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A guinea-pig hippocampal slice model of organophosphate-induced seizure activity

PATRICK K. HARRISON, ROBERT D. SHERIDAN, A. CHRIS GREEN, IAIN R. SCOTT & JOHN E.H. TATTERSALL

Biomedical Sciences, Dstl, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

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Corresponding author: P.K. Harrison, Biomedical Sciences, Dstl, Bldg 04, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK; Telephone: +44 1980 614683; Fax: +44 1980 613741; Email: pkharrison@dstl.gov.uk

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Abbreviations: OP, organophosphate; ACSF, artificial cerebrospinal fluid; AChE, acetylcholinesterase; ACh, acetylcholine; 4-AP, 4-aminopyridine; DFP, Diisopropylphosphorofluoridate; DMSO, Dimethyl sulfoxide; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine

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ABSTRACT:

Extracellular recording techniques have been used in the guinea-pig hippocampal slice preparation to investigate the electrophysiological actions of the organophosphate (OP) anticholinesterase, soman. When applied at a concentration of 100 nM, soman induced epileptiform activity in the CA1 region in approximately 75 % of slices. This effect was mimicked by the anticholinesterases, paraoxon (1 and 3 μM), physostigmine (30 μM) and neostigmine (30 μM), thus providing indirect evidence that the epileptiform response was mediated by elevated acetylcholine levels. Soman-induced bursting was inhibited by the muscarinic receptor antagonists atropine (concentrations tested: 0.1 to 10 μM), telenzepine (0.03 to 3 μM), AF-DX116 (0.3 to 300 μM) and biperiden (0.1 to 10 μM) and by the benzodiazepine anticonvulsants diazepam (3 to 30 μM) and midazolam (3 to 30 μM), but was not inhibited by the nicotinic antagonists mecamylamine (30 μM) and methyllycaconitine (300 nM). In contrast to soman-induced epileptiform activity, bursting induced by the K^+ channel blocker 4-aminopyridine (4-AP, 30 μM), the adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 30 nM) or perfusion with low Mg^{2+} buffer was insensitive to atropine (10 μM). The ability of muscarinic antagonists and benzodiazepines to inhibit soman-induced epileptiform activity is in accordance with the *in vivo* pharmacology of soman-induced seizures and suggests that the guinea-pig hippocampal slice preparation may provide a useful tool for the evaluation of novel anticonvulsant therapies for the treatment of seizures related to OP poisoning.

OP insecticides (e.g., paraoxon) and nerve agents (e.g., soman and sarin) exert their toxicity by irreversibly inhibiting AChE, leading to ACh accumulation and overstimulation of cholinergic pathways (Taylor, 1996). The symptoms and signs of OP intoxication are predominantly due to muscarinic receptor overstimulation and include miosis, bronchoconstriction, bradyarrhythmias, seizures and respiratory failure (McDonough and Shih, 1997; Brown and Brix, 1998; Lallement *et al.*, 1998).

Treatment for mild OP intoxication consists of a muscarinic receptor antagonist (atropine) and an oxime reactivator of OP-inhibited enzyme (e.g., pralidoxime). In moderate to severe OP intoxication a benzodiazepine (e.g., diazepam) may be added in an effort to control seizures and convulsions (Lallement *et al.*, 1998; Shih and McDonough, 1999; McDonough *et al.*, 2000). However, benzodiazepine treatment whilst efficacious in the majority of circumstances has scope for improvement: OP-induced seizures can sometimes reoccur despite treatment and, even when seizures are abolished, neuronal damage can still arise (Hayward *et al.*, 1990; Baze, 1993; Shih *et al.*, 1999). The undesirable side-effects of the benzodiazepine class of drugs (namely, sedation and respiratory depression) provide further impetus to identify more effective anticonvulsants for OP intoxication.

In animal experiments the resistance of OP-induced seizures to conventional antiepileptic drugs such as phenytoin and carbamazepine therapies is well-established (Shih *et al.*, 1999). However, muscarinic receptor antagonists are highly effective in terminating soman-induced seizures when administered early on (i.e., 5 min after seizure onset) but become less efficacious when treatment is delayed for more than 10 min and

can become ineffective when the delay is greater than 40 min (Shih *et al.*, 1999). This progressive resistance to antimuscarinic therapy is thought to reflect a time-dependent transition from cholinergic to non-cholinergic seizure mechanisms (Shih *et al.*, 1999; McDonough and Shih, 1997; Lallement *et al.*, 1998). These mechanisms include the recruitment of glutamatergic pathways as suggested by the observation that addition of NMDA antagonists (e.g., gacyclidine) to standard treatment regimes markedly improves the control and outcome of seizures (Lallement *et al.*, 1999; Raveh *et al.*, 1999). A similar profile of increased resistance to medical intervention due to recruitment of NMDA pathways has been proposed for *status epilepticus* (Rice and DeLorenzo, 1999).

Whilst the ability of AChE inhibitors and cholinergic agonists (e.g., pilocarpine; Turski *et al.*, 1989) to induce seizures *in vivo* is well known, the precise areas of the brain involved in the initiation and maintenance of OP-induced seizures are uncertain. However, the hippocampus is an area of particular interest as it is known to be important in the initiation and maintenance of epileptic seizures. Moreover, this structure has a large cholinergic innervation (Lewis *et al.*, 1967) and is damaged following OP-induced seizures (Hayward *et al.*, 1990; Baze, 1993).

The *in vitro* hippocampal slice preparation is widely used to investigate seizure activity and evaluate antiepileptic/anticonvulsant drugs (Oliver *et al.*, 1977) due to its ability to exhibit epileptiform activity. A variety of manipulations can induce this state including application of 4-AP (Perreault and Avoli, 1991), DPCPX (Alzheimer *et al.*, 1989) and alteration of external Mg^{2+} (Psarropoulou and Haas, 1989). Of particular relevance to the current

study is the report by Endres *et al.* (1989), who observed paraoxon-induced epileptiform activity in the CA1 region of guinea-pig hippocampal slices in which the CA3 region had been removed. Epileptiform activity has also been reported in the rat hippocampal slice following application of anticholinesterases although this has not been a universal finding (Cole and Nicoll, 1984; Lebeda and Rutecki, 1985; Williamson and Sarvey, 1985). Whilst Lebeda and Rutecki consistently saw the induction of epileptiform activity following application of the anticholinesterases diisopropylphosphorofluoridate (DFP) or soman, Cole and Nicoll observed the induction of epileptiform activity in only 20 - 30 % of slices following application of physostigmine, whilst Williamson and Sarvey failed to see any epileptiform activity following application of either DFP or physostigmine (although a secondary population spike was induced).

The aim of the present work was the development and characterisation of an *in vitro* model of OP-induced seizure activity in the hope of developing a useful tool for evaluating new therapies for OP poisoning. We have investigated the ability of soman to induce epileptiform activity in the guinea-pig hippocampal slice. Several other OP and carbamate anticholinesterases were also tested for their ability to induce epileptiform activity. The effects of several muscarinic and nicotinic antagonists and two benzodiazepines on the epileptiform activity were also evaluated. Finally, the effect of atropine on alternative forms of epileptiform activity was assessed. A preliminary report of this work has appeared elsewhere (Harrison *et al.*, 2000).

Methods

Guinea-pig hippocampal slices. Male Dunkin Hartley guinea-pigs (250 - 500 g) were anaesthetised with halothane and killed by decapitation. The brain was then removed and the left and right hippocampi removed. The hippocampus was then cut into temporal and septal sections. The temporal section was mounted on a tissue block and five to seven transverse slices (500 μm thick) prepared using a Vibratome (Intracel). The slices were stored at room temperature in an ACSF of the following composition (in mM): NaCl 118, KCl 3, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1, $CaCl_2$ 1.5, D-glucose 10 (gassed with 95% O_2/5% CO_2). Slices were left to recover for at least 1 h before transfer to a recording chamber (volume ≈ 1 ml) where they were submerged and continuously superfused (at 10 - 15 ml min^{-1}) with gassed ACSF at 31°C.

Electrophysiological recordings and application of compounds. After a further 30 min equilibration, an extracellular recording electrode (filled with 2 M NaCl; 1 - 5 MΩ resistance) was placed in CA1 *stratum pyramidale* in order to monitor neuronal activity. The Schaffer collateral/commissural pathway was then stimulated every 30s via a bipolar stimulating electrode (MCE 100, Harvard apparatus; stimulation pulse width ≈ 150 μs, amplitude ≈ 250 μA) and the recording electrode lowered into CA1 changed until the population spike amplitude was maximal. Stimulation was then terminated. Baseline activity was monitored for at least 30 min before application of anticholinesterase, DPCPX, 4-AP or low Mg^{2+} ACSF; any slices showing spontaneous epileptiform activity during this period were discarded. For the experiments with low Mg^{2+} ACSF, the slices were perfused with an ACSF of

the same composition but with $MgCl_2$ omitted. Anticholinesterases were applied for up to 60 min – if epileptiform activity had not appeared by this time the slice was classified as 'unresponsive', but only after slice viability had been confirmed by the appearance of epileptiform activity following exposure to 4-AP (30 µM). In slices in which epileptiform activity appeared, the activity was allowed to continue for 15 min before addition of the test compound. All compounds were applied by addition of appropriate volumes of concentrated stock solution directly into the perfusion reservoir (exchange and equilibration of added compounds with the recording chamber occured within 3 min). In a series of separate experiments neither 0.3% v/v DMSO nor 0.1% v/v ethanol (the maximum vehicle concentrations used) had any effect on epileptiform activity (data not shown).

Data analysis. Data were captured via a CED 1401 ADC interface controlled by Spike 2 software (Cambridge Electronic Design, UK). The analogue signal was digitised at 2 kHz. At the end of each experiment the digitised trace was manually analysed for burst characteristics (amplitude, duration and rate) using on-screen cursors. A burst was defined as a deflection from baseline noise lasting > 10 ms, with a magnitude of > 0.1 mV above baseline and separated from neighbouring "bursts" by a quiescent period of > 2 s. To determine the effect of the various treatments on epileptiform activity, the average burst rate in the 10 min epoch immediately before treatment application was compared with the burst rate 30 - 40 min after treatment application (throughout this paper bursting and epileptiform activity was used interchangeably).

Unless otherwise specified, summarised data are presented as mean ± s.e. mean, with significance determined using 1 way ANOVA with Dunnett's post-hoc test.

Drugs. All chemicals were obtained from Sigma-Aldrich Co Ltd (Poole, UK) or Tocris Cookson Ltd (Langford, UK) with the following exceptions: soman (pinacolyl methylphosphonofluoridate), purity > 95 %, approximately 5 mg/ml solution in isopropyl alcohol, was synthesised by the Chemistry Department, Dstl Porton Down; biperiden lactate ('Akineton'; α-bicyclo[2.2.1]hept-5-en-2-yl-α-phenyl-1-piperidinepropanol; Knoll) and midazolam hydrochloride ('Hypnovel'; 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo [1,5-a][1,4]-benzodiazepine; Roche) were obtained from Medical Supplies Agency (Andover, UK). Compounds were dissolved as follows: AF-DX 116 (11-(2-[(diethylamino)methyl]-1-piperidinyl acetyl)-5,11, dihydro-6H-pyrido 92.b-b) (1,4)-benzodiazepin-6-one; 100 mM in DMSO), 4-AP (4-aminopyridine; 30 mM in water), atropine sulphate (endo-(±)-α-(hydroxymethyl)benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester; 30 mM in water), biperiden lactate (supplied formulated at 5 mg/ ml), diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one; 30 mM in ethanol), DPCPX (8-cyclopentyl-1,3-dipropylxanthine; 30 mM in ethanol), mecamylamine hydrochloride (2-(methylamino)isocamphane; N,2,3,3-Tetramethylbicyclo(2.2.1)heptan-2-amine; 100 mM in water), methyllycaconitine citrate ([1α,4(S),6β,14α,16β]-20-Ethyl-1,6,14,16-tetramethoxy-4-[[[2-(3-methyl-2,5-dioxo-1-pyrrolidinyl) benzoyl]oxy]methyl]aconitane-7,8-diol citrate; 3 mM in water), midazolam hydrochloride (supplied formulated at 5 mg/ ml), neostigmine bromide (3-

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(N,N-Dimethylcarbamoyloxy)-N,N,N,-trimethylanilinium bromide ; 30 mM in water), paraoxan (O,O-Diethyl O-(4-nitrophenyl) phosphate; as supplied), physostigmine hemisulfate ((3aS-cis)-1,2,3,3a,8,8a-Hexahydro-1,3a,8-trimethylpyrrolo [2,3-b]indol-5-ol methylcarbamate hemisulfate; 30 mM in water) and telenzepine dihydrochloride (4,9-dihydro-3-methyl-4-[(4-methyl-1-piperazinylacetyl)]-10H-thienol[3,4-b][1,5]benzodiazepin-10-one; 30 mM in water).

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Results

The effect of soman on neuronal activity. Administration of soman (100 nM; a concentration which should rapidly abolish acetylcholinesterase activity, see Santos et al., 2003) resulted in epileptiform activity which could be recorded from the CA1 *stratum pyramidale* in 117/154 slices (Fig. 1). The characteristics of epileptiform activity are summarised in Table 1. Within an individual slice, the burst duration did not vary greatly. Once initiated, bursting continued for up to 4.5 h (the longest time recorded) and once induced the activity was unaffected by wash-out of soman. Application of the vehicle for soman (0.0004 % v/v isopropyl alcohol) did not induce bursting in 4 slices (data not shown).

The effect of atropine on soman induced bursting. Previous *in vivo* experiments have shown that soman-induced epileptiform activity can be reversed by the muscarinic receptor antagonist atropine. If the activity induced here is from inhibition of AChE (with resulting overstimulation of cholinergic pathways), it too should be sensitive to blockade of these receptors. In order to test this hypothesis, we investigated the effect of atropine on soman-induced activity. When applied 15 min after the development of soman-induced bursting, atropine (0.1 to 10 μM) inhibited bursting (Fig. 2). A significant reduction ($P < 0.05$) was seen at concentrations > 100 nM. However, even at the highest concentration tested (10 μM) bursting was never abolished, the burst rate being 23 ± 11 % of pretreatment (n = 5).

The effect of other anticholinesterase agents on hippocampal electrophysiology. In order to provide evidence that the bursting activity

was due to inhibition of AChE, the effect of three other anticholinesterases was investigated. Paraoxon, applied at 1 μM (4 slices) and 3 μM (3 slices), neostigmine (30 μM; 4 slices) and physostigmine (30 μM; 4 slices) also induced epileptiform activity which was sensitive to the muscarinic antagonist atropine (Fig. 3). We could not quantify any difference in the reversal produced by atropine between anticholinesterases.

The effect of muscarinic antagonists on soman-induced bursting. Several subtype-selective muscarinic receptor antagonists also affected soman-induced bursting. The compounds chosen were the selective M_1 antagonist telenzepine, the selective M_2 antagonist AF-DX 116 and the M_1 preferring antiparkinson drug, biperiden. Both telenzepine and biperiden reduced the rate of bursting in a concentration-related manner (Fig. 4). A significant effect ($P < 0.05$) was seen for telenzepine at concentrations of ≥ 100 nM and for biperiden at 10 μM.

AF-DX 116 was less potent, only producing a significant reduction ($P < 0.05$) in bursting at 300 μM.

Time dependent effect of atropine. A characteristic of nerve agent-induced seizures *in vivo* is that they become increasingly resistant to antimuscarinic intervention as the seizure continues. For this reason a series of experiments was undertaken to compare the effects of atropine (300 nM) when added 15 min or 75 min after the onset of epileptiform activity. The inhibition produced was not significantly different between the two time points ($P > 0.05$; unpaired, 2-tailed *t*-test). When added after 15 min of activity the burst rate was 46.0 ± 19 % of pretreatment whilst when added after 75 min of

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activity the rate was 49 ± 15 % of pretreatment (n = 5 slices at each time point).

The effect of nicotinic antagonists on soman induced bursting. In order to test whether the epileptiform activity might also involve nicotinic receptor activation, two selective antagonists, mecamylamine and methyllycaconitine (α7 selective) were tested. Neither 30 μM mecamylamine (n = 4) nor 300 nM methyllycaconitine (n = 4) had any significant effect compared to control ($P > 0.05$; unpaired, 2-tailed *t*-test): burst rates 30 - 40 min after addition of antagonist were 180 ± 24 % (mecamylamine), 111 ± 13 % (methyllycaconitine) *versus* 124 ± 11 % in vehicle-treated slices (Fig. 5).

The effect of benzodiazepines on soman-induced bursting. One of the most commonly used anticonvulsants in the treatment of OP poisoning is diazepam, which has previously been shown to terminate soman-induced epileptiform activity *in vivo*. Therefore, the effects of diazepam and another benzodiazepine anticonvulsant, midazolam, were investigated. Both anticonvulsants inhibited soman-induced bursting in a concentration-dependent manner (see Fig. 6), with a significant reduction being seen at concentrations ≥ 10 μM for both benzodiazepines ($P < 0.05$).

The effect of atropine on non-anticholinesterase induced epileptiform activity. In order to investigate whether inhibition of bursting by atropine was restricted to anticholinesterase-induced epileptiform activity, the effect of atropine on epileptiform activity induced by other means was studied. Application of 30 μM 4-AP (a blocker of K^+ channels), 30 nM DPCPX (an adenosine A_1 antagonist) or perfusion with low Mg^{2+} ACSF, all induced epileptiform activity. Table 1 summarises the characteristics of the

epileptiform activity induced by the different treatments with an example of each shown in Fig. 7.

The burst rate (as a percentage of pretreatment) following 10 μM atropine for 4-AP induced, DPCPX and low Mg^{2+} ACSF treatment was 112 ± 8 % (n = 5), 131 ± 13 % (n = 5) and 202 ± 69 % (n = 6), respectively. In contrast, the burst rate following 10 μM atropine for soman-induced bursting was 23 ± 11 %.

Discussion

The present study, which confirms and extends previous work from this laboratory (Wood and Tattersall, 2001), demonstrates that the irreversible anticholinesterase, soman, induces epileptiform activity in the guinea-pig hippocampus *in vitro*. This action was mimicked by the OP anticholinesterase paraoxon, by the carbamate anticholinesterases physostigmine and neostigmine, and was inhibited by muscarinic but not nicotinic antagonists. These findings provide evidence that epileptiform activity induced by anticholinesterases in the guinea-pig hippocampal slice is mediated by muscarinic receptor stimulation contingent upon inhibition of ACh hydrolysis and subsequent elevation of ACh concentration.

Although the site of initiation of anticholinesterase-induced epileptiform activity was not explored, work by others suggests that the CA1 region in isolation is capable of generating OP-induced epileptiform activity. Endres *et al.* (1989), using guinea-pig hippocampal slices in which the CA2/CA3 region had been removed, found that paraoxon elicited muscarinic receptor-mediated bursting in the CA1 region in ≈ 70 % of slices. The decision to leave the CA2/CA3 region intact in the slices used here was predicated on the fact that this region would contribute *in vivo* to any anticholinesterase response.

The proportion of OP-responsive slices in this study (≈ 75 %) is similar to that reported by Endres *et al.* (1989). Why some slices did not respond to OP application is unclear, but it is conceivable that the orientation of the hippocampus during preparation and the precise locale of the slice within the hippocampus (septal *versus* temporal) may determine the extent to which

cholinergic afferents are preserved and consequently the inherent excitability of individual slices (Lewis *et al.*, 1967; Lee *et al.*, 1990).

The ability of anticholinesterases to induce epileptiform activity or increase excitability has also been reported in rat hippocampal slices. Lebeda and Rutecki (1985) reported that the OP anticholinesterases, DFP (diisopropylfluorophosphate) and soman, induced atropine-sensitive epileptiform discharges in the CA3 region of rat hippocampus *in vitro*. Although Williamson and Sarvey (1985) failed to detect epileptiform activity in CA1 following application of DFP or neostigmine, they did find that anticholinesterases induced a secondary population spike.

In common with atropine, other muscarinic receptor antagonists reduced the frequency of soman-induced epileptiform bursting. The rank order of potency of these antagonists (telenzepine > biperiden >> AF-DX116) tentatively suggests that the effects of soman may be mediated by the action of endogenous ACh on M_1 muscarinic receptors. Multiple muscarinic subtypes are present within the hippocampus, with M_1/M_3 subtypes thought to be located predominantly postsynaptically and M_2/M_4 subtypes modulating transmitter release presynaptically (Levey *et al.*, 1991; Boddeke, 1991; McKinney *et al.*, 1993). ACh exerts muscarinic receptor-mediated excitatory actions within the hippocampus by various mechanisms: block of a Ca^{2+}-dependent K^+ current, block of the voltage-dependent M-current and block of a non-voltage-dependent resting K^+ current (Cole and Nicoll, 1984; Madison *et al.*, 1987). Previous reports support the idea that antagonists at the M_1 receptor might be more efficacious in the treatment of OP poisoning *in vivo*. Thus, pretreatment with the M_1 selective antagonist, pirenzepine, prevented

the initiation of seizures induced by carbachol in 91 % of rats compared to only 9 % for the M_2 selective antagonist, methoctramine (Cruickshank *et al.*, 1994).

In addition to a postsynaptic excitatory action via muscarinic receptors, soman-induced epileptiform bursting could also be mediated by suppression of GABAergic inhibition. Two opposing effects of cholinoceptor stimulation on $GABA_A$ receptor-mediated inhibition have been reported in the guinea-pig hippocampus (Behrends and ten Bruggencate, 1993): (a) a decrease in the frequency of miniature IPSCs and (b) a potentiation of spontaneous IPSCs. Santos *et al.* (2003), recording from rat CA1 neurones, obtained direct evidence for a muscarinic receptor-mediated effect of soman on inhibitory responses subserved by $GABA_A$ receptors: soman decreased the amplitude and frequency of spontaneous IPSCs and reduced the amplitude of evoked IPSCs. However, no effect on miniature IPSCs was observed (Santos *et al.*, 2003).

Of note in the present study was the failure to obtain a complete inhibition of soman-induced epileptiform discharges with muscarinic receptor antagonists, suggesting that a component of the epileptiform activity is mediated by a non-muscarinic mechanism. One potential candidate mechanism is nicotinic receptor stimulation. Although there are no reports in guinea-pig, it is well-established that nicotine can induce seizures in mice (Damaj *et al.*, 1999) and rats (Newman *et al.*, 2001). Nicotinic receptor agonists have also been reported to enhance on-going epileptiform activity induced by 4-aminopyridine, bicuculline and low Mg^{2+} perfusion in rat hippocampal slices (Roshan-Milani *et al.*, 2003). However, the failure of

mecamylamine and methyllycaconitine to inhibit bursting in our study argues against the involvement of nicotinic receptors in sustaining soman-induced activity.

One hypothesis for the failure to completely inhibit soman-induced bursting with muscarinic antagonists is that epileptiform activity, once initiated by overstimulation of muscarinic receptors, is maintained following recruitment of non-cholinergic pathways. Evidence for the secondary involvement of glutamatergic mechanisms in primates has been presented by Lallement *et al.* 1999, who reported that treatment with atropine, oxime and diazepam following soman-intoxication failed to control seizure activity, but if gacyclidine (a non-competitive NMDA antagonist) was also included, normal EEG activity was restored. McDonough *et al.* (2000), comparing the effects of a series of antimuscarinic drugs on soman-induced seizure activity in guinea-pigs, reported that the antiseizure potency of the antagonists decreased by ≈ 10 fold when treatment was delayed from 5 to 40 min. In an attempt to determine whether our slices showed a time-dependent change in sensitivity to atropine, a series of experiments was undertaken to compare the effect of atropine after 15 or 75 min of soman-induced epileptiform activity. However, we found no significant difference at these two time points. In this regard, then, our *in vitro* model does not mimic the pharmacology of soman *in vivo*. However, a confounding feature of the present slice model is the requirement to wait for the induction of a moderately stable baseline bursting rate upon which the effects of any intervention may be quantified. It is therefore conceivable that any adaptive change in the sensitivity of the bursting to atropine may have already occurred.

Two clinically used benzodiazepine anticonvulsants, diazepam and midazolam, were examined for their effect on soman-induced epileptiform activity. These drugs can terminate soman-induced seizures *in vivo* (Hayward *et al.*, 1990; McDonough *et al.*, 2000). Both diazepam and midazolam inhibited soman-induced epileptiform activity in the slice at concentrations ≥10 μM. These concentrations are consistent with plasma concentrations reported for diazepam to terminate soman-induced seizures in guinea-pigs (approximately 3 - 4 μM; Capacio *et al.*, 2001). However, comparisons between our *in vitro* data and *in vivo* studies of soman-induced seizures are complicated by the need *in vivo* to administer adjunctive therapy to keep the animal alive – this therapy, in itself, may have anticonvulsant actions. The concentrations of diazepam and midazolam used in our experiments are also comparable to those in other *in vitro* slice models of epileptiform activity. When epileptiform activity was induced by penicillin in guinea-pig hippocampal slices, diazepam (≈ 10 μM) was required to consistently block bursting (Schneiderman and Evans, 1986), while an IC_{50} of ≈ 12 μM was determined for midazolam to inhibit low-Ca^{2+}-induced bursting in the guinea-pig hippocampal slice (Ashton *et al.*, 1988). Overall, the data obtained with the benzodiazepines are in accord with the known *in vivo* pharmacology of OP-induced seizures and are comparable to other *in vitro* models of epileptiform activity.

The pharmacology of the epileptiform activity induced by soman and other anticholinesterases in the present study was clearly distinct from other forms. This difference is clearly demonstrated by the fact that atropine reduced bursting only in anticholinesterase-treated slices. This finding is

perhaps not surprising given the varied mechanisms by which these epileptogenic agents act (4-AP blocks K^+ channels, DPCPX prevents endogenous activation of inhibitory adenosine A_1 receptors, low Mg^{2+} enhances NMDA receptor/ion channel function and increases the probability of transmitter release).

In order to be considered as a useful model in which to evaluate the ability of anticonvulsants to switch off OP-induced epileptiform activity, the *in vitro* hippocampal slice preparation must relate to the *in vivo* situation. One characteristic of OP poisoning is the development of seizures (Shih and McDonough, 1999; Ohbu *et al.*, 1997). Here we have shown that application of the OP anticholinesterase, soman, as well as other structurally diverse anticholinesterases, can induce epileptiform activity in the guinea-pig hippocampal slice. The seizures induced by OPs *in vivo* can be treated with antimuscarinics. Here we have shown that application of antimuscarinic compounds inhibit soman-induced epileptiform activity. Clinically, seizures associated with OP poisoning are sensitive to treatment with benzodiazepine anticonvulsants (Ohbu *et al.*, 1997). Again, the epileptiform activity induced here was inhibited by both diazepam and midazolam. Consequently, the guinea-pig hippocampal slice may well prove a useful tool for the study of OP-induced seizures.

The next stage in the validation of the model will be to compare the efficacy of a wider range of anticonvulsants against OP-induced epileptiform activity in guinea-pig hippocampus *in vitro* with efficacy against OP-induced seizures in guinea-pig *in vivo*.

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Footnotes:

a) Unnumbered footnote; “This work was supported by the UK Ministry of Defence” T

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Table 1. Characteristics of different forms of epileptiform activity.

Values are means ± s.e. mean; n = 117 for soman, n = 5 for 4-AP, n = 5 for DPCPX and n = 6 for low Mg^{2+} ACSF.

	Characteristic			
	time to onset[a] (min)	**burst rate[b] (bursts/min)**	**amplitude[c] (mV)**	**duration[d] (ms)**
Soman	19.2 ± 1.4	2.6 ± 0.1	2.2 ± 0.1	585 ± 16
4-AP	4.8 ± 0.5	9.1 ± 0.9	2.6 ± 0.4	290 ± 38
DPCPX	12.8 ± 2.4	5.9 ± 0.6	2.4 ± 0.7	340 ± 23
Low Mg^{2+}	6.0 ± 0.8	2.9 ± 0.6	2.9 ± 0.4	1890 ± 897

[a] the time taken from the application of treatment until the establishment of epileptiform activity.

[b] the burst rate was defined as the average number of bursts/min in the 10 min epoch 5 - 15 min after epileptiform activity was established.

[c] the amplitude was defined as the maximum difference between the positive and negative deflection of the burst.

[d] the duration of a single epileptiform burst.

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Fig. 1. Application of the organophosphate nerve agent soman induces epileptiform activity in the guinea-pig hippocampal slice. The top trace is an example of the long lasting epileptiform activity induced by 100 nM soman (*horizontal bar*: 15 min; *vertical bar:* 1 mV). The bottom trace shows an individual burst from the above experiment on an expanded time base (*horizontal bar*: 100 ms; *vertical bar:* 1 mV).

Fig. 2. The effect of atropine on soman-induced epileptiform activity. The effect of atropine in a single slice is shown above (*horizontal bar*: 15 min; *vertical bar:* 1 mV). A summary *(bottom)* of the effects of atropine after 30 - 40 min exposure to the antagonist (*abscissa:* concentration in μM; *ordinate:* burst rate as a percentage of pretreatment). Each bar represents the mean (± s.e. mean) of 4 - 8 individual experiments. *, P = < 0.05; **, P = < 0.01.

Fig. 3. Inhibition of acetylcholinesterase induces epileptiform activity (*horizontal bar*: 10 min; *vertical bar:* 1 mV). The top trace shows an example of the epileptiform activity induced by the organophosphate, paraoxon, the middle and bottom traces by the carbamates physostigmine and neostigmine, respectively. In each case, epileptiform activity was sensitive to atropine.

Fig. 4. The effect of the muscarinic antagonists, telenzepine, AF-DX 116 and biperiden on soman-induced bursting. The traces *(left)* show individual experiments (*horizontal bar*: 10 min; *vertical bar:* 1 mV). The effects of the antagonists at 30 - 40 min on all slices tested is shown on the right (*abscissa:* concentration in μM; *ordinate:* burst rate as a percentage of pretreatment).

Each bar represents the mean (± s.e. mean) of 4 - 5 experiments. *, P = < 0.05; **, P = < 0.01.

Fig. 5. The effect of nicotinic antagonists on soman-induced epileptiform activity. The effect of mecamylamine on soman-induced bursting is shown above and the effect of methyllycaconitine shown bottom (*horizontal bar*: 15 min; *vertical bar:* 1 mV). A summary of the effects of the antagonists after 30 - 40 min exposure is shown on the right (*abscissa:* concentration in µM; *ordinate:* burst rate as a percentage of pretreatment). Each bar represents the mean (± s.e. mean) of 4 experiments.

Fig. 6. The effect of the benzodiazepines, diazepam and midazolam, on soman-induced bursting. The traces (left) show individual experiments with diazepam and midazolam (*horizontal bar*: 10 min; *vertical bar:* 1 mV). The graphs *(right)* summarise the effects after 30 - 40 min exposure to the benzodiazepines (*abscissa:* concentration in µM; *ordinate:* burst rate as a percentage of pretreatment). Each bar represents the mean (± s.e. mean) of 5 - 6 individual experiments for diazepam or 4 experiments for midazolam. *, P = < 0.05; **, P = < 0.01.

Fig. 7. Induction of epileptiform activity in the guinea-pig hippocampal slice by 4-AP, DPCPX and low Mg^{2+} ACSF and the lack of effect of atropine. Epileptiform activity can be induced by a variety of treatments (*horizontal bar*: 10 min; *vertical bar:* 1 mV). The top trace shows an example of the epileptiform activity induced by 4-AP, centre DPCPX and bottom by low Mg^{2+}

ACSF. Examples of individual bursts are shown on the right. The vertical bar represents 1 mV, the horizontal bar 100 ms for 4-AP and DPCPX and 400 ms for low Mg^{2+}.

Figure 1

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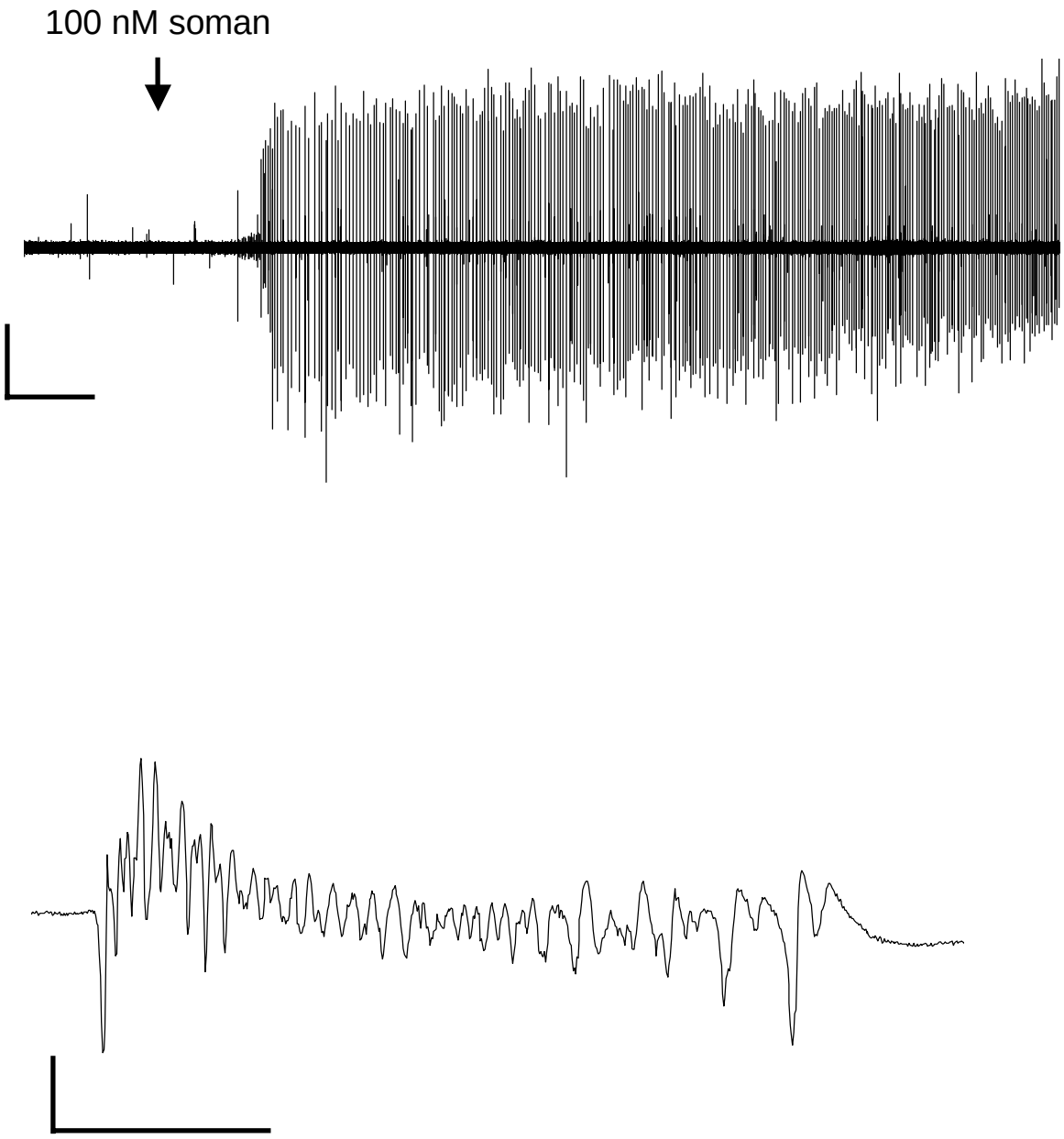

Figure 2

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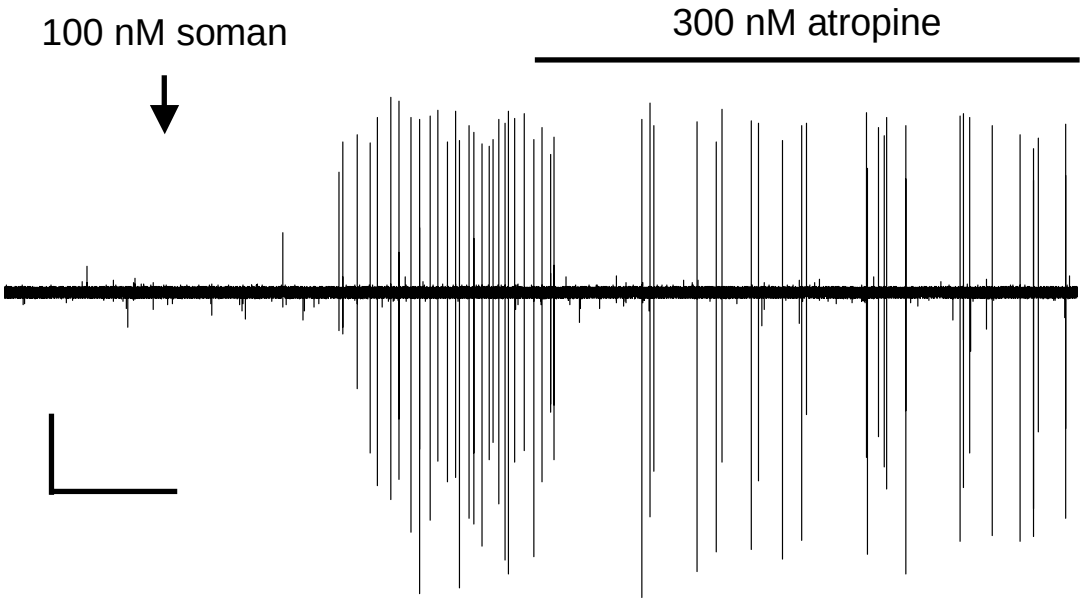

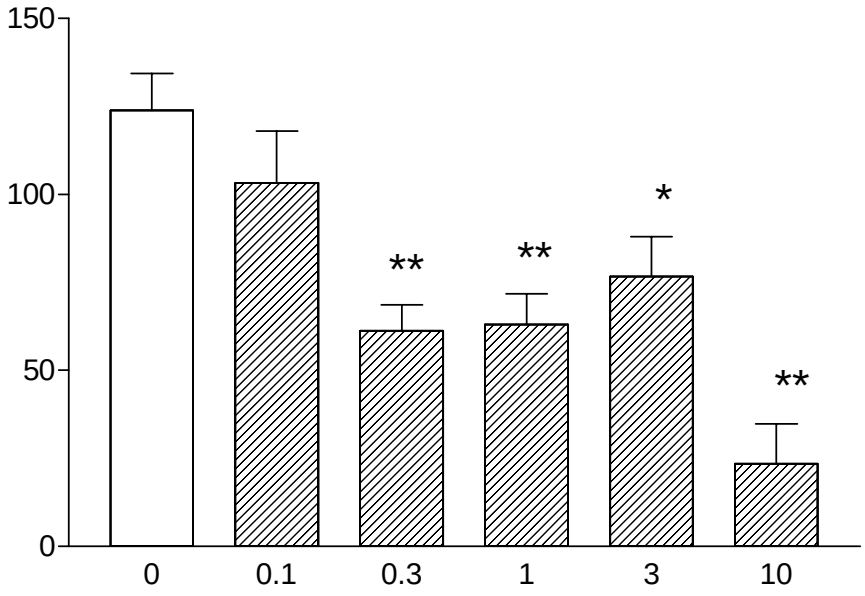

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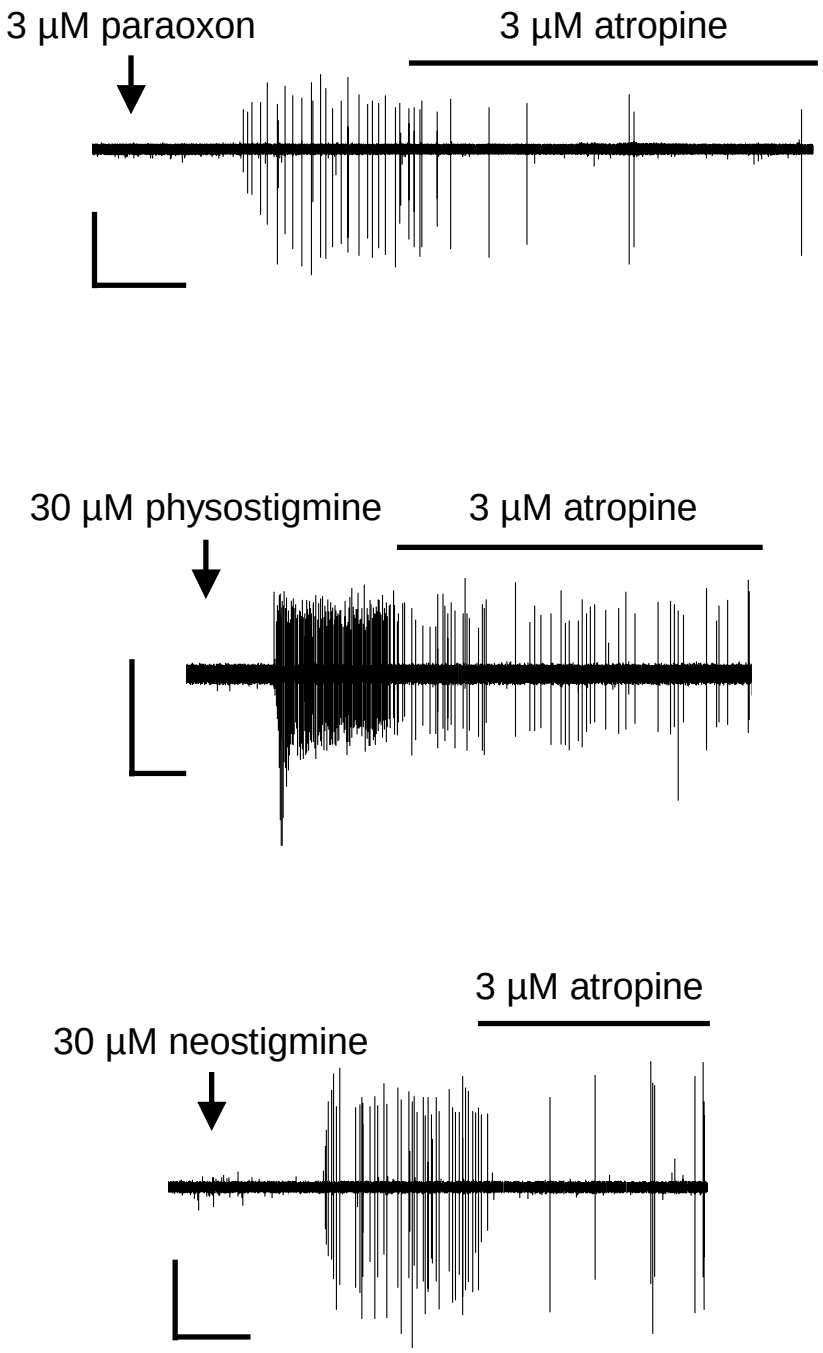

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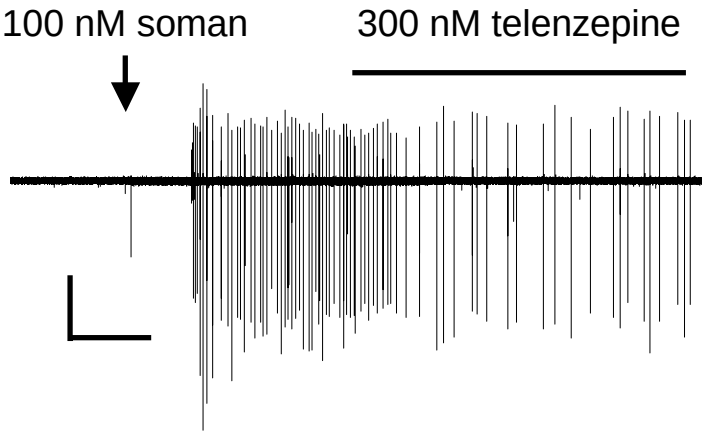

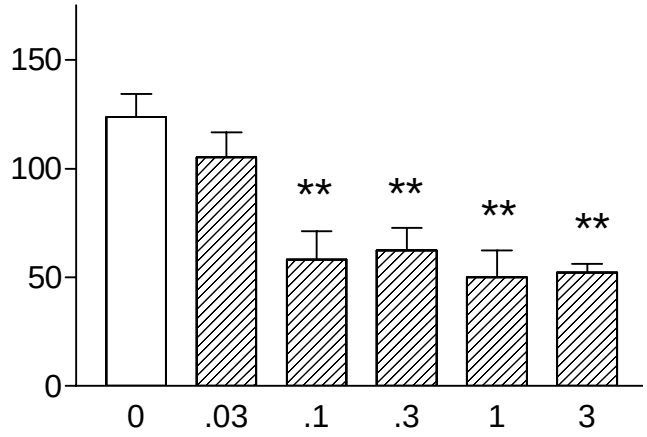

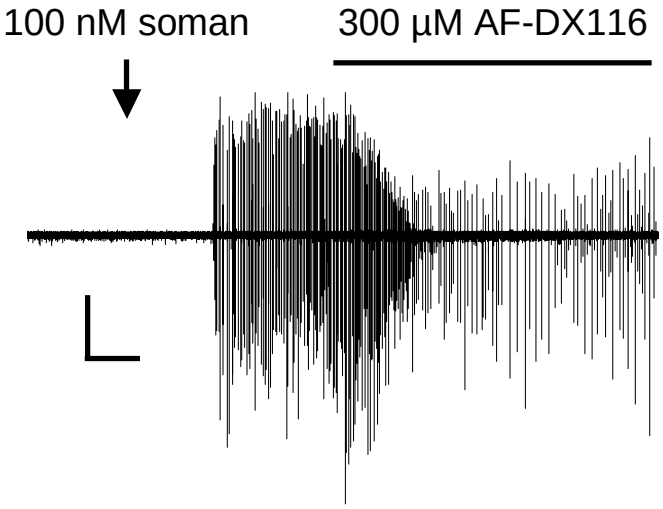

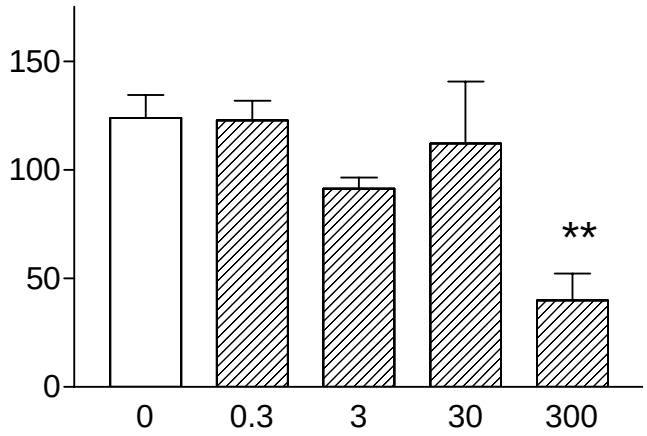

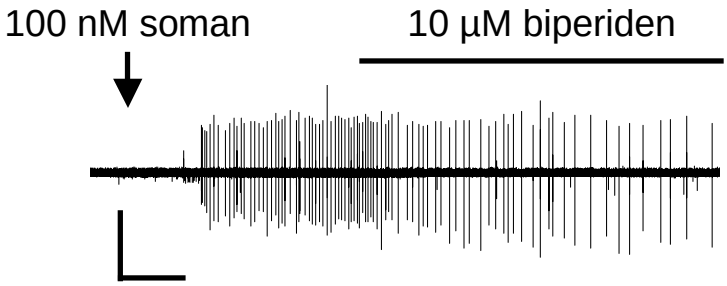

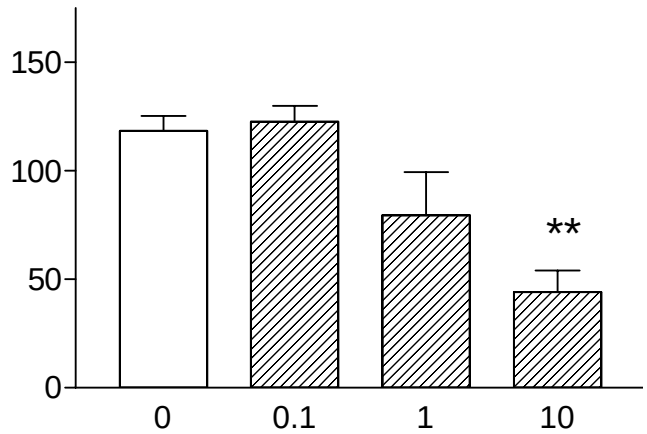

Figure 5

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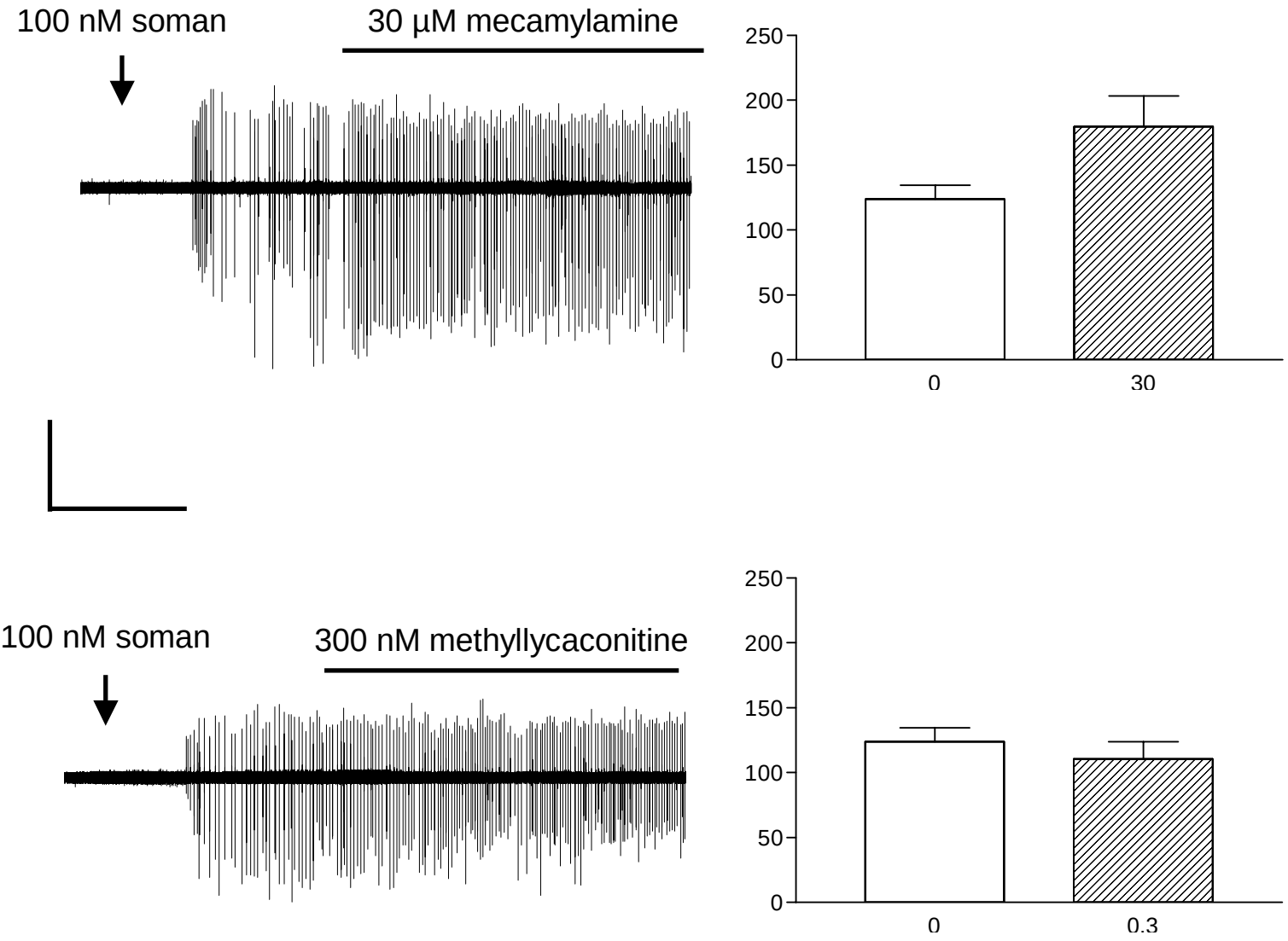

Figure 6

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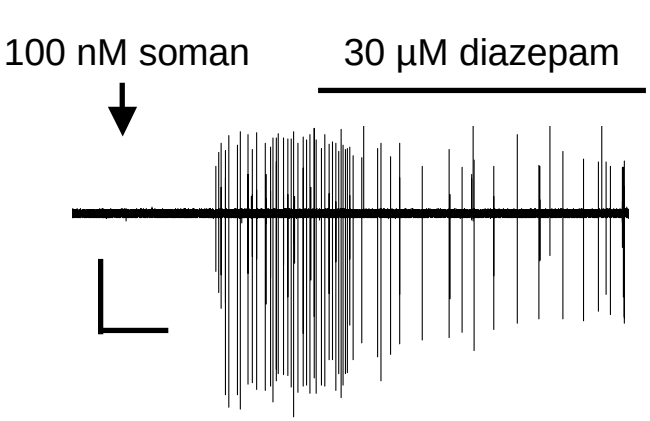

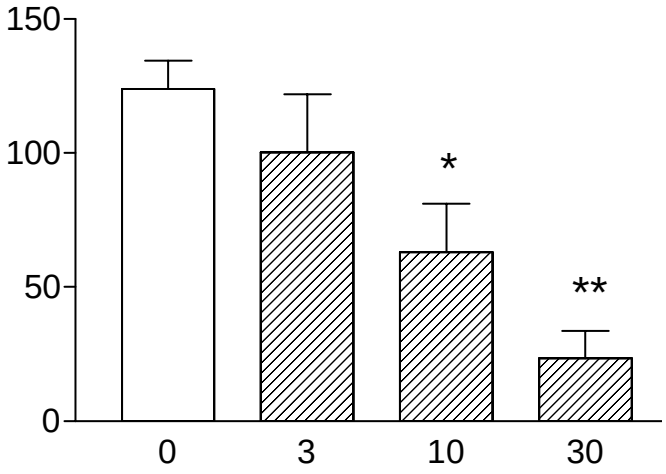

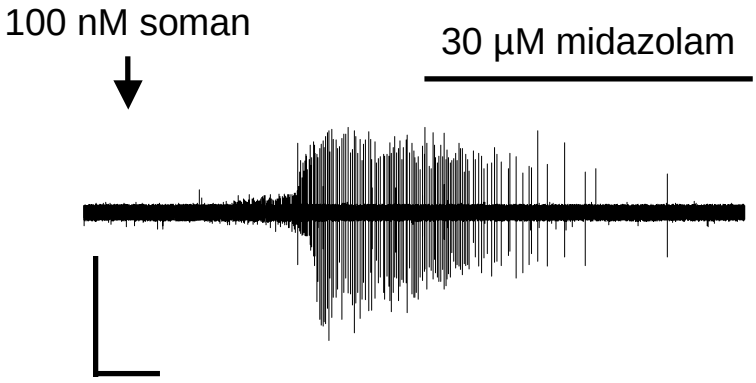

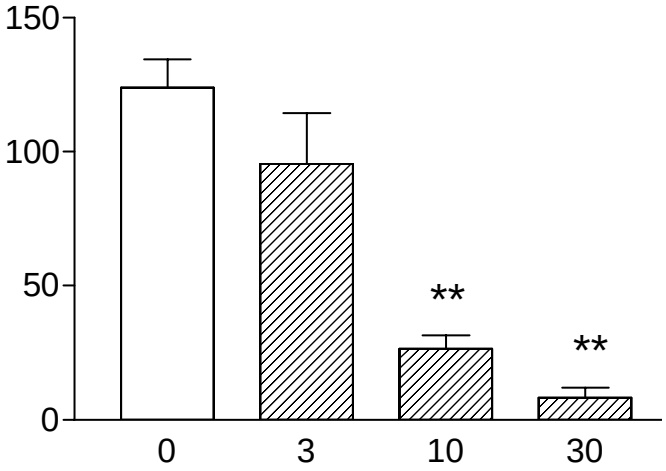

Figure 7

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