Ketamine and xylazine depress sensory-evoked parallel fiber and climbing fiber responses

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Running head: Anesthetics depress peripheral cerebellar responses

ABSTRACT

The last few years have seen an increase in the variety of *in vivo* experiments used for studying cerebellar physiological mechanisms. A combination of ketamine and xylazine has become a particularly popular form of anesthesia. However, because non-anesthetized control conditions is lacking in these experiments, so far there has been no evaluation of the effects of these drugs on the physiological activity in the cerebellar neuronal network. In the present study, we used the mossy fiber, parallel fiber and climbing fiber field potentials evoked in the non-anesthetized, decerebrated rat to serve as a control condition against which the effects of intravenous drug injections could be compared. All anesthetics were applied at doses required for normal maintenance of anesthesia.

We found that ketamine substantially depressed the evoked N3 field potential, which is an indicator of the activity in the parallel fiber synapses, (-40%), and nearly completely abolished evoked climbing fiber field potentials (-90%). Xylazine severely depressed the N3 field (-75%) and completely abolished the climbing fiber field (-100%). In a combination which is commonly used for general anesthesia (20:1), ketamine+xylazine injections also severely depressed the N3 field (-75%) and nearly completely abolished the climbing fiber field (-90%). We also observed that lowered body and surface temperatures (below 34 degrees C) resulted in a substantial depression of the N3 field (-50%). These results urge for some caution in the interpretations of studies on cerebellar network physiology performed in animals anesthetized with these drugs.

Keywords: parallel fibers, climbing fibers, granule cells, synaptic transmission, anesthetics

INTRODUCTION

As interest in *in vivo* cerebellar recordings recently have started to increase, it is important to evaluate the effects on the properties of the cerebellar circuitry of one of the most commonly used anesthetic combinations, i.e. ketamine and xylazine. Previous investigations on the effects of barbiturates on evoked responses (Körlin and Larsson 1970; Gordon et al. 1973) have shown that they severely or completely depress parallel fibre input, but not mossy fibre and climbing fibre inputs (Armstrong and Drew 1980; Ekerot and Larsson 1980; Garwicz and Andersson 1992; Ekerot and Jörntell 2001; this is also seen in the data of Garwicz 1997; Jörntell et al. 2000; Baker et al. 2001).

Ketamine has been shown to substantially or completely depress sensory-evoked responses in cells of the striatum (West 1998) and spinal cord (Hartell and Headley 1996). In the cerebellum, various neuron types have been reported to exhibit sensory-evoked responses under ketamine-xylazine anesthesia (see for example Chadderton et al., 2004; Loewenstein et al., 2005; Shin and De Schutter, 2006). However, so far the effect of ketamine and xylazine on sensory-evoked responses in the cerebellum has not been evaluated against a non-anesthetized control.

Here we use a non-anesthetized, decerebrate preparation of the rat to evaluate the effects of ketamine and xylazine, both in isolation and in combination, on sensory-evoked responses. We record the effects of acute and repeated injections primarily on the parallel fiber input, but also on the climbing fiber input. We strive to mimic the conditions under which a general anesthesia is maintained. To quantify the effects, we use the N3 field potential, which reflects the transmission

of mfEPSPs via the grc-pf to the pf synapses in the molecular layer (Eccles et al. 1967), and the climbing fiber field potential in the molecular layer.

MATERIALS AND METHODS

Preparation

Eleven adult Sprague-Dawley rats weighing 280-490 g were initially sedated with an intramuscular injection of ketamine (120-160 mg/kg, Ketaminol ® 100 mg/ml, Intervet International B.V., Boxmeer, Holland). Under local lidocaine anesthesia (Xylocain ®, 20 mg/ml, AstraZeneca, Södertälje, Sweden), a venous cannula was inserted into the jugular vein and a deep propofol (Diprivan ® 10 mg/ml, AstraZeneca, Södertälje, Sweden) anesthesia was induced and maintained by i.v. injections until the moment of decerebration. Propofol, which is a rapidly eliminated injection anesthetic (Cox et al. 1998), was administered continuously at 5-10 min intervals and usually 0.8-1 ml was administered before the decerebration. Recordings did not start until about three hours after the decerebration, and hence the discontinuation of the propofol anesthesia, which should be enough to allow for an essentially complete elimination of the propofol (Cox et al. 1998).

The level of anaesthesia before decerebration was characterized by constricted pupils and a complete muscle atonia before the administration of paralysing agents. Cannulae were inserted into the trachea, the right jugular vein and the right femoral vein and artery. The animals were artificially ventilated and given a continuous infusion (buffered Ringer-acetate and glucose solution). The blood pressure, end-expiratory CO2 and body temperature were continuously monitored and maintained within physiological limits (4·0-4·5 %, 80-150 mm Hg and 37·0-38·5°C, respectively). All wound areas were infiltrated by lidocaine. During recordings, the

animals were paralyzed with pancuronium bromide (Pavulon ® Organon Teknika B.V., Boxtel, Holland).

To access the brainstem, a small craniotomy was performed on the medial part of the left side of the skull, just rostral to the lateral suture. The dura overlying the occipital part of the cerebrum was cut before the blunt spatula was lowered towards the brainstem. Decerebration was carried out with a blunt spatula and a coiled metal wire that was driven through the brainstem. Transections were made at a rostral intercollicular level. Post-mortem examination verified a complete brainstem transection.

EEG recordings, obtained during the experiment after the decerebration, from the surface of the parietal cerebral cortex showed the characteristic patterns of deep sleep (Niedermeyer and Lopes da Silva 1993). Furthermore, the blood pressure and the end-expiratory CO2 remained stable throughout experiments, also on noxious stimulation.

The head of the animal was fixed in a frame by ear bars covered with lignocaine and by a nose ring. To increase the mechanical stability of the brain, cerebrospinal fluid was drained through a hole in the dura between the occipital bone and the first vertebra. A craniotomy was performed to expose the left posterior lobe of the cerebellar cortex and the overlying dura was cut.

A pool of cotton-in-agar was built around the cerebrum, cerebellum and brain stem. The pool was filled with warm paraffin oil to prevent the exposed parts from drying.

In one experiment, the rat was kept under a continuous ketamine+xylazine anesthesia. This animal was not decerebrated, but otherwise prepared as described above (see also Results).

Stimulation, recording and drug injections

A glass-insulated tungsten microelectrode (exposed tip 30-50 µm) was inserted through the surface of lobule VI. The electrode was parasagittally oriented and tilted about 30 degrees caudally. The electrode was advanced by a motorized micromanipulator and the depth from the surface was indicated on the electronic unit controlling the manipulator. Pairs of percutaneous needle electrodes, insulated except at their tips, were used for electrical stimulation of peripheral skin (100 µs shocks at 0·8 mA, 1 s interstimulus interval). Peripheral electrical stimulation of the ipsilateral snout evoked characteristic responses at the pial surfaces and in the successive molecular, Purkinje cell and granule cell layers (Eccles et al. 1967). Before data sampling, the electrode was adjusted to the depth within the molecular layer where the amplitude of the N3 field was maximal. After localization of the optimal site for recording small doses of either ketamine (Ketalar ® 50 mg/ml, Pfizer, Täby, Sweden), xylazine (Rompun ® 20 mg/ml, Bayer AB, Sweden) or a combination of the two were diluted in saline and administered intravenously while recording the field potentials. Pentobarbitone injections (PentobarbitalNatrium ® 60 mg/kg, Apoteket AB, Stockholm, Sweden) were also used in experiments and to sacrifice the animals after the termination of experiments. All data points are reported as mean+/-standard deviation. Single unit recordings were performed with a glass-insulated tungsten microelectrode (exposed tip ~5 µm) within the Purkinje cell layer. Purkinje cells were recognized by characteristic simple and complex spike firing (see Fig 9).

The experimental procedures were approved in advance by the local Swedish Animal Research Ethics Committee.

RESULTS

All recordings were made in lobule VI/crus 2, which is easily exposed and accessed. The specific recording site that we used was located just lateral to the medial border of a zone with c2 zone type of climbing fiber responses on electrical stimulation to the face/snout (Jörntell et al. 2000) (Fig. 1). This area also corresponds to the medial part of the c2 zone defined for caudal lobule VI/crus 2 by Baker et al. (2001). Among the exposed cortical areas, this area was found to have the largest N3 field potential responses on electrical stimulation of the snout.

Identification of mossy fiber-related field potentials

All recordings of N3 field potentials and climbing fiber field potentials were made by microelectrodes positioned at middle depth in the first or second molecular layer (ML) encountered from the surface. For the non-superficial recording sites, we continuously monitored the sequence of molecular, Purkinje cell and granule cell layers encountered during electrode tracking. These layers can be identified by their characteristic spontaneous and evoked activities (Eccles et al. 1967; Armstrong and Drew 1980; Jörntell et al. 2000). The N3 field potential is defined as being recorded in the ML as a subsequent response to mossy fiber activation. It primarily reflects the activation of parallel fiber synapses and the associated postsynaptic response in the ML (Eccles et al. 1967). As such, it is recorded as a negative field potential in the ML but as a positive field potential in the granule cell layer (GCL) (Fig. 2). As an additional identification criterion, all of our N3 field potentials had a response onset latency time of 3.8-5.0

ms. The fastest known response latency time for the only other peripherally evoked field potential response that have a negative polarity in the ML, the climbing fiber response, is around 8.0 ms (Jörntell et al. 2000). However, such fast climbing fiber responses occur only in the c1 zone. The climbing fiber field potentials that were recorded in the present set of experiments had a response latency time of 11-12 ms, i.e. response latencies previously found in the c2 zone (Jörntell et al. 2000).

With respect to the earliest part of the evoked response (0-8 ms), all our responses were of two types, either N3 field potentials without a pronounced preceding mossy fiber response complex (Fig. 2B) or N3 field potentials associated with a pronounced preceding mossy fiber response complex (Fig. 2D). N3 field potentials with preceding mossy fiber complexes might have been recorded above a part of the GCL where a strong local, snout-evoked mossy fiber input existed, whereas 'simple' N3 fields might have been recorded outside this mossy fiber input territory (cf. Ekerot and Larson 1980). In both cases, the negative N3 field potential was found to reverse to a P2 potential (Eccles et al. 1967) in the adjacent GCL. The synaptic field component of the preceding mossy fiber response complex (the N2 field potential in GCL recordings) was also found to reverse polarity between the two layers (Fig. 2D). As the N2 potential is recorded as a positive potential in the ML, this potential is henceforth referred to as the MF field potential (which represents the synaptic field potential of the mossy fiber input). The earliest part of the compound mossy fiber response is a field potential that signals the arrival of the afferent mossy fiber volley (responsible for the P1 and N1 field components in the granule layer), and caused a pure positive field in the ML. Before the P1 potential, there was an additional positive field potential that did not reverse polarity between the layers, and which could possibly reflect a

massive volley in the brainstem (cf. Armstrong and Drew 1980). Neither of these two earliest of field potential components was analyzed further.

It is well known that even though evoked mossy fiber and climbing fiber field potentials may be very large under barbiturate anesthesia, the N3 field potential component is essentially or completely absent (Körlin and Larsson 1970; Gordon et al. 1973; Armstrong and Drew 1980; Garwicz and Andersson 1992; Garwicz 1997; Jörntell et al. 2000; Baker et al. 2001; Ekerot and Jörntell 2001). In a previous study, it was shown in a non-anesthetized decerebrate preparation that depending on dose, i.v. administration of pentobarbitone in small doses can be used to obtain a graded elimination of the N3 field potential (Gordon et al. 1973). In similar experiments (N=8), we found that i.v. administration of about 10 mg/kg of pentobarbitone was enough to completely abolish the N3 field component (Fig. 2E, F), sparing only the underlying mossy fiber field potential. (Note also that even after a near total elimination of the N3 response, it's onset time and time-course is only marginally retarded, as shown in Fig. 2C). When the response under barbiturate anesthesia was subtracted from the control response, there remained a more or less pure N3 field component (Fig. 2G) similar to that observed in evoked responses with a 'simple' N3 field potential (Fig. 2B). The underlying mossy fiber mossy fiber field potential, recorded after high doses of barbiturate (10 mg/kg or more) at the end of experiments, remained essentially unchanged in time-course even a short time after the heart stopped beating when the animal was given an overdose. In cases in which the preceding mossy fiber complex was prominent, the underlying mossy fiber field potential recorded after high barbiturate doses was used as a template against which the N3 field was measured (cf. Fig. 2E-G). Hence all N3 fields analyzed had a well-defined start-point and peak amplitude (see Fig. 2G). In many cases, the N3 field potential was followed by a positive-going field potential component, the P3 field potential (Fig.

2G). The P3 field potential has been ascribed to the inhibitory synaptic action of the cells activated during the N3 field component (Eccles et al. 1967). In line with this notion, large compound EPSP responses, with a high probability of firing the cell, have been observed in the inhibitory interneurons of the ML during the N3 field response phase (Jörntell and Ekerot 2003). Altogether, the results of this analysis (Fig. 2) are very similar to those of Armstrong and Drew (1980) in the decerebrate and pentobarbitone-anesthetized rat.

The effects of anesthetics on the evoked field potentials

In order to provide a functional validation of the anesthetic doses used, we used one rat that was not decerebrated, but continuously maintained under ketamine/xylazine anesthesia (20:1). The anesthesia was supplemented by intravenous injections as required to prevent the occurrence of spontaneous muscle activity and to keep the animal in a relaxed state. The level of anesthesia was further indicated by an absence of withdrawal reflexes to noxious pinch and a stable blood pressure. Over a five hour period, the required doses were 6.5+0.33 mg/kg when administered at 10 min intervals, or 13+0.65 mg/kg when administered at 20 min intervals. This test was crucial as the anesthetic doses required in a preparation like the present, where physiological parameters of the preparation such as blood pressure, ventilation and body temperature are controlled and maintained within physiological limits has not been investigated previously for experiments of this type.

Figure 3 illustrates an experiment in which a series of injections of ketamine+xylazine was administered according to a scheme that would suffice to maintain the rat under a general anesthesia. The first injection was larger mimicking induction of the anesthesia. Subsequently, maintenance doses were administered at the same rate as they would have been during a standard

experiment. Both the MF and N3 field potentials were depressed by the injections. However, whereas the effect on the MF field was weak and non-persistent, the N3 field potential was initially severely depressed and then persistently reduced by nearly 50% after a number of repeated injections (Fig. 3A). The effect on the evoked climbing fiber responses was even more dramatic (Fig. 3B; identification of climbing fiber responses were made as described by Jörntell et al. 2000). The raw traces in this panel also illustrate the near complete loss of all the field potential components that normally followed the MF field potential. For comparison, an optimal anesthesia administered through slow-delivery pathways such as intramuscular or intraperitoneal pathways would most likely correspond to the intermediate time-points between consecutive intravenous injections.

Figure 4A summarizes the reductions, and early time-course of recovery, of the control N3 field potential on injections of ketamine, xylazine or a combination of the two at a concentration ratio of 20:1. Ketamine by itself clearly had a substantially smaller effect on the N3 field than when combined with xylazine or xylazine by itself. The effects of xylazine also lasted for a substantially longer time, as illustrated in Fig. 4B, C.

A summary of the average depressions during a time period of 20-40 s recorded 30-120 s after drug injections is shown for both N3 and CF field potentials in Fig. 5. Most of these depressions were recorded after the first drug injections of each experiment, although some cases also represent the second or third injections of an experiment. In the latter cases, the field potentials (N3 and CF) were allowed to recover before the new drug injection and there were no differences in the magnitude of the depressions for consecutive injections. The injections included in this data set all fell within relatively narrow concentration ranges (see legend). In addition to the

depressions of the field potentials, injections of either drug were always accompanied by an audible reduction in background noise (not shown). In the majority of cases (17/21) presented in Fig. 5 there was essentially no change in the MF field potential after the injections (-1.1+/-3.64 % (mean +/- standard deviation)). In some cases (4/21), there was a slight reduction (>5%) in the preceding MF field potential. To further preclude the possibility that the effects observed on the N3 field potential were due to changes in precerebellar synaptic relays, we also tested the anesthetic-induced depressions on the responses evoked by direct activation of mossy fiber afferents from a region dorsal to the location of the lateral reticular nucleus, close to the inferior cerebellar peduncle. As shown in Fig. 6, there was a distinct depression of the N3 field potential (-63%+/-17%, N=4; ketamine+xylazine given at 13.6+/-2.8 and 0.68+/-0.12 mg/kg, respectively) whereas the MF field remained unchanged (within +/- 5 % of the original value).

Experiments with repeated injections

In some experiments, we followed the N3 field potential continuously across a number of i.v. injections of the anesthetics (Fig. 7). This illustrated the synergistic and cumulative effects of the two drugs, i.e. note the substantial additional reduction of the N3 field obtained by the second ketamine injection long after a preceding xylazine injection in Fig. 6. It may also again be noted that the MF field potential was not reduced even though there was a substantial reduction in the N3 field after administration of both types of anesthetics.

Temperature effects on the N3 field

Apart from anesthetics, the condition of the preparation is another critical factor for the amplitude of the N3 field potential (see discussions in Garwicz and Andersson 1992; Ekerot and Jörntell 2001). In order to illustrate this in a reversible way, we manipulated the temperature of the

cerebellar surface. Under normal experimental conditions, the animal's body temperature is maintained at 37.0-38.5 degrees C by a heating system. To test if changes in temperature have any effect on the N3 field potential, the heating system was temporarily turned off in four experiments. The body temperature fell to below 34 degrees within half an hour (room temperature 25 degrees) and, unless the heating system was turned on again, would continue to fall towards room temperature. We replaced the paraffin oil that usually covers the cerebellar surface with warm saline (37 degrees). When the body temperature had fallen to 32-34 degrees, there was a substantial reduction of the N3 field potential (Fig. 8). Note that since saline is a good heat conductor, it is likely that the saline assumed a temperature somewhere in between the body and room temperatures and the actual temperature at the cortex may therefore have been lower than 32-34 degrees in these experiments. The reduction of the N3 field potential in these four experiments was 45-55%. The reduction was accompanied by a marked slowing of the response.

Reduction in evoked Purkinje cell activity after ketamine+xylazine administration.

The reduction in the N3 field potential reflects the transmission of mfEPSPs via the grc-pf to the pf synapses in the molecular layer (Eccles et al. 1967). How does this reduction affect sensory-evoked unitary activity? To test this we recorded evoked simple spike activity in Purkinje cells before and after ketamine+xylazine administration. In this case evoked responses were defined as the simple spike activity evoked between 4-20 ms after the facial stimulation. The result was expressed as the ratio between the preceding baseline activity (200 ms prestimulus) and the evoked activity. As shown in Fig. 9, Purkinje cell responses were substantially depressed by ketamine+xylazine, in this case by 62%. Overall, the reductions in evoked simple spike activity were 57+/-26% (N=5 Purkinje cells) for ketamine+xylazine injections at average concentrations of 12 and 0.6 mg/kg, respectively.

DISCUSSION

We have shown that the transmission of evoked parallel fiber synaptic input in the molecular layer of the cerebellar cortex can be substantially depressed both by ketamine and by xylazine and by a combination of the two drugs. This effect was seen even though the preceding MF field potential was essentially unchanged, which localizes the main effect to the grc-pf-synapses. The effect on the climbing fiber field potentials was even more dramatic with reductions in the range of 80-100%.

The experiments were performed in a preparation in which we continuously monitored and maintained blood pressure, end-expiratory CO_2 and body temperature, factors which otherwise might affect the amplitude of the field potentials (see Fig.7; cf. Garwicz and Andersson 1992). The doses required to supplement a normal ketamine+xylazine anesthesia under these conditions were studied in a separate experiment, and our doses fell within this range. Dosage for the single applications of ketamine and xylazine, respectively, was more difficult to calibrate but the high doses of ketamine (22 mg/kg) could be compared to the 120-160 mg/kg (intramuscular injection) required for the initial sedation of the animals (see Methods). Importantly, whereas a normal induction of anesthesia requires an initial, very large bolus dose which is supplemented by subsequent, smaller doses, our summarized data (Fig. 5) shows the effects of isolated, supplement-sized, smaller doses in animals which had not been given induction doses. Hence, under a normal, maintained anesthesia the effects might be expected to be larger than those reported here. It could be argued that our decerebrate preparation, since it involves transection of the descending cerebral input to the important mossy fiber source in the pontine nuclei, does not fully reflect the normal activity state of the cerebellum. This may be so, but if anything, a loss of

an important mossy fiber input would seem to lessen the control response and hence underestimate the effects of the anesthetic. We would also like to underscore that the cerebellar network in the decerebrate preparation is capable of producing well-timed behavioural responses, i.e. the classical conditioned response (see Hesslow et al., 2000; Jirenhed et al., 2007).

What does the N3 field potential reflect in terms of neuronal activity? The N3 field potential follows the mossy fiber volley and the synaptic mossy fiber field potential evoked by electrical stimulation of ascending mossy fiber pathways (Eccles et al. 1967). The N3 field potential is abolished when inhibition is evoked by a local stimulation electrode on the surface of the cerebellar cortex, whereas the mossy fiber field potentials are unaffected by such inhibition. These and other findings lead Eccles et al. (1967) to the conclusion that the N3 field potential is an extracellular reflection of parallel fiber spike activity and postsynaptic responses in Purkinje cells and interneurons. This conclusion is supported by the near exact coincidence of the N3 field potential and the earliest evoked EPSPs in molecular layer interneurons (Jörntell and Ekerot 2003). Accordingly, the reduction in the N3 field response after ketamine+xylazine injections was accompanied by a change in the evoked spike responses in Purkinje cells (Fig. 9).

The experiment with direct mossy fiber activation (Fig. 6) illustrates that mechanisms in the cerebellar cortex can account for major reductions of the N3 field after ketamine+xylazine injections. Moreover, in most cases (17/21) where sensory-evoked responses were studied, the quantified field potential depressions (Fig. 5) were recorded without accompanying changes in the MF potential. In these cases, it is likely that the reductions of the N3 potentials observed were primarily due to a reduction of the granule cell/parallel fiber excitability and/or parallel fiber synaptic activity. Nevertheless, it cannot be completely excluded that depressions occurred at

other synaptic relays in the brainstem, i.e. the trigeminal nucleus. Indeed, in Fig. 3, in which the initial dose was substantially higher than those used for quantifying the depressions, a reduction of more than 25% of the MF potential was observed, indicating that brainstem sites of depression most likely is important at least for higher doses of ketamine/xylazine than those used in our systematic study (Fig. 5). On the other hand, also in the experiment illustrated in Fig. 3 the depression of the N3 field persisted after the MF field depressions had waned. These results seem to be at odds with a study of ketamine-effects on sensory evoked responses in trigeminal cells, where lower doses of ketamine resulted in a decreased responsiveness of trigeminal cells (Cairns et al., 1999). However, different sets of afferents were activated in that we used electrical stimulation of whisker and facial hair inputs whereas Cairns et al. studied trigeminal cells with tooth pulp inputs and also some cells activated by air puffs to the face. Both of these inputs is likely to have a lower safety margin of transmission, and hence a higher sensitivity to anesthetic agents, than the electrical stimulation of the skin as used in our paper. Furthermore, it was mainly the later parts of the trigeminal response of Cairns et al that were reduced; the initial part of their responses, which should underlie the N3 field recorded in our study, were not depressed to the same extent.

Ketamine acts on the NMDA receptors (Anis et al. 1983; Yamamoto et al. 1990) but also on the voltage-gated sodium and potassium currents (Schnoebel et al. 2005) as well as nicotinic (Scheller et al. 1996) muscarinic (Hustveit et al. 1995) and opioid receptors (Smith et al. 1997). The antagonizing effect on NMDA receptors, which contribute to the later phases of the mf-grc EPSPs (Silver et al. 1992; Cathala et al. 2000), could of course explain part of the N3 field reduction, although this would seem to be contradicted by an absence of substantial reductions in the MF field. The effects of ketamine on the voltage-gated channels are substantially reduced

spike amplitudes and spike firing rates to controlled excitation in dorsal horn neurons (Schnoebel et al. 2005) at ketamine concentrations of about 100 μm. The average initial whole body concentrations after the ketamine injections in our experiments were 51 μM (ketamine, normal) or 94 μM (ketamine, high), although pharmacokinetics and differential tissue distribution may cause the actual concentration in the brain to deviate up or down from these values. If the effect on voltage-gated channels is a factor behind the ketamine-induced N3 field depressions, the results would seem to imply a differential safety margin of action potential propagation between mossy fibers and parallel fibers as the MF field potentials were largely unchanged. Note that the presynaptic NMDA receptors on parallel fiber synapses are not likely to contribute to the field potential depressions since Casado et al. (2002) found that blocking of presynaptic NMDA channels (i.e. by ketamine) would lead to an increase in the parallel fiber responses.

It is known that xylazine is an alpha-2-adrenoceptor agonist. However, the mechanisms by which it might affect neuronal activity in the cerebellum are not well understood. In other neuron types, alpha 2-adrenoceptor agonists are known to elicit an outward potassium current (Pralong and Magistretti 1995; Sonohata et al. 2004) and/or to block high-threshold voltage-sensitive calcium channels of the N and P/Q types (Li et al. 1998; Timmons et al. 2004). In ketamine and xylazine anesthetized rats, as compared to decerebrates, hindlimb alpha-motoneurones have a substantially more hyperpolarized membrane potential and therefore require more current for spike initiation and rhythmic discharge (Button et al. 2006). If this also applies to cerebellar granule cells under ketamine and xylazine anesthesia, it could be enough to explain the reductions in the N3 field potential observed here.

All of the mechanisms suggested above for the granule cells could of course also contribute to a reduced excitability in the inferior olivary cells. In particular, if alpha 2-adrenoceptors exist on inferior olivary cells, a reduced inferior olivary excitability could result from blockage of high-threshold calcium channels, which may be important for the spike generation in these neurons (Llinas and Yarom 1981a,b). In addition, inferior olivary excitability is under the control of the inhibitory cerebello-olivary pathway (reviewed by Bengtsson and Hesslow 2006). If ketamine and xylazine results in decreased spiking activity in the Purkinje cells and/or an increase in the number of firing pauses, there would be a relative release of the inhibitory input to the nucleo-olivary inhibitory neurons which in turn would cause them to fire more intensely and thus reduce olivary excitability.

It should be noted that a dramatic or sometimes near complete reduction of the N3 and CF field potentials does not necessarily imply that the peripheral responsiveness to parallel and climbing fiber inputs is completely removed. The amplitudes of field potentials rely on a synchronous activation of afferents. Hence, a small field potential can signify a lack of input and/or a lack of synchrony in the input. A reduced excitability in the afferent cells could lead to a complete transmission block of peripheral input, or it could lead to a delayed and less synchronized activation. Hence, at the first input stage of the afferents, i.e. at the PF and CF input to Purkinje cells and ML interneurons, some input might be expected to remain despite the reduction in field potentials. Moreover, in many previous experiments using ketamine-xylazine in the study of peripheral responses in the cerebellum, intramuscular or intraperitoneal administration have been used which result in a more sustained anesthesia. In comparison, minimal acceptable anesthetic levels, i.e. blood concentration of the anesthetic, would probably correspond to some intermediate time-point between two consecutive injections, when field depressions are

submaximal (cf. Figs 3 and 4). Under ideal conditions this would be the anesthetic level reached after intramuscular or intraperitoneal administration. Accordingly, many reports have shown that sensory-evoked responses are present also under ketamine/xylazine anesthesia (see for example Brown and Bower, 2001; Loewenstein et al., 2005; Lu et al., 2005; Shin and De Schutter, 2006). However, from a network physiology point of view, a reduced or desynchronized input may be equally problematic. For example, an altered excitability in the inferior olivary cells change the overall background firing frequencies of Purkinje cells (Bengtsson et al, 2004). The present data add to previous reports that ketamine/xylazine profoundly alters the spiking behavior of different types of neurons, e.g. by transforming relatively regularly spiking neurons to intrinsically bursting neurons or neurons with bi-modal (up-down states) firing patterns (Steriade et al. 2001; Destexhe et al. 2003; Mahon et al. 2003; Steriade 2004; Schonewille et al. 2006). Hence, ketamine/xylazine anesthesia is likely to substantially change the activity of the cerebellar neuronal network, in particular activity driven by extracerebellar and peripheral input.

GRANTS

This work was supported by the Swedish Research Council (project no. K2005-04X-14780-03A), the Segerfalk Foundation, the Swedish Medical Society, Crafoordska stiftelsen and Magn. Bergwalls Stiftelse.

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Fig. 1. Location of recordings.

A: View of the exposed part of the cerebellar cortex from one experiment. A cross indicates the insertion point of the recording microelectrode. *B:* Basic 3D model of the rat cerebellum, viewed from above, slightly from the left and from behind, to illustrate the approximate location of the recording points. The reconstruction was based on the outlines of serial sagittal sections of the cerebellum (cut at 60 μm, every third section was included, all other sections were discarded, flocculus is only partially reconstructed). PV, paravermal vein; PM, paramedian lobule; L simplex, lobulus simplex.

Fig. 2. Evoked field potentials due to mossy fiber activity.

A: Sketch illustrating the recording locations illustrated in *B* and *D-G*. *B:* 'Simple' N3 response recorded in the ML and corresponding P2 potential recorded in the adjacent GCL (see sketch). *C:* Subtotal depression of a N3 field potential by a large dose of barbiturate (8 mg/kg). Note that the onset and peak times of the N3 field (indicated by dotted lines) were shifted by less than 1 ms even though the peak amplitude was reduced by 85%. *D:* Compound mossy fiber-parallel fiber response evoked in the ML and corresponding potentials recorded in the GCL. Vertical dashed lines are shown to facilitate comparison of the timing of events in the two layers. Field potentials are defined according to Eccles et al (1967). Calibrations apply to *B-G*. *E:* Abolition of the N3 field potential component recorded after an injection of pentobarbitone (10 mg/kg). *F:* Overlay of the response evoked before and after the pentobarbitone injection. *G:* 'Pure' N3 field potential component obtained after subtraction of the response evoked in pentobarbitone from the response evoked in the non-anesthetized animal.

Fig. 3. Effects of ketamine+xylazine on N3 and CF field potentials.

A: Peak amplitudes of MF and N3 field potentials recorded before and after injections of a ketamine+xylazine mixture at 33+1.7 mg/kg (large arrow) or at 9.8+0.5 mg/kg (small arrows). Averaged raw traces (N=50-100 sweeps) are shown at bottom (and superimposed to the right). Each point in the graphs represents an average of the data values from 5 adjacent data points. *B:* From the same experiment, the effects of ketamine+xylazine injections on the CF field potential. The inset sweeps are 5 superimposed raw traces illustrating the difference in variability of the N3 and CF field potentials in the control condition and their near complete absence after ketamine+xylazine injections. Ket, ketamine; Xyl, xylazine.

Fig. 4. Effects on the N3 field of ketamine, xylazine and ketamine+xylazine.

A: Averaged time-voltage curves of the evoked N3 field immediately after injections of anesthetics as indicated. Each data point from each experiment was calculated as an average of 20 data values. The values were subsequently averaged across experiments (N=5 for each data set). *B, C:* Samples of time-voltage recovery curves of the N3 field following injections of ketamine (12 mg/kg) and xylazine (2.6 mg/kg), respectively, in the same experiment. Ket, ketamine; Xyl, xylazine.

Fig. 5. Summary of field potential depressions.

Summary of remaining evoked N3 and CF field potential responses after injections of drugs as indicated. All responses were measured as averages of 20-40 responses recorded 30-120 s after the drug injections. Responses are expressed as ratios of the average of 20-40 control responses recorded before the injection. The doses injected (and number of tests) were for ketamine:

12.1+/-1.4 mg/kg (N=6); ketamine, high dose: 22.0+/-6.5 mg/kg (N=4); combined ketamine and xylazine injections: 10.6+/-1.8 and 0.53+/-0.08 mg/kg (N=6); xylazine 2.6+/-1.1 mg/kg (N=5).

Figure 6. N3 field reductions in responses evoked by direct mossy fiber stimulation

A, Control response, response after ketamine-xylazine injection (12 and 0.6 mg/kg, respectively), response after recovery and response after barbiturate injection (10 mg/kg). The latter response was used to show the time-course of the underlying MF field response when the N3 field was essentially abolished (cf. Fig. 2). B, Superimposed traces from A.

Fig. 7. Repeated injections of ketamine and xylazine.

N3 field potential depressions recorded during repeated injections as indicated. Data display as in Fig *3A.* Ketamine (Ket) doses were 13 mg/kg and the xylazine (Xyl) dose was 2.5 mg/kg. Note that the mossy fiber synaptic field potential (mf) was essentially unchanged during this time. The final pentobarbitone (Barb) injection was lethal (>50 mg/kg).

Fig. 8. The N3 field potential is reduced when temperature is lowered.

A: N3 field recorded at a body temperature of 37 degrees C with paraffin oil on the surface. *B:* N3 field at 34 degrees C with saline on the surface. *C:* As the body temperature recovered (to 37 degrees), and the saline was replaced with warm (37 degrees) paraffin oil again, the N3 field regained its previous amplitude and time course. *D: A-C* superimposed. All data are averages of 20-100 sweeps.

Figure 9. Ketamine-xylazine induced reduction in evoked Purkinje cell responses.

Peristimulus histograms of simple spike responses (SSp) before, immediately after, and 35 min after ketamine-xylazine injection, when the N3 field had recovered. Numbers inside diagrams indicate the response amplitude expressed as a ratio of the preceding baseline activity. Insets at top show superimposed simple spikes and complex spikes, which identify the cell as a Purkinje cell. Bin width in histograms, 2 ms.

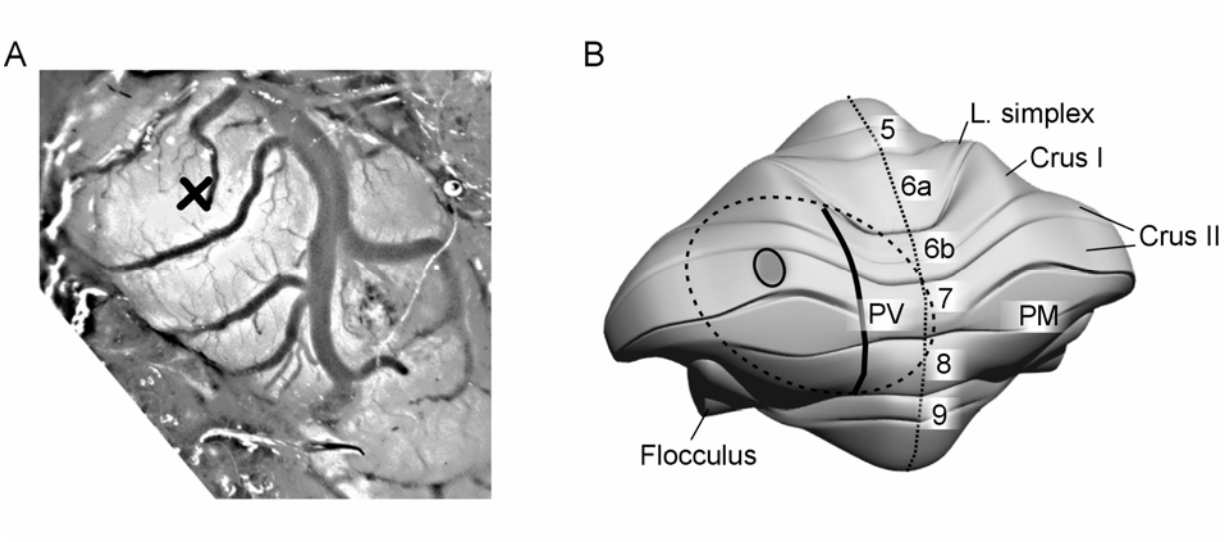

Figure 1

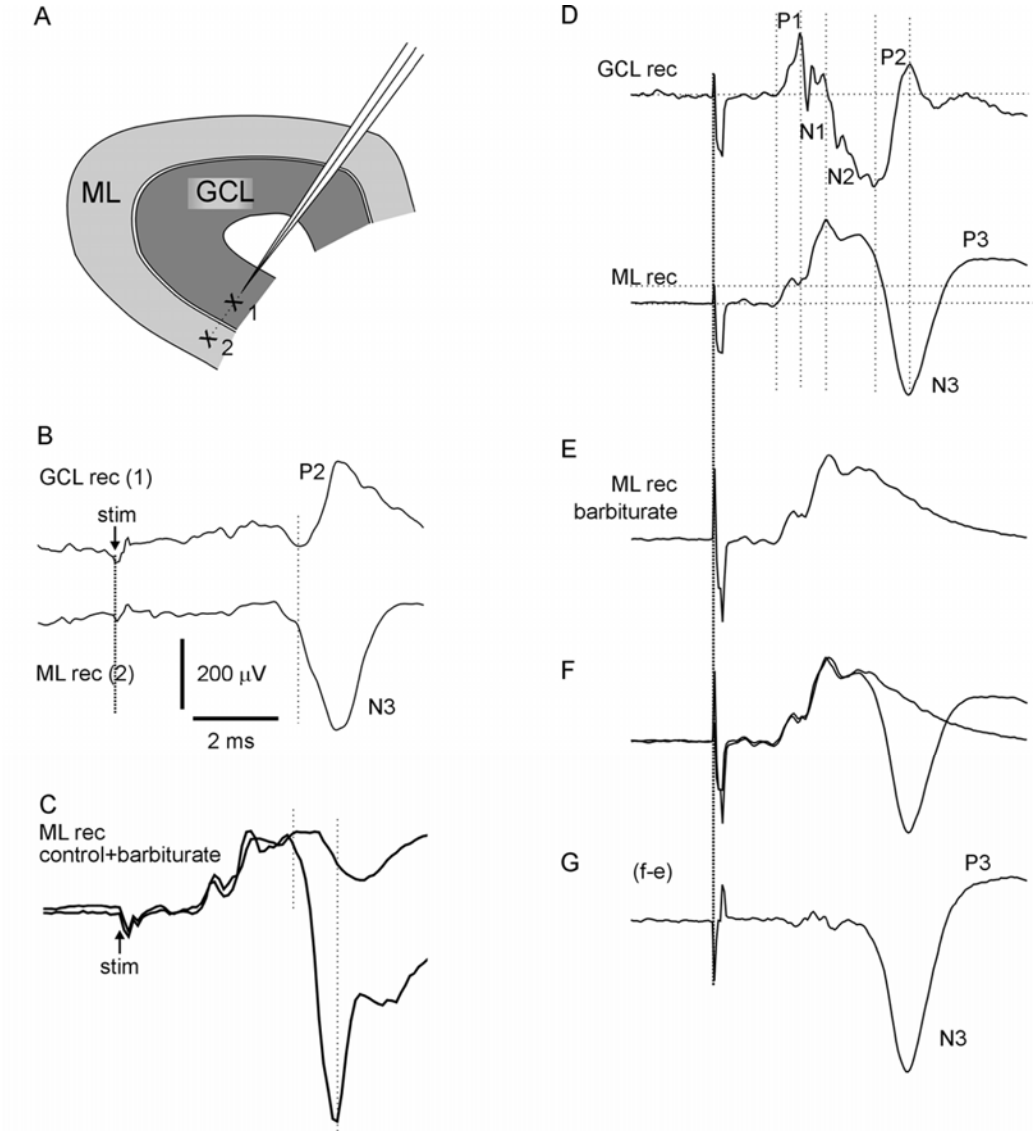
A
ML
GCL
1
2
B
GCL rec (1)
P2
stim
ML rec (2)
200 μV
2 ms
N3
C
ML rec
control+barbiturate
stim
D
P1
GCL rec
P2
N1
N2
P3
ML rec
N3
E
ML rec
barbiturate
F
G
(f-e)
P3
N3

Figure 2

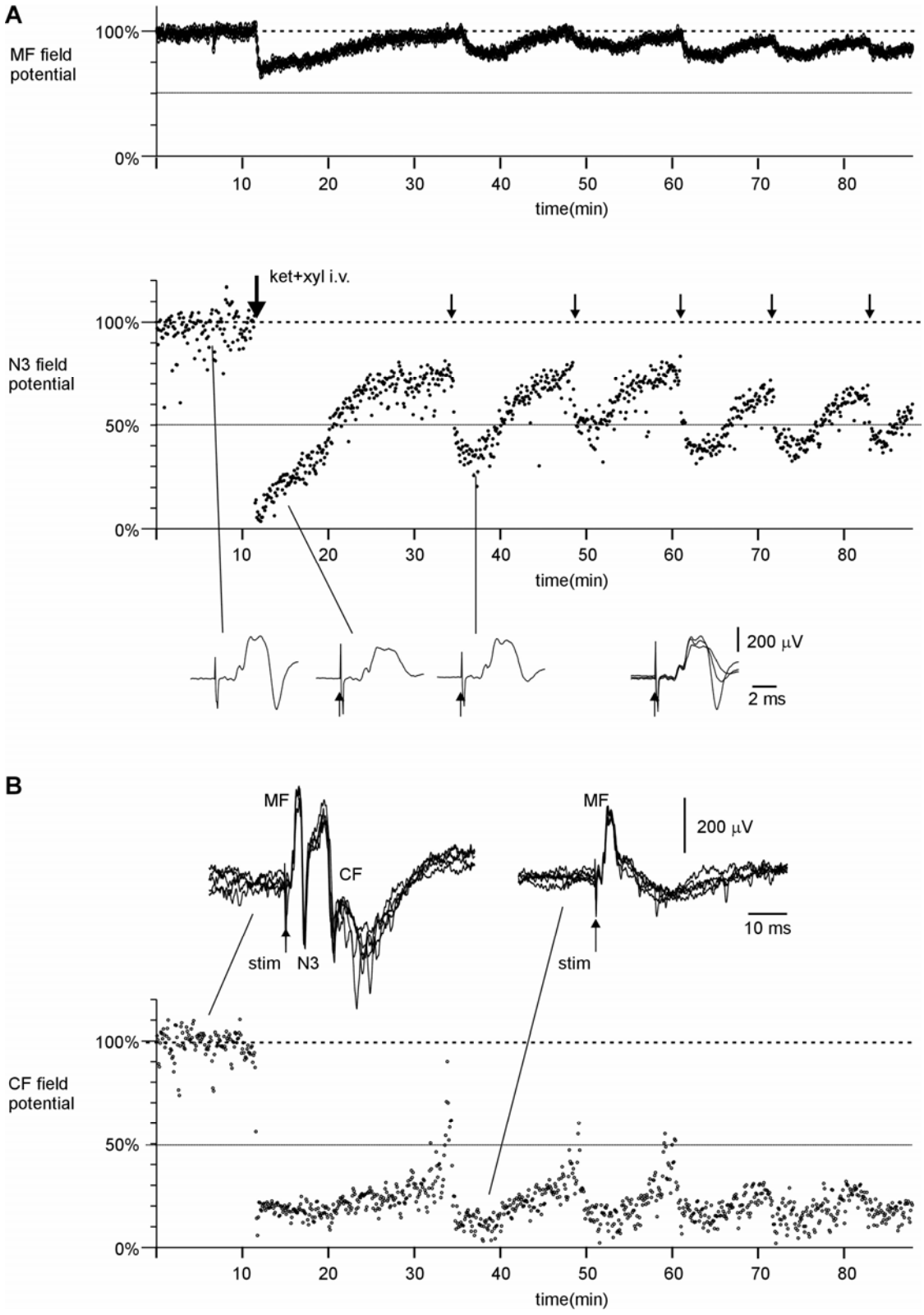
A
MF field potential
100%
0%
10
20
30
40
50
60
70
80
time(min)
ket+xyl i.v.
N3 field potential
100%
50%
0%
time(min)
200 µV
2 ms
B
MF
CF
stim
N3
MF
200 µV
10 ms
stim
CF field potential
100%
50%
0%
time(min)

Figure 3

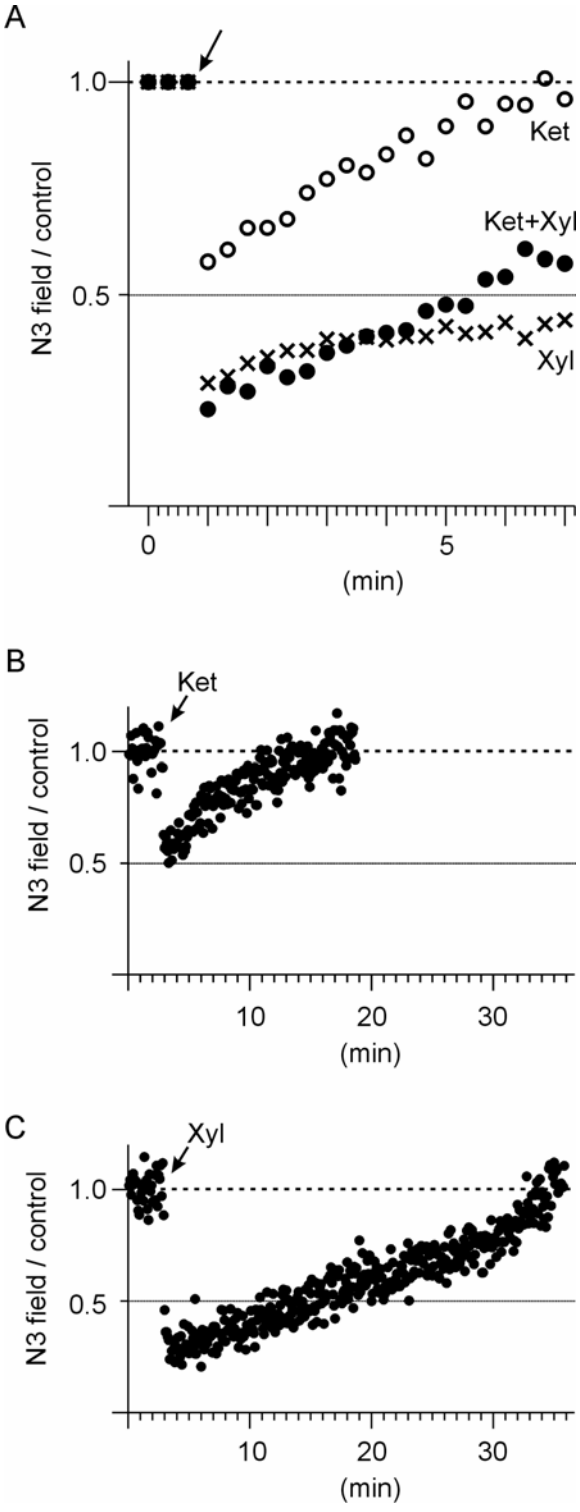
A
1.0
0.5
N3 field / control
Ket
Ket+Xyl
Xyl
0
5
(min)
B
Ket
1.0
0.5
N3 field / control
10
20
30
(min)
C
Xyl
1.0
0.5
N3 field / control
10
20
30
(min)

Figure 4

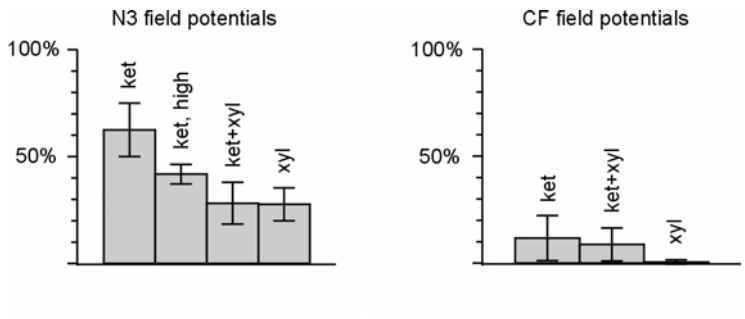
N3 field potentials
100%
50%
ket
ket, high
ket+xyl
xyl
CF field potentials
100%
50%
ket
ket+xyl
xyl

Figure 5

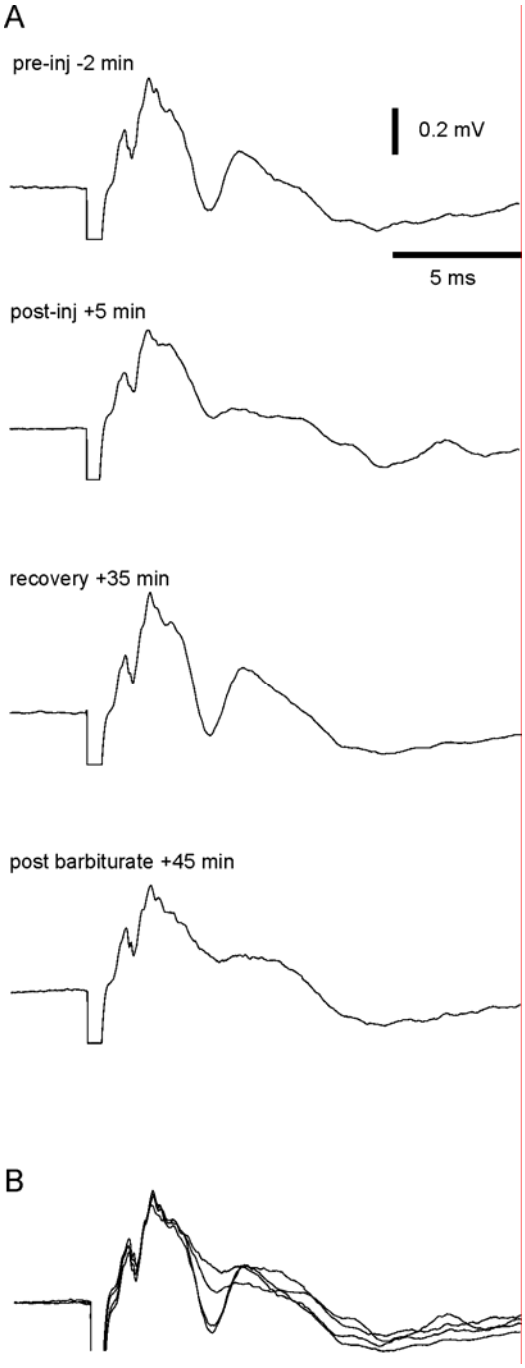
A
pre-inj -2 min
0.2 mV
5 ms
post-inj +5 min
recovery +35 min
post barbiturate +45 min
B

Figure 6

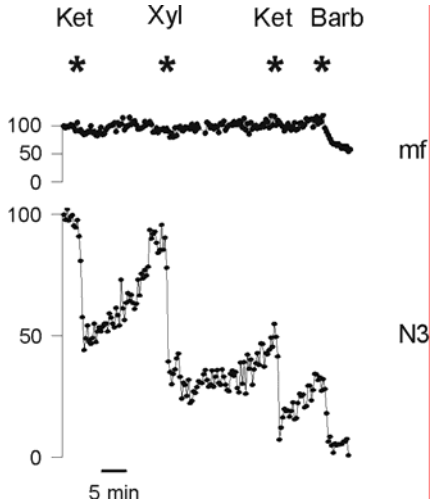

Figure 7

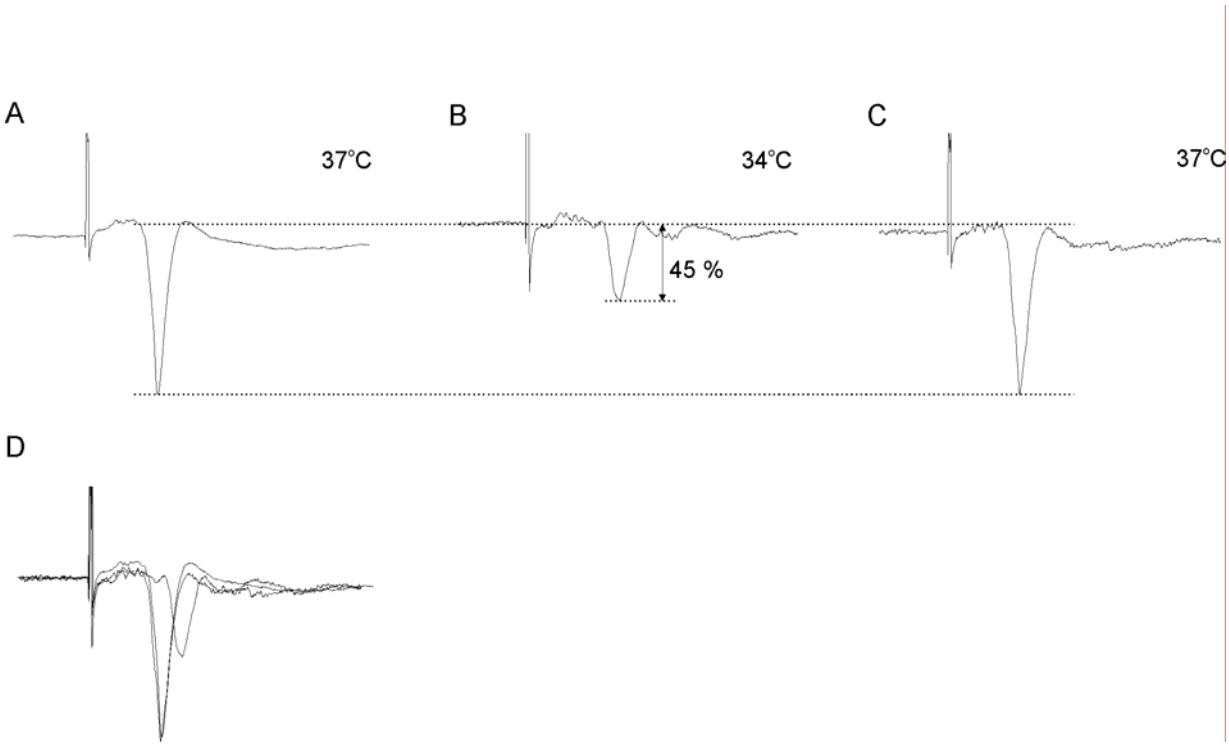

Figure 8

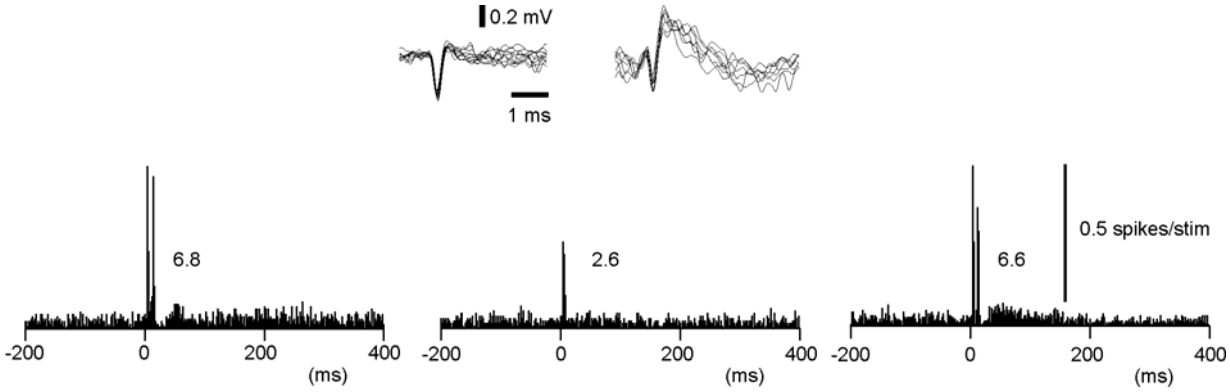

Figure 9