

The perirhinal cortex of rats: An intricate area for
microinfusion of anticonvulsants against soman-induced
seizures

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Abstract

Microinfusion of anticonvulsants into the perirhinal cortex through 1 guide cannula in each hemisphere only invades a small area of this seizure controlling site in rats exposed to soman. The purpose of the present study was to examine whether infusions made through 2 cannulas in each perirhinal cortex may produce more efficacious anticonvulsant action against soman intoxication than the use of 1 cannula only in rats infused with the ionotropic antagonists procyclidine and caramiphen or the metabotropic glutamate modulators DCG-IV and MPEP. The results showed that the mere presence of indwelling double cannulas caused proconvulsant effect in response to subsequent systemic administration of soman. Both the control and caramiphen groups with double cannulas had significantly shorter latencies to seizure onset than the corresponding groups with single cannula. Procyclidine resulted in anticonvulsant efficacy, even in rats with double cannulas. In rats that received twin infusions of DCG-IV or MPEP, the anticonvulsant impact was very high, inasmuch as a majority of the rats in each group was protected against seizure activity. Drugs possessing powerful anticonvulsant potency can apparently counteract the proconvulsant effect of double cannulas, and some can even gain enhanced anticonvulsant capacity when invading a larger area of the perirhinal cortex. Perirhinal EEG recordings (electrodes in indwelling cannulas) in a separate set of rats not exposed to soman or drugs showed no differences in basal electrical activity (total power 0.5-25 Hz or the theta band 4-12 Hz) between groups with single or double cannulas. The intrinsic excitability and synaptic connectivity of the perirhinal cortex may be associated with the proconvulsant impact observed in rats with double cannulas when exposed to soman.

Keywords: Anticonvulsants; Microinfusions; Perirhinal cortex; Soman; Seizures

1. Introduction

In preclinical epilepsy studies, seizure controlling areas have been identified by means of lesion studies and microinfusion studies (Löscher and Ebert, 1996). Some of these areas are the substantia nigra, area tempestas, perirhinal cortex, and posterior piriform cortex (Gale, 1988; Halonen et al., 1994). The anterior perirhinal cortex and posterior piriform cortex act as critical links in the propagation of epileptiform activity in limbic structures generated from microinfusion of bicuculline into the area tempestas (Halonen et al., 1994). In nerve agent research, it has been shown that microinfusion of procyclidine or NBQX into the perirhinal cortex ensures anticonvulsant efficacy against seizures subsequently induced systemically by soman, whereas a corresponding effect is obtained by scopolamine or muscimol in the posterior piriform cortex (Myhrer et al., 2010a). In addition to ionotropic glutamate receptor antagonists, also metabotropic glutamate modulators (DCG-IV ((2*S*,2'*R*,3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine), MPEP (2-Methyl-6-(phenylethynyl)pyridine hydrochloride)) cause anticonvulsant impact against soman intoxication when microinfused into the perirhinal cortex (Myhrer et al., 2010b). These findings probably indicate that there is an increase of glutamatergic activity in the perirhinal cortex already during the cholinergic phase of nerve agent poisoning, and that the perirhinal cortex is a potential site for recruiting the glutamatergic phase of the 3-phase model of McDonough and Shih (1997).

Microinfusions made through a single cannula in each perirhinal cortex probably affect a comparatively small fraction (about 7%) of the entire structure (Myhrer et al., 2010a). Lesions of the perirhinal cortex comprising an average of 74% prevent convulsions in 38% of the rats in response to a convulsant dose of soman (Myhrer et al., 2008a). Enhancement of the anticonvulsant efficacy might have been achieved if the infusions had affected a larger area of the perirhinal cortex, because microinfusions into the area

tempestas affecting almost the entire region can prevent seizures in up to 75% of the animals, whereas lesions (comprising an average of 74%) in the same area prevent convulsions in 43% of the cases (Myhrer et al., 2007, 2008,b). In principle, pharmacological manipulation of transmitter activity may have more powerful anticonvulsant impact than mere disruption of neuronal pathways. The anterior part of the perirhinal cortex that is the site of origin of the direct projections to the frontal cortex (McIntyre et al., 1996) appears as the most strategic region to affect pharmacologically and served as the target area in the previous microinfusion studies (Myhrer et al., 2010a,b).

Procyclidine (6 µg/µl) produces anticonvulsant effect when microinfused into the perirhinal cortex, whereas caramiphen (10 µg/µl) yielding similar glutamatergic and cholinergic antagonism does not (Myhrer et al., 2010a). The latter finding may imply that higher doses of caramiphen might have resulted in anticonvulsant impact. However, variations in solubility of chemical substances and the small volume used in microinfusion studies (0.25-1 µl) limit the doses that can be applied. In our hands, procyclidine can maximally be dissolved in 0.9% saline to 6 µg/µl. Both procyclidine (6 µg/µl) and caramiphen (10 µg/µl) cause anticonvulsant effects against soman poisoning when infused into the area tempestas in which cholinergic antagonism is supposed to be the crucial action of these drugs (Myhrer et al., 2008b). Hence, 2 doses of caramiphen were used to test whether single infusion of 20 µg/µl into the perirhinal cortex may cause anticonvulsant efficacy and whether twin infusions of 10 µg/µl may produce similar effect.

The purpose of the present study was to examine whether drugs invading a larger area of the perirhinal cortex through 2 cannulas in contrast to 1 cannula may result in more powerful anticonvulsant effects against soman intoxication. The drugs used for this comparison were the ionotropic antagonists procyclidine and caramiphen and the metabotropic glutamate modulators DCG-IV (mGlu2/3 receptor agonist) and MPEP

(mGlu5 receptor antagonist). Prevention of seizures/convulsions or increased latency to onset of seizure activity was used as measures of anticonvulsant efficacy. The perirhinal cortex is known to kindle faster than any other brain structure and is highly influential in the development and maintenance of temporal lobe seizure activity (Kelly and McIntyre, 1996; McIntyre and Kelly, 2000). In order to determine whether the presence of 2 versus 1 cannula in the perirhinal cortex might influence the basal intrinsic neuronal activity perirhinal EEG recordings were performed in 2 additional groups of rats.

2. Materials and methods

2.1. Animals

A total of 86 male Wistar rats from a commercial supplier (Taconic Breeding Laboratories, Denmark) weighing 300-330 g (about 90 days old) at the time of surgery were used as subjects. The experiments were carried out according to EC Directive 86/609/EEC for animal experiments and approved by the National Animal Research Authority. Twelve groups of rats (N=6-7) received bilateral single or double microinfusions of drugs or vehicle into the perirhinal cortex. Two groups of rats (N=4) received electrodes in the perirhinal cortex. The animals were housed individually and had free access to commercial rat pellets and water. The rats were handled individually 4 days preoperatively and 4 days postoperatively, being allowed to explore a table top (80 x 60 cm) for 3 min per day. The climatized vivarium (21 °C) was illuminated from 0700 to 1900 h.

2.2. Surgery

The rats were anesthetized i.p. with diazepam (10 mg/kg) and fentanyl fluanisone (2 mg/kg). Lidocain liniment was applied to the periost. The rats were implanted

stereotaxically (flat skull) with guide cannulas aimed at perirhinal cortex in both hemispheres. The guide cannula (25 gauge) was 0.5 mm in diameter and cut to a length of 11 mm. For rats provided with electrodes through implanted cannulas, the guide cannula (23 gauge) was 0,8 mm in diameter to allow penetration of the electrodes (cf., later). The upper part of the cannula was roughened in order to improve the grip of the dental cement (Durelon; ESPE, Seefeldt, Germany), which was anchored to the skull by 2 steel screws. The point of insertion was for the anterior position 3.5 mm behind bregma and 6 mm lateral to the midline. The cannula was lowered in an angle of 15° (end pointing laterally) 6 mm from the top of the skull. The point of insertion for the posterior position was 5.5 mm behind bregma and 6.5 mm lateral to the midline. The cannula was lowered in an angle of 14° (end pointing laterally) 6 mm from the top of the skull. A cannula 0.3 mm in diameter and 12 mm long (30 gauge) was fitted into the guide cannula and protruded 1 mm beyond the latter one. The infusions were made by means of a microinjection pump (Model CMA 100, Carnegie Medicine AB, Stockholm, Sweden). To prevent plugging of the indwelling cannulas, smaller cannulas (30 gauge) with a cut and bent top were inserted to a depth of 10 mm.

The rats implanted with electrodes in the perirhinal cortex were anesthetized as described above, and they were provided with single or double cannulas in the sites described above. The electrodes were made from insulated silver thread of 0.3 mm in diameter (Johnson Matthey Metals Ltd., USA) each soldered to a male golden pin component (220-PO2100 Bunker Ramo, Amphenol North America, USA). The electrodes were inserted into the cannulas localized in the anterior position with 1 mm of the end protruding the indwelling cannula. Only the tip of the electrode was bared of insulation. The golden pins were fitted into a plastic component fixed with a screw (ground) and

dental cement. A female plug was used to connect the golden pins in the plastic component with the polygraph. The rats were allowed to recover 7 days before experimentation.

2.3. *Histology*

The perirhinal cortex was defined as areas 35 and 36 of Brodmann (Burwell, 2001). After decapitation, the brains were removed and stored in 10 % formalin and dehydrated before being embedded in paraffin. Coronal sections were 5 μm thick and stained with hematoxylin and eosin.

2.4. *Drug administration*

Doses of drugs previously tested in microinfusion studies were applied. The doses used were: procyclidine hydrochloride 6 $\mu\text{g}/\mu\text{l}$, caramiphen edisylate 10 or 20 $\mu\text{g}/\mu\text{l}$, DCG-IV 1 $\mu\text{g}/\mu\text{l}$, and MPEP 0.1 $\mu\text{g}/\mu\text{l}$ (Myhrer et al., 2010a,b). The drugs were dissolved in 0.9% saline, and they were purchased from Sigma-Aldrich. Saline (0.9%) was used as vehicle. All drugs were given in 1 μl over 1 min while the rats were gently held, and the cannula remained in position for an additional $\frac{1}{2}$ min before retraction. Bilateral injections were carried out simultaneously. In the case of double infusions, they were carried out twice in rapid succession (the anterior first). Twenty min following microinfusions the rats received 1.3 x LD_{50} of soman subcutaneously that causes seizures in all rats, and the lethality is 100 percent (Sterri et al., 1980). The injection volume of 1 μl was used to ensure optimal anticonvulsant impact of the drugs. Infusion of 1 μl of 4% methylene blue in saline (0.9%) into the temporal or entorhinal cortices invades an area of about 1 mm^3 (Myhrer and Andersen, 2001) as an indication of spreading in brain tissue. The computed volume of the perirhinal cortex is about 14 mm^3 (Myhrer et al., 2010a).

2.5. EEG

The screws used for anchoring the indwelling cannulas served as electrodes. The screw in the left hemisphere was lowered 1 mm into the parietal cortex. The contralateral screw (in the skull) served as ground. The rats were connected with the recording polygraph (Grass Model 79E) with alligator clips and leads. Baseline EEG activity was monitored 15 min before injection of drugs. EEG recordings were made during convulsions or incapacitation in response to soman. Seizure activity was defined as continuous high amplitude rhythmic spike or sharp wave activity. The rats were situated in their home cages (50 x 30 x 15 cm) during the recordings.

The perirhinal EEG recording system was composed of a classical EEG recorder (Grass Model 79E) connected to a microcomputer equipped with a Labjack U12 A/D converter (Labjack Corporation, Lakewood, CO, USA). Digitization and analysis were accomplished with "in house" programs. Signals were recorded with a program written with the DAQFactory software package (Azeotech Inc, Ashland, OR, USA) and spectral analysis was performed off-line with a program written in Origin 8 Labtalk (Originlab, Northampton, MA, USA). "Total" EEG power (0.5-25 Hz) in absolute values in arbitrary units proportional to μV^2 and "partial" power in the theta frequency band (4-12 Hz) were calculated in-line through a Fast Fourier Transformer executing program. The rats were grounded to the recording polygraph from a screw in the skull. The leads were connected with a five-channel swivel that allowed the rats to move freely. Recordings were made 7 days following surgery during a period of 15 min when the rats were situated in their home cages. During the recording period, the rats characteristically expressed walking, rearing, grooming, and rest.

2.6. Observation of animals

Each rat was observed for overt behavioral changes and signs of intoxication. In the rats that convulsed, hypersalivation was seen from moisture of the lips and nose.

Unconsciousness was determined by loss of both righting and corneal reflexes. The rats were observed for convulsions/seizures and visible signs of intoxication continuously up to 40 min.

2.7. Statistics

Anticonvulsant effects of the drugs were defined as prolonged latency from injection of soman to onset of seizures/convulsions or prevention of seizures. In order to combine the data from these 2 reaction categories a latency criterion was used for the nonconvulsing rats. One min more than the longest latency among the convulsing rats was set as criterion for full anticonvulsant effect. The use of this criterion prevented normal distribution of data, and nonparametric statistics were required. For this reason, the scores had to be expressed in median and range. Overall analyses were carried out with two-way analysis of variance (ANOVA) or one-way Kruskal-Wallis ANOVA. Group comparisons were made with Dunn's multiple comparison test, Mann-Whitney *U* test, or *t* test. Computations were made with the Prism statistical software program (GraphPad Software CA, USA).

3. Results

3.1. Histology

The tracks from the guide cannulas were well marked by dead tissue in the cortical areas above the perirhinal cortex. The smallest damage was made by the sharp end of the cannulas in the perirhinal cortex. Acceptable sites of infusion were reconstructed in hematoxylin and eosin stained sections to be within the perirhinal cortex for all rats

included in this study (Fig. 1). The anterior level for localization of cannulas was common for all groups, whereas the posterior level was only used in rats with 2 cannulas in each hemisphere. In the 2 additional groups of rats equipped with electrodes in the anterior guide cannula, tracks from the guide cannulas were found to be localized in appropriate positions. No neuropathology was detected in the piriform cortex, hippocampal CA1, or amygdala in nonconvulsing rats.

3.2. Effects of pharmacological agents

When infusions were made through 2 cannulas versus 1 cannula, the latency to seizure onset either decreased (saline, caramiphen), remained unchanged (procyclidine), or increased (DCG-IV, MPEP) (Fig. 2). Two-way ANOVA revealed a significant interaction between groups and treatment category (cannula) ($F(1,5) = 24.77, p = 0.0302$).

Among the rats with single cannula, 3 rats did not convulse (Table 1). Onset of seizure activity was never observed later than 25 min after soman intoxication. Hence, the criterion for complete anticonvulsant effect was set to 26 min. One-way ANOVA revealed a reliable treatment effect ($H(6) = 27.33, p < 0.0001$). Dunn's test showed that significantly longer latencies relative to the control group were seen for the procyclidine group ($p < 0.05$) and the DCG-IV and MPEP groups ($p < 0.01$). The DCG-IV group also displayed reliably longer latency than the caramiphen 20 μ g group ($p < 0,05$).

Among the rats with double cannulas, 9 rats did not convulse (Table 2). Seizure onset was not seen later than 28 min after soman exposure. Hence, the criterion for complete anticonvulsant effect was set to 29 min. One-way ANOVA showed a significant treatment effect ($H(6) = 29.64, p < 0.0001$). Reliable differences in latencies were seen between the saline group and the procyclidine group ($p < 0.05$) and the DCG-IV and MPEP groups ($p < 0.01$). The DCG-IV and MPEP groups also had significantly longer latencies

than both caramiphen groups ($p < 0.05$). The control group and the caramiphen groups with double cannulas displayed shorter latencies to seizure onset than corresponding groups with single cannula (Tables 1 and 2). This decrease in latency was significant with U test ($p < 0.05$). The increase in latency with double cannulas versus single cannula seen for the DCG-IV and MPEP groups was also significant ($p < 0.05$).

Before the criterion for full-blown tonic-clonic convulsions/seizures was achieved the rats displayed clonic convulsions that evolved to tonic convulsions with the hind legs splayed out and rigid muscles. Rats that did not seize displayed a short period (10-20 min) of incapacitation about 10 min after soman exposure. When convulsions were prevented, no epileptiform activity was seen during incapacitation or 24 h following challenge with soman (data not shown). Convulsing rats either died spontaneously, or they were euthanized within 40 min after onset of convulsions. Nonconvulsing rats were euthanized 5 days after soman poisoning.

3.3. EEG recordings

In 2 additional groups of rats (not exposed to soman or drugs) provided with guide cannulas and electrodes, the basal neuronal activity in the perirhinal cortex did not seem to increase with double cannulas relative to single cannula (Table 3). No significant differences between the groups with single versus double cannulas were found for total EEG power (0.5-25 Hz) or theta power (4-12 Hz) with unpaired t test ($P > 0.05$). During walking and rearing, rudimentary theta waves were seen in rats with perirhinal electrodes (Fig. 3A). For the sake of comparison, Fig. 3B shows theta waves from the hippocampal region in a rat from a previous study (Myhrer et al., 2006). The polygraphs applied in the latter study and the present one had different chart speeds. In the rats subjected to

microinfusions and soman, parietal cortical electrodes reflected global seizure activity concomitant with convulsions (data not shown).

4. Discussion

The results from the present study showed that the mere presence of 2 bilateral indwelling cannulas in the perirhinal cortex resulted in proconvulsant effect during soman intoxication. When twin infusions versus single infusions were used, the latency to seizure onset either decreased (saline, caramiphen), remained unchanged (procyclidine), or increased (DCG-IV, MPEP) in response to soman. The most potent anticonvulsants apparently counteracted the proconvulsant effect of double cannulas. The proconvulsant impact of double cannulas is probably not attributable to enhanced basal activity in the perirhinal cortex, because perirhinal recordings of total power or the theta band did not differ between groups with double or single cannula.

The perirhinal cortex emerges as a particularly excitable structure, because microinfusion of a high dose of MPEP (1 µg/1 µl) causes proconvulsant action (Myhrer et al., 2010b). Furthermore, the perirhinal cortex is a highly seizurogenic area, because microinfusion of soman into this structure evokes full-blown tonic-clonic convulsions in 75% of the rats (Myhrer et al., 2010a). However, even if the presence of double cannulas elicited proconvulsant effect, it proved possible to achieve enhanced anticonvulsant efficacy by invading a larger area of the perirhinal cortex (about 20% including interaction areas) with drugs possessing high anticonvulsant potency through single perirhinal infusion (DCG-IV, MPEP). The single infusion of procyclidine was effective, and the anticonvulsant impact was maintained by twin infusions. However, both doses with single infusion of caramiphen were negative, and proconvulsant influence of double cannulas was reflected in twin infusions.

Because microinfusion of the NMDA antagonist ketamine or the muscarinic antagonist scopolamine has no anticonvulsant action in the perirhinal cortex (Myhrer et al., 2010a), blocking of NMDA receptors probably has to be supported by the antimuscarinic impact of procyclidine to achieve anticonvulsant effect. Increasing the dose of caramiphen to 20 µg/µl did not enhance the anticonvulsant capacity. Procyclidine has a far more powerful capability to antagonize a lethal dose of NMDA in mice than caramiphen (Raveh et al., 1999). Procyclidine inhibits the phencyclidine site at the NMDA receptor very potently (Reynolds and Miller, 1988) in a concentration-dependent manner (Myhrer et al., 2004), whereas caramiphen appears to bind to the Zn²⁺ site at the NMDA receptor (Raveh et al., 1999). Caramiphen has been reported to block soman-evoked down-regulation of [³H]AMPA binding to forebrain membrane preparations (Raveh et al., 2002). However, caramiphen does not interact directly with AMPA receptors, because AMPA-evoked currents in the basolateral amygdala are not affected by caramiphen (Figueiredo et al., 2011). In the latter study, GABA-evoked currents were seen to be facilitated by caramiphen. This additional effect cannot be expected to enhance the anticonvulsant capacity of caramiphen in the perirhinal cortex, because the potent GABAergic agonist muscimol is without impact in this structure (Myhrer et al., 2010a). Procyclidine and caramiphen also differ in their antimuscarinic properties, since procyclidine binds to M1-M4 receptors (Waelbroeck et al., 1992) and caramiphen binds to M1 receptors (Hudkins et al., 1993). The perirhinal cortex in rats is well provided with muscarinic M1 and M2 receptors (Spencer et al., 1986). A likely explanation of the difference between procyclidine and caramiphen in the perirhinal cortex may be that the critical combination of glutamatergic and cholinergic antagonism is more powerful in procyclidine than caramiphen.

A large body of evidence from multiple experimental paradigms strongly suggests that glutamatergic systems are engaged or recruited at an early stage of nerve agent poisoning, i. e., seconds after exposure (Weissman and Raveh, 2008). The perirhinal cortex may be a likely site for recruiting the glutamatergic phase of the 3-phase model. Anticonvulsant action of NBQX microinfused into the perirhinal cortex (Myhrer et al., 2010a) implies that in addition to NMDA and mGlu receptors also AMPA receptors play central roles in this structure. Therefore, an increase in glutamatergic activity already during the cholinergic phase appears to occur. This notion is in agreement with the evidence presented by Weissman and Raveh (2008). The present data showed that full protection against soman-induced seizures can effectively be achieved by pure modulation of glutamatergic activity. Perirhinal twin infusions of DCG-IV prevented seizures in 83% of the rats. The protection rate is 38% in rats with perirhinal lesions affecting 74% of the structure (Myhrer et al., 2008a). It appears that pharmacological manipulation of transmitter activity can have more powerful anticonvulsant impact than mere disruption of neuronal pathways.

Perirhinal EEG recordings revealed no differences in the total power (0.5-25 Hz) or theta band (4-12 Hz) between groups provided with single or double cannulas. Each electrode was placed inside an indwelling cannula and protruded the latter. The cannulas containing electrodes were localized in the anterior perirhinal cortex in which the efferents to the frontal motor cortex have their site of origin (McIntyre et al., 1996). This placement of electrodes allowed recording of theta rhythm associated with running, walking, and rearing in both groups. However, the theta waves were not as distinct as those seen in the hippocampal region (Fig. 3). A high proportion of perirhinal neurons have been reported to participate in the hippocampal-entorhinal theta activity in rats (Muir and Bilkey, 1998).

Since only the small, sharp ends of the indwelling cannulas intruded the perirhinal cortex, there is no obvious reason why double cannulas elicited proconvulsant effect. Metals (cobalt, zinc, antimony, iron, alumina cream) can slowly develop seizures (Velišek, 2006), but stainless steel has not been reported to induce such reaction. It has been reported, however, that vascular damage can result from mere implantation of electrodes in the hippocampus, and local neurochemical changes can affect areas distant from the lesion site (Boast et al., 1976). Thus, the proconvulsant impact seen from double cannulas may be attributable to a larger extent of vascular microlesions than that produced by a single cannula in the excitable perirhinal cortex. Moreover, the indwelling cannulas in the neocortical areas above the perirhinal cortex produced an additional neuronal insult. The projections from the anterior part of the perirhinal cortex to the frontal motor cortex run through the cortical region in which the indwelling cannulas were localized (cf., McIntyre et al., 1996). The damage caused by double cannulas may influence the excitability in the perirhinal cortex and/or the motor frontal cortex. It is not within the scope of the present study to examine whether the presence of multiple guide cannulas may cause similar proconvulsant actions in response to soman in other parts of the brain.

Kindling (spaced, repeated low-intensity electrical stimulation) is one of the most common models of complex partial seizures with secondary generalization and has been used extensively to model temporal lobe epilepsy (McIntyre and Kelly, 2000). In temporal lobe slices from kindled rats, it was shown that the circuitry of the perirhinal cortex has a greater propensity to develop and sustain epileptiform activity than the amygdala and piriform cortex (Kelly and McIntyre, 1996). In whole rat experiments, the fastest kindling rates (number of stimulations to first stage-5 convulsion) in the temporal lobe are, in descending order, the perirhinal cortex, amygdala, entorhinal cortex, ventral hippocampus, and dorsal hippocampus. The latency from stimulation to onset of stage 5 clonic phase of

the kindled convulsions also parallels the kindling rates (McIntyre and Gilby, 2008). The above findings are in compliance with the notion that the perirhinal cortex with its extensive projections to the frontal motor network ultimately drives the brainstem spinal cord motor networks evolving clonic-tonic convulsions (McIntyre et al., 1996; McIntyre and Gilby, 2008). Thus, the intrinsic excitability of the perirhinal cortex and its close anatomical connection with the motor system may explain why the presence of double cannulas in this structure or its efferent projections produces proconvulsant effect in rats poisoned with soman.

In conclusion, the presence of 2 guide cannulas in the perirhinal cortex induced proconvulsant impact during intoxication by soman. Inasmuch as microinfusion through 1 cannula only invades an area about 7% of the perirhinal cortex (Myhrer et al., 2010a), the real potency of the perirhinal cortex as a seizure controlling site may not be fully demonstrated. However, very potent anticonvulsants like DCG-IV and MPEP can increase their efficacy markedly when invading a larger area of the perirhinal cortex by twin infusions. The obstacle encountered may be associated with this structure's intrinsic excitability and synaptic connectivity making it highly influential in the development of temporal lobe seizures (Kelly and McIntyre, 1996).

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Figure legends

Fig. 1. Reconstruction of coronal sections showing anterior (A) and posterior (B) levels of guide cannulas aiming at the perirhinal cortex (Brodmann's areas 35 and 36; Burwell, 2001). Infusion cannulas and electrodes protruded 1 mm beyond the end of the guide cannulas. The sections are adapted from the atlas of Paxinos and Watson (2005). Peri ctx = perirhinal cortex.

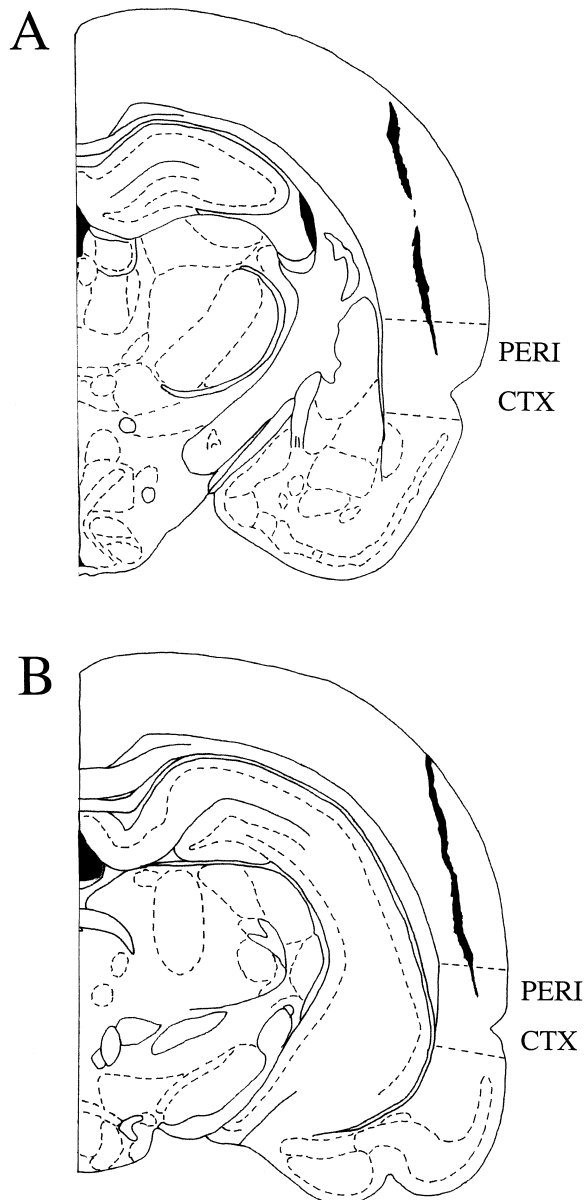


Fig. 2. Median latency to seizure or nonseizure criterion after soman intoxication in rats microinfused bilaterally with anticonvulsants through single or double cannulas in the perirhinal cortex.

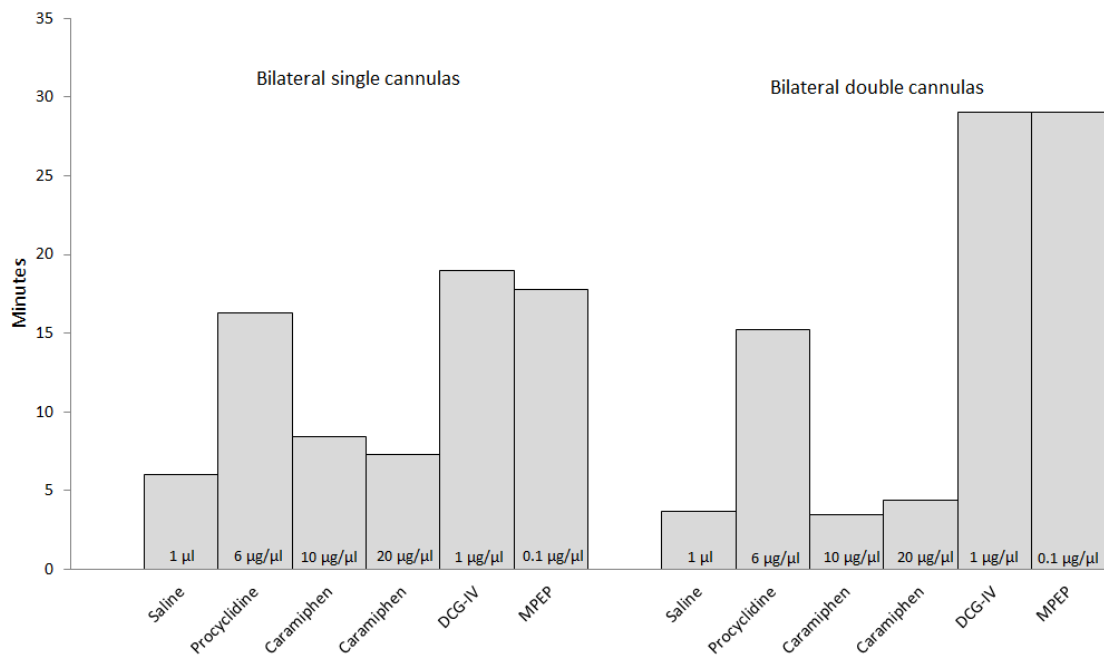
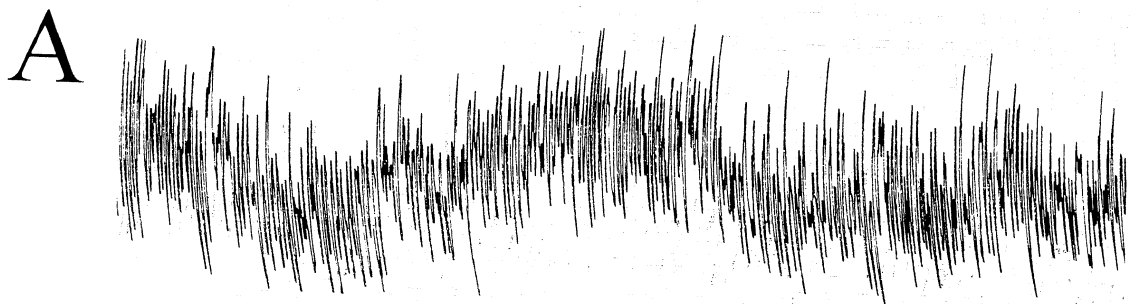
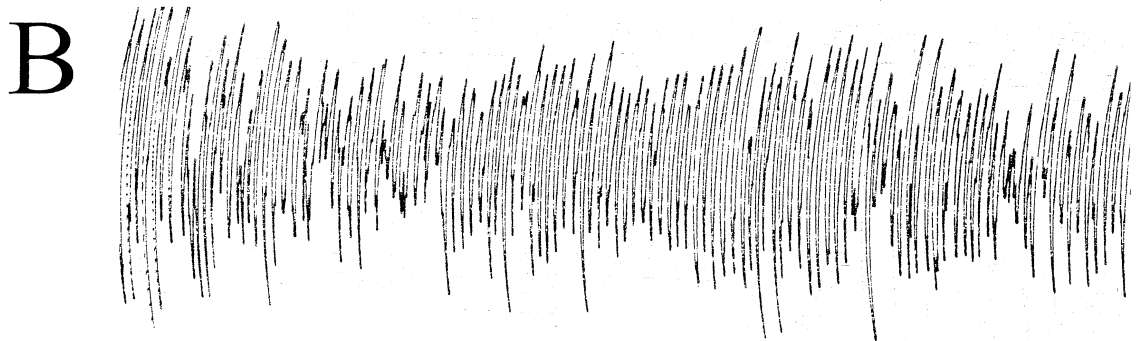


Fig. 3. Theta waves during walking recorded from electrodes through the anterior cannulas in a rat with double cannulas aimed at the perirhinal cortex (A). Theta waves during walking from the hippocampal region in a rat included in a previous study (Myhrer et al., 2006) (B). The recordings in A and B have been carried out with different polygraphs and with different chart speeds.



6 sec



6 sec

Table 1

Anticonvulsant effects of single bilateral microinfusions (1µl) of ionotropic antagonists and modulators of metabotropic glutamate (mGlu) receptors into the perirhinal cortex of rats intoxicated by soman (1.3 x LD₅₀)

Drug	Dose	Receptor	N	Latency (min) to		Nonconvulsing	
				seizure/nonseizure	Median	Range	N
Saline	-----	-----	7	6.0	3.2 - 9.0	0	0
Procyclidine	6 µg	M1-M4+	7	16.3*	8.3 - 25.0	0	0
		NMDA					
Caramiphen	10 µg	M1 NMDA	7	8.4	4.5 - 12.5	0	0
Caramiphen	20 µg	M1 NMDA	7	7.3	4.4 - 12.0	0	0
DCG-IV	1 µg	mGlu2/3	7	19.0**	10.4 - 26.0	2	29
MPEP	0.1 µg	mGlu5	7	17.8**	9.0 - 26.0	1	14

Significantly different from saline-treated control group * $p < 0.05$, ** $p < 0.01$.

Table 2

Anticonvulsant effects of double bilateral microinfusions (1 μ l) of ionotropic antagonists and modulators of metabotropic glutamate (mGlu) receptors into the perirhinal cortex of rats intoxicated by soman (1.3 x LD₅₀)

Drug	Dose	Receptor	N	Latency (min) to		Nonconvulsing	
				Median	Range	N	Percent
Saline	-----	-----	6	3.7	3.0 - 6.0	0	0
Procyclidine	6 μ g	M1-M4+ NMDA	6	15.2*	6.0 - 18.3	0	0
Caramiphen	10 μ g	M1 NMDA	6	3.5	3.3 - 7.3	0	0
Caramiphen	20 μ g	M1 NMDA	6	4.4	3.2 - 6.9	0	0
DCG-IV	1 μ g	mGlu2/3	6	29.0**	28.0 - 29.0	5	83
MPEP	0.1 μ g	mGlu5	6	29.0**	15.0 - 29.0	4	67

Significantly different from saline-treated control group * $p < 0.05$, ** $p < 0.01$.

Table 3

Mean results of bilateral perirhinal EEG recordings from freely moving rats provided with single or double guide cannulas aimed at the perirhinal cortex. Electrodes had been inserted through the cannulas in the anterior position (Fig. 1A) in both groups

Group	<i>N</i>	Total power (0.5-25 Hz)		Theta power (4-12 Hz)	
		Mean (S.E.M.)	Mean Hz	Mean (S.E.M.)	Mean Hz
Single cannula	4	198±19.3	4.0	115±17.7	6.1
Double cannulas	4	186±54.7	4.5	106±17.1	6.0

