The construction and evaluation of a device for mechanomyography in anaesthetized Göttingen minipigs

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Abstract

Objective—To devise a method for assessing evoked muscle strength on nerve stimulation [mechanomyography (MMG)] in the anaesthetized minipig.

Study design—Prospective observational.

Animals—Sixty male Göttingen minipigs weighing 10.5–26.0 kg.

Methods—After cadaveric studies, a limb fixation device was constructed which allowed the twitch responses of the pelvic limb digital extensor muscles to be measured by force-displacement transduction in response to supramaximal train-of-four (TOF) stimulation of the common peroneal nerve. The device was tested in 60 minipigs weighing 10.5–26.0 kg positioned in dorsal recumbency.

Results—The technique recorded the MMG of the common peroneal-pelvic limb digital extensor nerve-muscle unit for up to 12 hours during which twitch height remained constant in 18 animals in which single twitch duration was <300–500 ms. In 42, in which twitch duration was >300–500 ms, 2 Hz nerve stimulation caused progressive baseline elevation (reverse fade) necessitating a modified signal capture method for TOF ratio (TOFR) computation. However, T1 was unaffected. The mean (range) of the TOFR in pigs with reverse fade was 1.2 (1.1–1.3).

Conclusions and clinical relevance—The technique allowed MMG recording in unparalysed pigs in response to TOF nerve stimulation and revealed a hitherto unreported complication of MMG monitoring using TOF in animals: reverse fade. This complicated TOFR calculation.

Keywords

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Introduction

The measurement of force generated by muscles (mechanomyography) of the appendicular skeleton may be necessary in anaesthetized laboratory pigs for the evaluation of drugs or toxins affecting motor neuronal, neuromuscular or muscular function. For example, mechanomyography (MMG) has been used in anaesthetized, malignant hyperthermia-susceptible pigs for screening neuromuscular blocking drugs for their propensity to trigger the condition (Clutton et al. 1986a,b; McGrath et al. 1986). When isometric muscle contraction is studied, a means of limb fixation is necessary to ensure the constant spatial relationship between the appendage and the transducer. This is important when high stimulation frequencies may cause equipment slippage, or prevent muscles from returning to their pre-stimulation length. However, the means of limb fixation must not impede the action of muscles under test as this will reduce evoked 'twitch' strength and produce spurious results. When the ulnar nerve–carpal flexor MMG was studied in pigs (Clutton et al. 1986a,b; McGrath et al. 1986; Richards et al. 1989) limb fixation was achieved with fibre-glass casts which probably affected muscle action.

The goal of the current study was to devise and evaluate an MMG technique using the TOF stimulus pattern applied to the common peroneal–pelvic limb digital extensor nerve-muscle unit anaesthetized Göttingen minipigs.

Materials and methods

The project was approved by the Home Office under project licence 60/3757. The need for surgical access to the ventral neck (for jugular venous and common carotid arterial cannulation) precluded the use of the previously described (Clutton et al. 1986a,b; McGrath et al. 1986;Richards et al. 1989) ulnar–carpal flexor nerve-muscle unit (NMU). Consequently, amputated pelvic limbs of six minipig cadavers were dissected to determine: 1) the anatomical course of the common peroneal nerve and 2) prominences on the lateral and medial surfaces of the limb which could serve as fixation points. These points were selected on the basis that their penetration by transdermal fixation pins would not interfere with muscle contraction and the subsequent dorsiflexion of the digit, but would immobilize the joint(s) proximal to the metatarso-phalangeal joint. The lengths between likely fixation points were measured in the largest and smallest cadavers using a steel ruler. These were (from and to respectively): 1) the lateral femoral condyle (A; Fig. 1) and the tibial crest (B; Fig. 1); 2) the tibial crest and the lateral (tibial) malleolus (C; Fig. 1); 3) the lateral malleolus and the distal extremity of metatarsal IV (D; Fig. 1).

On the basis of cadaver studies, a limb fixation device was constructed. This consisted of three 'H'-shaped frames (Fig. 2) constructed of 12.5 mm diameter stainless steel rod joined together by one central (9.5 mm diameter) and two (12.5 mm diameter) side braces. The connectors were 360° rotation connectors accommodating 9.5 and 12.5 mm rods (Harvard Apparatus; MA). The fixation pins had to fit easily and adjustably into the limb fixator, be single-use only and therefore inexpensive. These requirements were met using 2.1 mm × 38.1 mm (14 gauge 1.5 inch) aluminium hub hypodermic needles (Kendall Monoject Tyco Healthcare UK Ltd, UK) encased in two sections of 12.5 mm diameter stainless steel rod

bored to accommodate the needle and its hub, and machined so that the two components screwed together, encasing the needle tightly. Construction of the needle-barrel assemblies are shown in Fig. 3. Each assembly, which was 7 cm long, was fixed to the vertical posts of the frame using 12.5 mm × 9.5 mm closed apparatus connectors (Harvard Apparatus). The relationship of the limb to the fixation frame is shown in Fig. 2. The adjustment of the 360° rotation connectors allowed modification of the angle of the posts relative to each other, whilst adjustment of the 12.5 mm × 9.5 mm closed apparatus connectors allowed fixation pin movement through arcs approximately perpendicular to the long axis of the limb.

After purchase from the breeder and supplier (Elegaard, Denmark) the pigs were housed in colonies of 9–26 animals for periods of 4–10 weeks under specified (Home Office) conditions. Only pigs judged to be healthy on the basis of: normal food and water intake (and hence growth rate) physical appearance and physical examination (including rectal temperature) were studied.

Pigs were anaesthetized with ketamine 5 mg kg^{-1} and midazolam 0.5 mg kg^{-1} mixed in the same syringe and injected intramuscularly. When profound sedation was present some 10–12 minutes later, anaesthesia was induced with isoflurane carried in a 1:2 O_2:N_2O mixture delivered by Hall's mask attached to a Bain breathing system. When the jaw was relaxed, the laryngeal mucosa was desensitized with a lidocaine spray and the trachea intubated. Anaesthesia was maintained thereafter using isoflurane (end-tidal concentrations of 1.5–2.0%) based on cranial nerve and other reflexes, cardiovascular variables and the bispectral index. End-tidal concentrations of CO_2 and isoflurane were recorded throughout anaesthesia whilst core and rectal temperatures were monitored using appropriately positioned thermistor probes. Normothermia was preserved by operating in ambient temperatures of 22–24 °C, with the pigs enveloped in a hot air blowing system (Bair Hugger; Arizant UK Limited, UK). Whilst the temperature of the skin overlying *m. tibialis cranialis* was not monitored, the exposed limb was covered with an insulating layer of 'bubble-wrap'.

The limb fixation device was applied approximately 60 minutes after the induction of anaesthesia. A 2 mm diameter hole was drilled in the tip off the outer claw and fixed to the isometric force displacement transducer (Harvard Apparatus) using USP 1 Metric 4 surgical steel wire (Steelex monofilament Braun Aesculap AG & CO KG, Germany). Muscle preload was not measured in every case but ranged from 1.72 to 2.20 Newtons (N) (175-–xis. It was also sufficient to result in minimum movement on nerve stimulation. The transducer was calibrated at 0, 4.90 and 9.81 N (0, 0.5 and 1.0 kg).

The common peroneal nerve was stimulated using the train of four (TOF) stimulus pattern delivered from a peripheral nerve stimulator (Bard Biomedical, NY). Four square-wave stimuli each with a pulse duration of 200 ms were delivered at 2 Hz every 15 seconds (Fig. 4a). A miniature electrode (Harvard Apparatus) was attached to the nerve and connected to the cathode of the nerve stimulator. The anode was connected to a 21 gauge hypodermic needle inserted through and under the skin over the distal end of the femur (Fig. 1). The four evoked responses (twitches; T) were displayed, measured and recorded electronically by a purpose-built data-logging system (Vetronic Services Ltd). Supramaximal stimulation was achieved by increasing the delivered current in 5-10 mA steps every 12–15 seconds until the

twitch response was a stable maximum. Thereafter, the responses studied were those elicited by currents >20% of those producing these maxima. Signals from the transducer were amplified and conditioned (Harvard Transducer Amplifier; Harvard Apparatus) before passing to a 10-bit Analogue to Digital Converter (Vetronic Services Ltd) sampling at 500 second^{-1}. Samples were passed through a digital pulse detection algorithm to measure the instantaneous height of each twitch. Twitches were measured from the starting low point (trough) to the ending high point (peak). Data from the Analogue to Digital converter was sent to a PC (BCS Genesis, UK) where the data were displayed on screen. The detection algorithm searched for a four pulse stream of minimum height and with a clear window of duration such that a missing pulse was recorded as zero height. On the detection of the 4th pulse TOFR was calculated and displayed. All pulse trains, whether complete or not, were recorded on the computer's hard drive for later analysis.

The study objective was to assess the system's performance, i.e. stability, in monitoring neuromuscular transmission and so no attempt was made to record the effects of neuromuscular blocking agents in the current study.

Results

In the larger cadaver (dead weight 22 kg) the lengths between the lateral femoral condyle and the tibial crest, the tibial crest and the lateral (tibial) malleolus, the lateral malleolus and the distal extremity of metatarsal IV were 4.5, 9.5 and 7.5 cm respectively. In the smaller animal (dead weight 17 kg) the lengths between these same points were 4.0, 8.6 and 7.0 cm.

Cadaver dissection revealed that the common peroneal nerve coursed distocranially beneath the tibial part of the biceps femoris muscle within a groove bordered cranially by *m. extensor digiti IV* and caudally by *m. soleus*. Consequently, it could not be stimulated using transcutaneous electrodes without causing direct stimulation of *m. biceps femoris* and so a cut-down technique was adopted. This involved making an 8 cm skin incision centred approximately 4 cm caudal to the tibial crest, in a direction parallel to the extended femoro-tibial joint. The underlying biceps femoris muscle, which is about 1 cm thick in 25 kg animals, was cut and its fibres separated by blunt dissection. This revealed an underlying groove containing the common peroneal nerve in its proximal margins (Fig. 1).

In pilot studies the distal cathodal and proximal anodal electrodes were positioned approximately 4 mm apart but appeared to become 'short-circuited' after several hours – presumably by inflammatory exudate resulting from the 'cut-down' technique. In the main study the electrodes were reconfigured with the cathodal lead positioned around the nerve and the anode connected to a hypodermic needle inserted through the skin over the distal end of the femur (Fig. 1) some 3–5 cm away.

Three potential fixation points were identified on each aspect of the limb. Medial fixation points were the medial surface of the medial femoral condyle, the *caput tali* and the distal extremity of metatarsus III (Fig. 2). Corresponding sites on the lateral limb were the lateral surfaces of the lateral femoral condyle, *os calcaneous* and the distal extremity of metatarsus IV.

Figure 5a is representative of the TOF waveform recorded In 18 animals in which relaxing muscle achieved the same resting position before and after all four stimuli were delivered, i.e., in which the contraction–relaxation cycle was <300–500 ms (depending when contraction began in the 200 ms stimulation period – see Fig. 4b). However, in 42 animals the cycle was longer (Fig. 5b). This generated traces in which the fourth twitch (T4) height exceeded the first (T1) and produced 'reverse fade' in which TOFR >1.0. The mean (range) of the TOFR in pigs with reverse fade was 1.2 (1.1–1.3). Such reverse fade occurring in paralysed cases would spuriously increase the TOFR and create the impression that a less intense neuromuscular block was present. That signal 'ramping' occurred at 2 Hz was confirmed by applying a 1 Hz stimulus and measuring period duration in 12 of the 42 animals showing the anomaly. In a sample trace taken from a representative animal (Fig. 6) the contraction–relaxation cycle ranged from 534 to 801 ms. However, the signal recognition programme recognised the rising baseline in computing the TOFR and so 'ramping' did not affect the value registered. In the majority of animals, TOFRs remained unchanged for periods of more than 12 hours, including those showing reverse fade. This confirmed the device's stability. In an unknown, but small number of cases, marked fade caused by slippage of the wire knots connecting the transducer and the toe was observed. Pigs were euthanatized whilst anaesthetized at the conclusion of each study.

Discussion

Limb fixation was achieved with a fibre-glass cast when the ulnar–carpal flexor NMU was studied in pigs using MMG (Clutton et al. 1986a,b; McGrath et al. 1986; Richards et al. 1989). This created at least three problems: 1) the cast took time to set which prolonged anaesthesia; 2) it could only be removed on destruction – making it a single-use item; and 3) it probably affected muscle movement. The device described in the current study was re-useable; only four hypodermic needles had to be removed from the barrels, discarded and replaced between pigs. Furthermore, its construction allowed it to be rapidly adapted to pigs of differing sizes. No attempt was made in the current study to test it on non-miniature pigs, but the basic pattern could be adapted for larger (or smaller) animals. The anatomy of the fixation points precluded any probability that the described device impaired muscle movement and so signal quality was optimal. In contrast to limb casts, the current device could be set up and measuring twitches in <10 minutes.

When the limb fixation device was attached to the whole animal (rather than the amputated pelvic limb) it was found that adequate tarso-metatarso joint immobility could be achieved without the proximal femoral fixation points, i.e., only points LM, MM, LD and MD were required (Fig. 2). However, the proximal post was retained in the current trial because it enhanced the device's overall stability and rigidity. In future, frame rigidity could be achieved using a single horizontal 12.5 mm diameter stainless steel rod fixed to the side braces with two closed apparatus connectors. By obviating the need for the proximal post and the femoral condylar fixation points, such a modification would probably reduce post-operative discomfort.

Signal stability was highly satisfactory, with TOFRs remaining unchanged for periods >12 hours in animals showing both 'normal' and reverse fade. Marked fade was encountered in a

small number of experiments but was readily attributable to slippage in the wire knots connecting the transducer to the toe.

A major disadvantage of the limb fixator described is its suitability only for animals positioned in dorsal recumbency. Considerable modification would be necessary if the MMG were to be measured in animals in lateral or sternal recumbency.

Reverse fade is a common phenomena in unparalysed human patients in which acceleromyography is used to quantify twitch magnitude (Viby-Mogensen et al. 1988; Harper et al. 1994; Loan et al. 1995; Dahaba et al. 2002; Kopman et al. 2002). It probably arises because the non-relaxed free-moving appendage (thumb) does not return to exactly the same position after each stimulus (Loan et al. 1995). However, it seems unlikely that this occurred in the current study because reverse fade is eliminated by preloading (Viby-Mogensen et al. 1988; Harper et al. 1994;Dahaba et al. 2002) which, being a prerequisite of isometric mechanomyography, was applied in the current study using forces of 1.72–2.20 N.

The 'staircase' phenomenon which refers to the enhanced contractile response of muscle to repeated indirect stimulation is a well-recognized phenomenon (Ritchie & Wilkie 1955; Krarup 1981; Engbaek 1996) which complicates neuromuscular blockade monitoring and may generate spurious results when neuromuscular blocking agents are compared critically (Kopman et al. 2001). However, it is unlikely that the 'ramping' or baseline elevation observed in the current study was related to the staircase phenomenon for two reasons: 1) T1 remained constant in all animals studied – and over prolonged periods; 2) TOF stimulation does not alter TOF ratios in human beings (Kopman et al. 2001) nor dogs (Martin-Flores et al. 2011) at stimulation frequencies which increase T1 to 158% and 118% of control, respectively.

The abnormal train of four pattern observed in some pigs probably occurred because the contraction–relaxation cycle of the common peroneal–digital extensor NMU exceeded 300–500 ms. The duration of a muscle's contraction–relaxation cycle depends on the same ultrastructural and biochemical properties that allows muscle classification into 'slow' type 1 oxidative, and 'fast' type II anaerobic fibres (Hopkins 2000). Given the reverse fade observed in 42 pigs in the current study, it is possible that the muscle investigated –*m. tibialis cranialis*– contains a high proportion of slow fibre types. However, this is difficult to confirm; despite an extensive literature review using common search engines, no information on the slow *versus* fast properties of the fibres of this muscle in pigs or any other quadruped species was retrieved.

It is difficult to calculate the duration of contraction–relaxation cycles that cause progressive elevation of consecutive twitches because contraction may begin at anytime during the 200 ms stimulation period. When contraction begins at the onset of stimulation (Fig. 4b plot a) contraction–relaxation cycles >500 ms are required for baseline elevation in the following twitch. In contrast, when contraction occurs at the end of stimulation (Fig. 4b, plot b) contraction–relaxation cycles just >300 ms will produce similar effects. A high speed recording system may have elucidated events in the current study but such equipment was unavailable.

As there appeared to be no mechanical impediment to limb relaxation it is concluded that the NMU is inherently non-elastic, at least at the preloads applied. The use of greater preloads could have accelerated relaxation but by over-stretching the muscles may have impaired contractile function.

The TOF 'ramping' observed in the current study is unimportant when only T1 measurements are required. However, single twitch monitoring is of limited clinical benefit because it requires control twitch strength to be established before neuromuscular blockade is produced. This contrasts with the TOF stimulation pattern which allows responses to be quantified without force transduction, because the first twitch is the control for the others and can be gauged by palpation or observation of evoked twitches. However, accurate determination of the TOFR depends on a stable baseline and so a modified signal capture method – as was used here – is necessary when this is not present and the TOFR is required.

It is tempting to speculate that the detection of fade in NMUs in which the contraction–relaxation cycle exceeded 300–500 ms might be possible when more than four stimuli are applied at frequencies <2 Hz. This would require programmable stimulators rather than proprietary devices but in theory might allow the most desirable property of TOF nerve stimulation – the ability to quantify neuromuscular blockade by determining the TOF count – to be retained. However, the clinical relevance and meaningful interpretation of responses to train-of five (or greater) stimulation patterns would first require that the relationship between count, fade and the height of T1 in response to the novel pattern be established (Ali et al. 1970). In order to develop a stimulation pattern that could yield reliable and reproducible measurements research would also need to focus on standardizing other aspects of nerve stimulation known to affect the measurement of the magnitude and the time course of neuromuscular block, e.g., pulse width, stimulation frequency, pattern, current intensity and duration of pre-relaxant control stimulation (Maddineni et al. 1993; McCoy et al. 1995; Fuchs-Buder et al. 2007).

As pigs did not recover from anaesthesia there is no way of knowing the level of post-operative discomfort the technique may have caused. This would have at least three origins: 1) the 'cut-down' surgery required for nerve location; 2) prolonged pelvic limb hyperextension; and 3) the insertion of 14 gauge needle tips through the skin and periosteum at the four fixation points. In recovery experiments using the MMG described, systemic non-steroidal anti-inflammatory drugs or extradural opioids would probably provide adequate comfort. In extreme cases extradural local anaesthetics could be used.

Conclusion

The technique described allowed the evaluation and recording of the MMG of the common peroneal-pelvic limb digital extensor NMU in anaesthetized minipigs with body masses of 10.5–26.0 kg positioned in dorsal recumbency. Stimulation frequencies of 2 Hz caused baseline shifts in most pigs which means modified stimulation patterns or signal detection methods are required for TOFR determination. However, changes in T1 during TOF stimulation are unaffected. The assembly was stable for periods of up to 12 hours.

Acknowledgments

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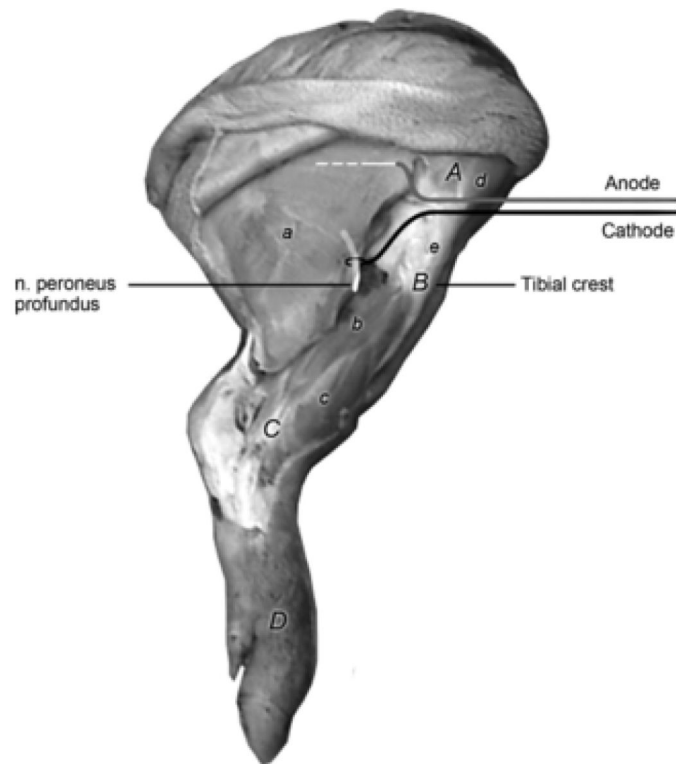
Figure 1.
Dissection diagram of the right pelvic limb (lateral aspect) showing: 1) The anatomic loci measured in cadaver studies to determine size requirements for the limb fixation frame. (A) lateral femoral condyle; (B) tibial crest; (C) lateral (tibial) malleolus; (D) distal extremity of metatarsal IV. 2) the deep peroneal nerve and location of stimulating electrodes for mechanomyography. The proximal portion of the nerve is shown lying deep to the biceps femoris muscle. (a) *m. biceps femoris*; (b) *m. extensor digiti IV*; (c)*m. tibialis cranialis*; (d) *m. vastus lateralis*; (e) straight patellar ligament.
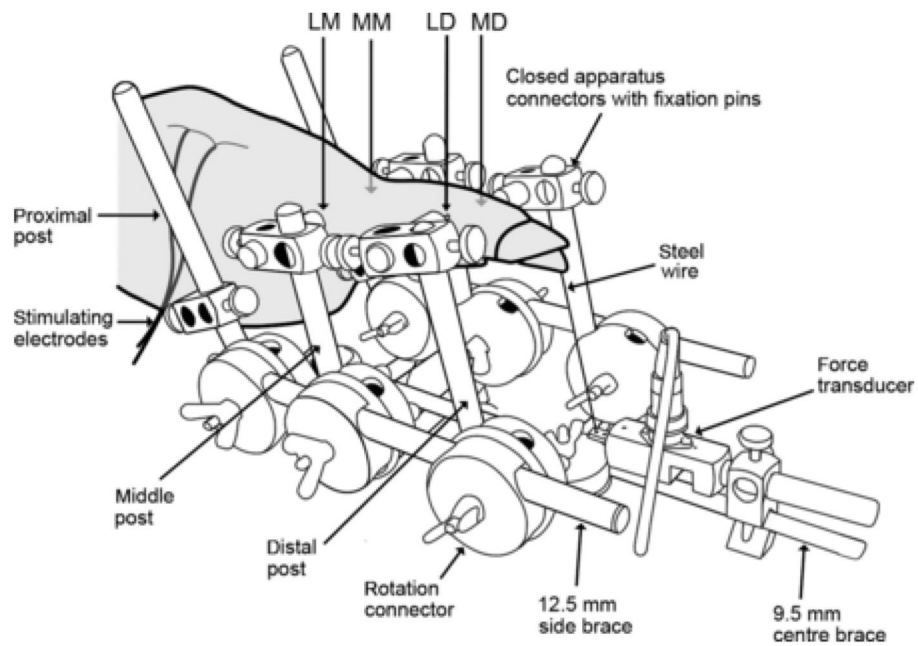
Figure 2.
Diagram showing the limb fixation frame for mechanomyography in anaesthetized minipigs positioned in dorsal recumbency. The lateral middle (LM), medial middle (MM), lateral distal (LD) and medial distal (MD) fixation points correspond to the *caput tali*, the distal extremity of metatarsus III, the *os calcaneous* and the distal extremity of metatarsus IV respectively.
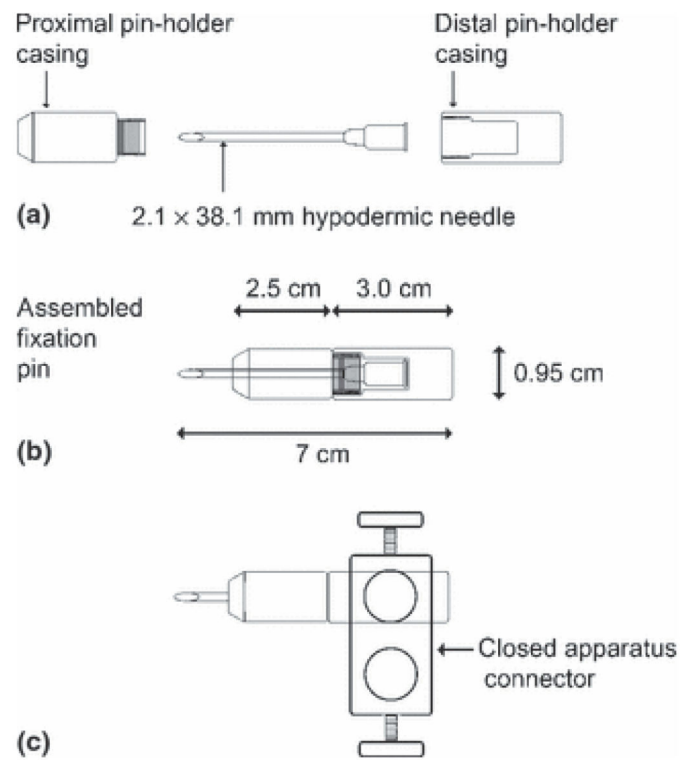
Figure 3.
Diagram showing construction of the pin holder (a) the fixation pin (b) and the fixation pin – closed apparatus assembly (c).
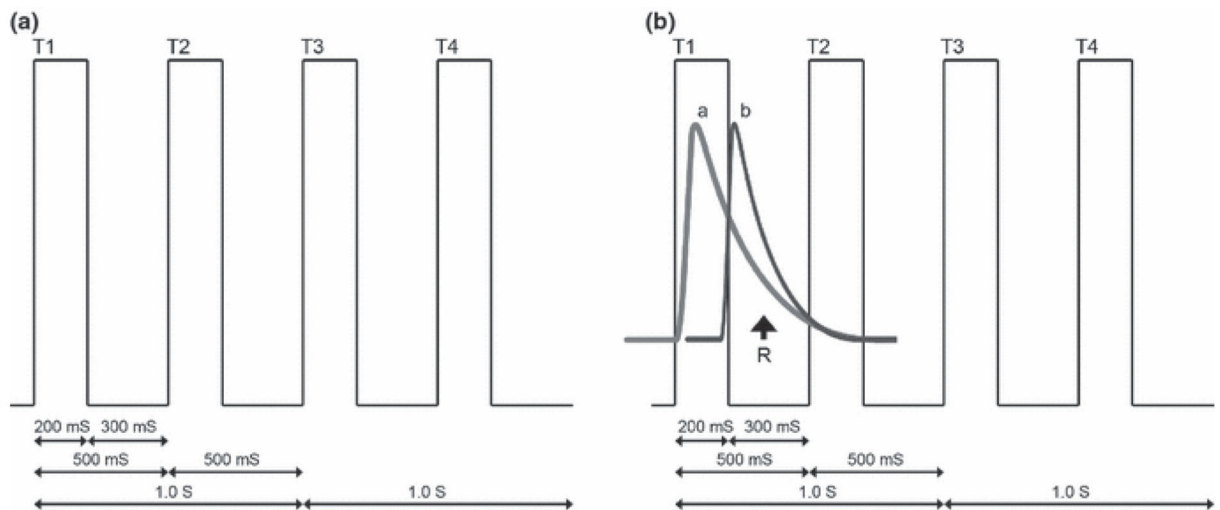
Figure 4.
(a) The train of four (TOF) stimulation pattern. (b) The TOF stimulation pattern with contraction–relaxation cycles beginning at the onset (plot a) and at the end (plot b) of the 200 ms stimulation period. Plot a illustrates that contraction–relaxation cycles >500 ms are required for baseline elevation in succeeding twitches. Plot b indicates how contraction–relaxation cycles >300 ms can produce the same effect.
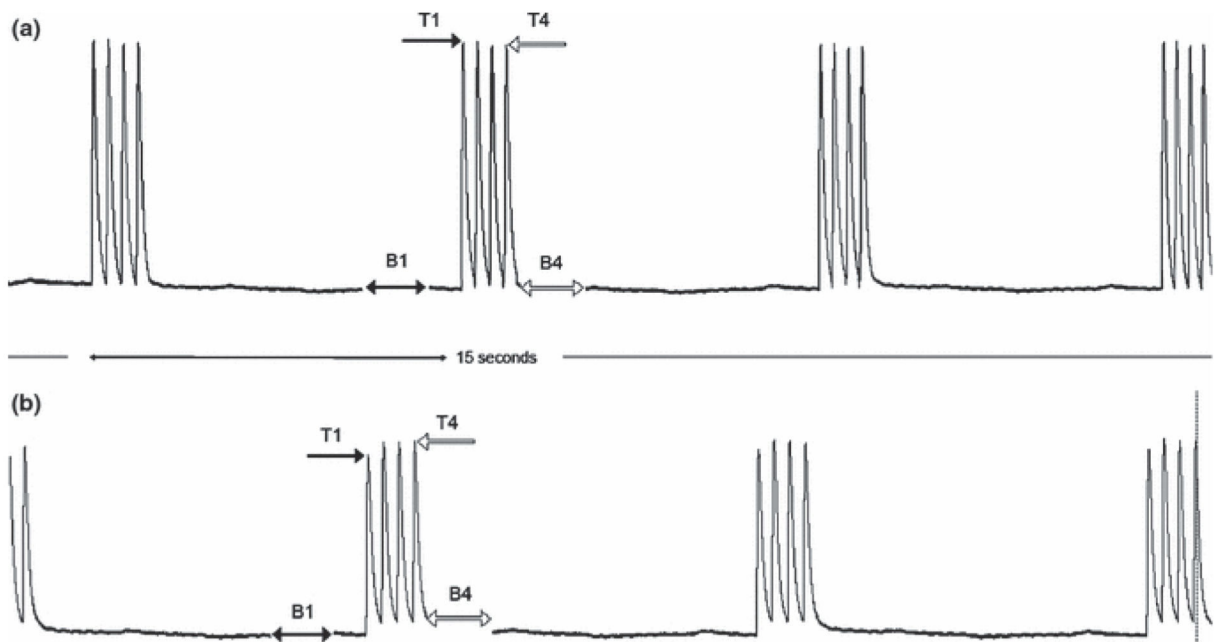
Figure 5.
a) A train of four mechanomyogram taken from an atypical pig with four contraction–relaxation cycles <300–500 ms. The pre-stimulation baseline (filled arrow; B1) is at a similar level to the post-stimulation baseline (clear arrow B4) whilst T4 (clear arrow) is only slightly <T1 (filled arrow). b) A train of four mechanomyogram taken from an animal with four contraction–relaxation cycles >300–500 ms. The post-stimulation baseline (clear arrow; B4) is elevated compared to the pre-stimulation baseline (filled arrow B1) and corresponds with a T4 (clear arrow) elevated with respect to T1 (filled arrow) representing 'reverse fade'.
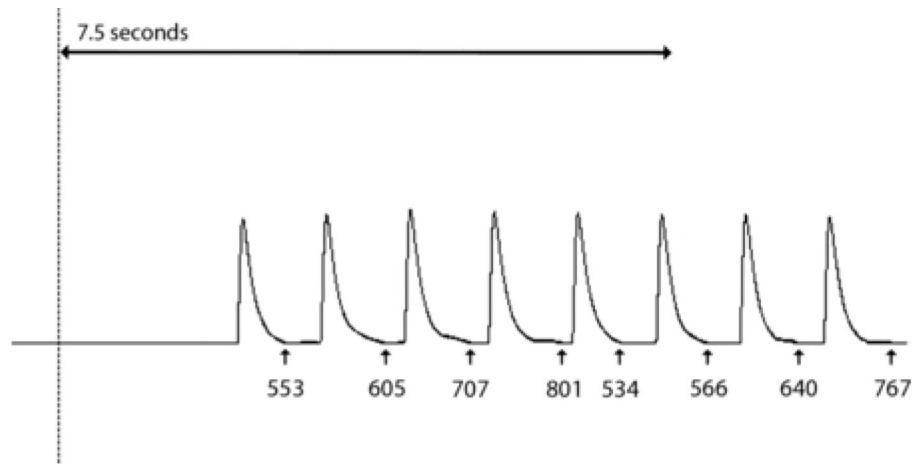
Figure 6.
The mechanomyogram recorded during single twitch stimulation (1 Hz) of the common peroneal-pelvic limb digital extensor muscles in an anaesthetized minipig showing reverse fade. The contraction–relaxation cycle of each twitch is indicated in ms. The arrow indicates the time at which baseline was restored with each twitch.