal cells was compared initially by use of microiontophoresis. The application of the three agonists kainate, quisqualate and NMDA by microiontophoresis readily produced excitation of all cells

Figure 1 (a) Ratemeter records of the responses of a neurone in the CA1 pyramidal layer to the iontophoretic application of kainate (Kain) with a current of 32 nA, quisqualate (Quis) with a current of 22 nA and N-methyl-D-aspartate (NMDA) with a current of 36 nA. The currents were adjusted to produce plateaux of firing rate of approximately 30 Hz. (b and c) Show the ejecting currents required in control (b) and colchicine-lesioned (c) rats to elicit plateau firing of cells in the CA1 (open columns) or CA3 (hatched columns) regions of hippocampal slices. Each column shows the mean current (*n* = number in column) and bars represent s.e.mean. ** *P* < 0.05.

tested in both regions of the hippocampus. Figure 1 illustrates the type of responses obtained by adjusting the ejecting currents so as to induce a plateau of firing rate at about 30 Hz, and also summarizes the iontophoretic currents required to elicit these plateaux. It is clear that the currents required for NMDA and quisqualate are similar in CA1 and CA3 pyramidal cells, but the sensitivity to kainate is approximately 5 times greater in the CA3 region. Indeed the difference in sensitivity to kainate was the only significant difference noted between the two regions.

Superfusion of the slices with medium containing kainate at various concentrations in the range 0.01 to $10\ \mu\text{M}$ caused concentration-related increases of the firing frequency of both spontaneously active and initially silent units. In the CA1 region excitation was produced by perfusion with kainate at threshold concentrations (the smallest concentration eliciting an observable increase of firing rate) ranging from 0.8 to $3.5\ \mu\text{M}$ (mean $2.1 \pm 0.41\ \mu\text{M}$, *n* = 6). However, in the CA3 region a threshold excitation was elicited by concentrations in the range 0.05– $0.40\ \mu\text{M}$ (mean $0.24 \pm 0.06\ \mu\text{M}$, *n* = 6) (Figure 2).

Above these threshold levels the dose-response relationships for kainate superfusion were similar on both populations of pyramidal cells and appeared to be parallel (Figure 3). However, no attempt was made to seek maximum responses, because of the problems of achieving this with a potent compound which can cause overdepolarization or excitotoxicity on repeated applications of high concentrations.

Microiontophoresis on colchicine-lesioned slices

Destruction of the mossy fibre projection from the dentate gyrus to CA3 pyramidal cells was essentially complete when viewed by light microscopy in most of the animals treated with colchicine. The histologically observable damage in a typical lesioned hippocampus is illustrated in Figure 4. In slices taken from such animals the threshold range for eliciting CA3 cell excitation by kainate superfusion was 0.35– $2.5\ \mu\text{M}$.

Figure 2 (A, a-d) Ratemeter records of the activity of a CA3 neurone in response to superfusion with kainate; (a) threshold of $0.25 \mu\text{M}$, (b) $0.5 \mu\text{M}$, (c) $1 \mu\text{M}$, (d) $5 \mu\text{M}$. (B) Record showing epileptiform bursts of activity of a cell in the CA3 region of a different slice, induced by kainate ($0.25 \mu\text{M}$) in the absence of any overt change of single cell firing. The scales shown apply to all records.

The mean value of $1.64 \pm 0.35 \mu\text{M}$ ($n = 6$) was significantly greater than that obtained from intact slices ($P < 0.05$).

Kainate-induced epileptiform activity

Not all slices from which an excitatory response to kainate had been recorded went on to develop epileptiform bursts of

Figure 3 Partial dose-response curves for the excitation caused by kainate of neurones in the CA1 region ($\square$), neurones in the CA3 region of control rats ($\bullet$) and neurones in the CA3 region of colchicine lesioned animals ($\circ$). The ordinate scale indicates the total spike count during the response to 2 min superfusion of kainate. Points indicate the mean and vertical bars show s.e.mean for 4-6 slices. * Significantly different from control slices at $P < 0.05$.

Figure 4 Photomicrograph of a section through the hippocampus of a rat treated with colchicine ($2.5 \mu\text{mol}$) 14 days previously. In comparison with the untreated side, the lesioned hippocampus is seen to lack the granule cell layer of the dentate region, which normally projects to the CA3 layer of pyramidal cells. The scale bar represents 1 mm.

cell firing. Equally, some cells which did not show an immediate increase of firing rate in response to kainate were involved in epileptiform bursts of activity, which arose from a few seconds to several minutes after the kainate superfusion. An example of such a cell is illustrated in Figure 2. The concentrations of kainate eliciting such bursts therefore did not exactly parallel the concentrations causing excitation. On 8 control slices bursts occurred only following kainate applications at a mean concentration of $1.19 \pm 0.16 \mu\text{M}$. In colchicine-treated slices this value rose significantly to $6.25 \pm 1.25 \mu\text{M}$ ($n = 4$; $P < 0.05$).

Antagonism by kynurenic acid

Figure 5 illustrates the responses of a neurone in the CA1 region excited by each of the three excitants tested. Superfusion with kynurenic acid at a concentration of $200 \mu\text{M}$ decreased sensitivity to all three agents. On some cells NMDA responses were antagonized to a relatively greater extent than kainate and quisqualate, but no concentration of kynurenic acid was found which would produce any reduction of NMDA responses without affecting the other two agonists to some extent. At the concentration illustrated ($200 \mu\text{M}$) all three agonists could be fully antagonized on all the neurones tested. At the lower concentration of $100 \mu\text{M}$ responses to all three

Figure 5 Records of the firing rate of a neurone in the CA1 pyramidal cell region in response to the iontophoretic application of N-methyl-D-aspartate (N) 24 nA, kainate (K) 40 nA and quisqualate (Q) 28 nA. (a) Shows control responses, (b) the non-selective blockade of all three agonists by kynurenic acid $200 \mu\text{M}$, and (c) illustrates recovery 6 min later.

Figure 6 (a) Records of the firing rate of a neurone in the CA3 region of a normal hippocampus in response to the iontophoretic application of N-methyl-D-aspartate (NMDA, N) 35 nA, quisqualate (Q) 18 nA, and kainate (K) 35 nA. Superfusion of the slice with kynurenic acid 200 μ M blocked selectively the responses to NMDA. (b) Shows recovery 5 min later.

agents were reduced partially – kainate by $52 \pm 11\%$ ($n = 10$), quisqualate by $40 \pm 4\%$ ($n = 12$) and NMDA by $58 \pm 10\%$ ($n = 12$).

Figure 6 illustrates the responses of a neurone in the CA3 region. In this case kynurenic acid at a concentration of 200 μ M produced a total blockade of the responses to NMDA without affecting sensitivity to kainate ($92 \pm 16\%$ of control,

$n = 9$) or quisqualate ($98 \pm 11\%$, $n = 10$). Even at a concentration of 1 mM kynurenic acid had no significant effect on the kainate or quisqualate responses.

Colchicine-lesioned slices

The sensitivity of a CA3 neurone in a colchicine lesioned hippocampus is illustrated in Figure 7. In this case it is now clear that even at a concentration of 200 μ M kynurenic acid was able to reduce sensitivity to kainate as well as NMDA. At lower concentrations of 150 μ M or 100 μ M the antagonism of both compounds was usually partial, NMDA responses being reduced to $47 \pm 8\%$ ($n = 6$) and $79 \pm 10\%$ ($n = 8$), respectively. However, no clear differences of sensitivity between NMDA and kainate were apparent, since kainate responses were also now reduced to $39 \pm 11\%$ ($n = 6$) and $72 \pm 5\%$ ($n = 8$), respectively. Quisqualate responses on the other hand were reduced by about 25% on 3 of the cells tested at 200 μ M kynurenate, but remained unchanged by kynurenate even at 1 mM on 6 others (overall reduction of $8 \pm 12\%$, $n = 9$).

Discussion

Kainic acid binding sites are widely distributed throughout the CNS. Their occurrence in organisms as primitive as *Hydra* (London *et al.*, 1980) suggests a fundamental role in cellular function. Of the many studies which have examined kainate receptor distribution in the mammalian brain, most are agreed that these sites are localized to synaptic regions (Foster *et al.*, 1981) and that by far the greatest density of binding is restricted to the stratum lucidum in the CA3 area of the hippocampus (Foster *et al.*, 1981; Unnerstall & Wamsley, 1983; Greenamyre *et al.*, 1985; Monaghan *et al.*, 1985).

Kainate sensitivity

This localization may account for the greater sensitivity of CA3 cells to the excitatory and burst inducing effects of superfused kainate observed in the present study. However, de Montigny *et al.* (1987) have shown previously that, using microiontophoretic applications, the CA3 neurones were approximately 80 fold more sensitive to kainate excitation than cells in the CA1 region, while no differences were noted in the sensitivity to acetylcholine or glutamate. This was not the case in the present work, where a difference of sensitivity of around 5 fold was seen with microiontophoretic applications of agonists.

The explanation for this difference is not clear, but the variability of iontophoretic ejection characteristics is notorious (Stone, 1985). In addition, the use of a separate recording electrode in the present work, protruding 40–50 μ M beyond the iontophoretic barrels, may have resulted in a different cellular distribution of the ejected compounds relative to the recording tip. A comparable difference of sensitivity was obtained for CA1 and CA3 pyramidal cells when kainate was applied directly into the superfusing medium at known concentrations. The difference in sensitivity with this technique was between 10 fold at threshold concentrations and 4 fold at the maximum concentrations tested (Figure 2).

Presynaptic kainate receptors

A great deal of evidence suggests that the excitatory, toxic and convulsant effects of kainate are mediated at least partly by actions on presynaptic terminals. Thus colchicine deafferentation of the CA3 pyramidal cells greatly reduces the toxic effects of kainate on those cells (Okazaki & Nadler, 1988). The ability of kainate to induce epileptiform bursting in hippocampal slices (Westbrook & Lothman, 1983; Fisher & Alger, 1984; Neuman *et al.*, 1988) is similarly diminished after colchicine lesions (Okazaki *et al.*, 1988). In addition, kainate

Figure 7 (a) Records of the firing rate of a neurone in the CA3 region of a hippocampus taken from a colchicine-treated rat. Excitation is seen in response to the iontophoretic application of N-methyl-D-aspartate (NMDA, N) 30 nA, kainate (K) 30 nA and quisqualate (Q) 20 nA. (b) Superfusion with kynurenic acid 200 μ M antagonised the responses to NMDA and kainate while having little effect on sensitivity to quisqualate. (c) Shows recovery 5 min after the addition of kynurenate.

has been shown directly to elicit a release of endogenous amino acids *in vivo* (Young *et al.*, 1988), from olfactory cortex (Collins *et al.*, 1983) and hippocampal synaptic terminals (Ferkany & Coyle, 1983; Connick & Stone, 1988) and from astroglia *in vitro* (Lehmann & Hansson, 1988), and a release of radiolabelled D-aspartate from cerebral cortex, striatum and cerebellum (Potashner & Gerard, 1983; Gallo *et al.*, 1989). On this basis it has been concluded that the kainate binding which results from mossy fibre lesions probably reflects the loss of receptors on the presynaptic terminals. The deaf-ferentation of CA3 pyramidal cells may also induce post-synaptic changes which could contribute to the change in kainate sensitivity.

After mossy fibre lesions de Montigny *et al.* (1987) found CA3 cells to be only 2.6 times more sensitive to kainate than CA1 cells, a 30 fold decrease in CA3 cell sensitivity. In the present study a significant diminution in sensitivity of only 5 fold was obtained when tested by microiontophoresis. This may be another reflection of kainate gaining access to different regions of the cell surface in the present work because of the electrode arrangement noted above.

It is interesting to note that Sawada's group has claimed that two distinct types of CA3 cell response can be observed with kainate, a fast response obtained with kainate application into the stratum oriens or stratum radiatum, and a slower response obtainable only in the stratum lucidum (Sawada & Yamamoto, 1984). It is possible therefore that these qualitatively different responses may involve the mossy fibre terminals to different degrees, and that their relative contributions to the excitatory responses seen by de Montigny *et al.* (1987) and the present author may account for our quantitative differences. However, it must be stated that no obvious or consistent variations comparable to those described by Sawada and Yamamoto (1984) were observed in the present work in the latency or duration of kainate responses in stratum pyramidale, probably because the durations of application were far longer than the 200 ms pulses used in the intracellular recording experiments (Sawada & Yamamoto, 1984).

Kynurenic acid

Undoubtedly the dominant new finding of the present work was the different degree of antagonism by kynurenic acid of kainate-induced excitation of CA3 cells in control and colchicine lesioned slices. The non-selective nature of the antagonism of excitatory amino acids by kynurenate was noted in the original study of this activity (Perkins & Stone, 1982), and a similar non-specificity has since been found in most regions of the neuraxis (Stone & Burton, 1988). There are several examples where kynurenate is a more effective antagonist of NMDA than of kainate or quisqualate (Ganong *et al.*, 1983; Herrling, 1985; Elmslie & Yoshikami, 1985), a finding which probably owes much to the ability of kynurenate to displace glycine from the strychnine-sensitive glycine site on the NMDA receptor complex (Birch *et al.*, 1988; Mayer *et al.*, 1988). The differentiation between NMDA and other receptors in these studies was only relative, and increasing the dose of kynurenate was usually able to block other amino acid agonists. This may be a consequence of the ability of kynurenate to interact directly with glutamate binding sites (Danysz *et al.*, 1989).

In most such areas the major excitant activity of all three primary amino acid agonists is thought to involve post-synaptic receptors. The main exception is the CA3 region where, as illustrated in Figure 6, kynurenate has a relatively selective ability to antagonize NMDA responses, even at high

(mM) concentrations in the superfusing medium. This represents a marked contrast with CA1 neurones where no such selectivity is apparent.

In view of the likely involvement of presynaptic receptors in the control responses to kainate in CA3, the mossy fibre projection was therefore removed in order to reassess the selectivity of kynurenate antagonism on a relatively pure population of CA3 postsynaptic receptors. The results show that under these circumstances the selectivity towards NMDA is lost, responses to both NMDA and kainate being susceptible to blockade at similar doses of the antagonist.

It was notable that responses to quisqualate remained largely unaffected by kynurenate, an observation made also by Ganong & Cotman (1986). This may indicate some subtle variations in the properties of the quisqualate receptor in different regions of the CNS, but will require careful quantification. Preliminary experiments in which quisqualate was administered directly into the superfusion medium show that quisqualate responses are more variable and less reproducible with this method than are responses to kainate. This makes quantification and further analysis difficult; it may be more appropriate to approach this problem by use of d.c. field potential responses of a population of CA3 and CA1 neurones. Alternatively the quisqualate resistance may be a reflection of some non-specificity in the action of quisqualate at non-amino acid receptors. It would be of interest to repeat some of this work using the more selective quisqualate analogue DL- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). However, it should be emphasized that kainate has been found to be more sensitive to kynurenate antagonism than quisqualate in other regions of CNS, such as spinal cord (Evans *et al.*, 1987) and cerebellar granule cells in culture (Gallo *et al.*, 1987).

The present results suggest that the presynaptic receptors for kainate, participating in kainate responses in control but not lesioned slices, are relatively resistant to the blocking action of kynurenate. The postjunctional receptors, studied in isolation after mossy fibre removal, appear to behave as do postjunctional sites in most other regions of CNS including the CA1 region. This implies that the presynaptic and postsynaptic receptors for kainate can be discriminated pharmacologically, a proposal voiced previously by Sawada *et al.* (1988). Kynurenic acid may be a useful tool in this regard, able to antagonize the postsynaptic but not the presynaptic kainate receptors.

This conclusion makes it likely that the presynaptic kainate receptors release a substance which acts on kynurenate-insensitive receptors to cause excitation and raises questions concerning the identity of that substance. Since the endogenous amino acids glutamate and aspartate have not been tested in this study it is quite possible that these could be released and act on kynurenate resistant sites. Glutamate responses have been retained in previous studies after blockade of NMDA and non-NMDA receptor populations in olfactory cortex (Surtees & Collins, 1985; see Stone & Burton, 1988). Precedent also exists in the brainstem, where kynurenate blocks synaptic transmission and responses to kainate, AMPA and NMDA but not the effects of glutamate (Leone & Gordon, 1989). Alternatively other endogenous compounds such as cholecystokinin which, together with its receptors, is known to be highly localized to the CA3 region (Innis *et al.*, 1979; Vanderhaegen *et al.*, 1980; Zarbin *et al.*, 1983) and causes a powerful depolarization of pyramidal cells (Dodd & Kelly, 1981) could be released.

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