



Influence of tetanic stimulation on the staircase phenomenon and the acceleromyographic time-course of neuromuscular block: a randomized controlled trial

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Abstract

During neuromuscular monitoring, repeated electrical stimulation evokes muscle responses of increasing magnitude ('staircase phenomenon', SP). We aimed to evaluate whether SP affects time course and twitches' values of an acceleromyographic assessed neuromuscular block with or without previous tetanic stimulation. Fifty adult patients were randomized to receive a 50 Hz tetanic stimulus (S group) or not (C group) before monitor calibration. After 20 min of TOF ratio (TOFr) stimulation rocuronium was administered. Onset time of block (primary endpoint), recovery of T1 to 25%, TOFr to 0.9, and recovery index were compared. We also compared T1 and TOFr at baseline, post-stimulation, and during recovery from block. Moreover the correlation between T1 at maximum recovery and (a) baseline T1 and (b) post-stimulation T1 along with T1/TOFr ratio during recovery were evaluated. After stimulation median T1 increased (32%) in group C and decreased (16%) in group S ($P=0.0001$). Onset time (Median [IQR] in seconds) was 90 (29–77) vs. 75 (28–60) in C and S group ($P=0.002$). Time [Mean (SD) in minutes] to normalized TOFr 0.9 were 70.13 (14.9) vs. 62.1 (21.2) in C and S groups ($P=0.204$). TOFr showed no differences between groups at any time point. T1 at maximum recovery showed a stronger correlation with post-stabilization T1 compared to baseline. ($\rho=0.80$ and $\rho=0.85$, for C and S groups.) Standard calibration does not ensure twitch baseline stabilization and prolongs onset time of neuromuscular block. TOF ratio is not influenced by SP.

Keywords Perioperative monitoring · Neuromuscular blockade · Neuromuscular blocking agents · Perioperative medicine

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1 Introduction

Neuromuscular blockade (NMB) is an important part of general anesthesia, however, monitoring of NMB is underused for several reasons, including difficulty in interpreting results [1, 2]. Repeated indirect electrical low-frequency stimulation of a motor nerve, even at supramaximal intensity [3], results in evoked muscle responses of increasing amplitude. This is known as the staircase phenomenon (SP) [4, 5]. The SP has been observed with acceleromyography (AMG), mechanomyography or phonomyography, but not with electromyography [6–8]. To the best of our knowledge, there are discrepancies regarding the clinical importance of the SP on the onset and offset of neuromuscular block. Moreover, the influence of stimulation frequency and type of muscle stimulated on the magnitude of SP remains to be fully elucidated [9, 10].

While achieving twitch stability for 2–5 min prior to administration of neuromuscular blocking agents (NMBAs) is recommended in research protocols [11], and applying a

short high-frequency stimulation [e.g. a 50 Hz tetanic stimulus for 5 s] is advocated to shorten this otherwise time-consuming (up to 20 min) procedure [6, 12, 13], there are no defined strategies for obtaining a stable baseline during calibration of the accelerometer in a clinical setting.

We hypothesized that if the SP is prevented by a prior tetanic stimulation, onset and recovery times of neuromuscular blockade would be shorter than if a tetanic stimulation is not applied. The main objective of our study was to evaluate the time course of rocuronium with our without previous tetanic stimulation. Secondary objectives were to evaluate specific monitor-related aspects such as sensitivity and supramaximal current.

2 Methods

This randomized controlled trial was performed at the Consercio Hospital General Universitario de Valencia, Valencia (Spain) and Hospital de Manises, Valencia (Spain), between February 2013 and March 2014. Approval for this study was granted by both Institutional Review Boards (protocol number: 86/2009 and 0816/2013 respectively). This trial was registered with ClinicalTrials.gov (Identifier: NCT03146767).

After obtaining written informed consent, patients > 18 year old with an American Society of Anesthesiologists physical status I–II [14], undergoing surgery not involving the airway, and with an expected duration longer than 90 min were recruited. Exclusion criteria included pregnancy, diseases or medications known to interfere with neuromuscular transmission, chronic renal or hepatic failure, allergy to medications used in the study, hemodynamic instability or potentially large intraoperative blood loss.

2.1 Anesthesia management

All patients were monitored with continuous electrocardiography, noninvasive blood pressure, pulse oximetry, capnography, a respiratory gas analyser (Dräger Medical Systems, Lübeck, Germany or GE Healthcare, Chicago, Illinois, USA) and Bispectral index (BIS Vista, Covidien, Dublin, Ireland). After premedication with intravenous (iv) atropine $0.01 \text{ mg} \cdot \text{kg}^{-1}$, fentanyl 0.1 mg , and midazolam $0.03 \text{ mg} \cdot \text{kg}^{-1}$, general anesthesia was induced and maintained with propofol (to achieve a BIS between 40 and 60) and fentanyl boluses as required. No inhalational anesthetic agents were used. Patients lungs were ventilated to maintain an end tidal carbon dioxide (EtCO_2) 35–40 mmHg. An upper body forced air warming blanket (Mistral-air plus, The 37° Company, Amersfoort, The Netherlands) was used for normothermia. Skin temperature was recorded and maintained above 32°C , in the monitored arm on the thenar eminence

by a built-in thermometer probe of the neuromuscular monitor.

2.2 Neuromuscular monitoring

Acceleromyography is based of the second law of motion: since force equals acceleration times mass, if mass is constant acceleration is directly proportional to force [15]. An accelerometer (TOF-Watch SX; Organon, Dublin, Ireland) was placed on the volar surface of the thumb distal to the interphalangeal joint to assess the adductor pollicis muscle response to ulnar nerve stimulation. The negative distal stimulating electrode was placed 1 cm proximally to the wrist on the radial side of the flexor carpi ulnari muscle and the positive one 2–3 cm more proximally. A constant preload was applied to the monitored thumb while the other fingers were strapped to the surgical table arm.

2.3 Study design and data collection

Subjects were randomly assigned to two groups with a computer-generated list by a sequence of pseudorandom numbers. For group C (control group), calibration of the AMG was set using the built-in calibration function (CAL 2 [16]) of the monitor, -that automatically determines supramaximal current, sensitivity of the accelerating sensor-, and sets the response of T1 to 100%. Thereafter, 2 Hz TOF stimuli were applied every 15 s for 20 min. Then, rocuronium bromide $0.6 \text{ mg} \cdot \text{kg}^{-1}$ was injected iv in 5 s through a fast running infusion of normal saline which was placed on the non-monitored arm.

Subjects in group S (study group) received an initial tetanic stimulation (50 Hz for 5 s, 50 mA current intensity); thereafter, the AMG monitor calibration and TOF stimulation period was the same as in group C.

All calibration procedures were performed immediately after induction of anesthesia.

Acceleromyography data were downloaded to a computer using dedicated software [TOF-Watch SX Monitor program version 2.5 for Windows (Organon, Dublin, Ireland)].

The following variables were recorded (Fig. 1):

- Monitor's sensitivity (adimensional number on a scale from 1 to 512) and supramaximal current (in milliamperes, mA) as set by the automated calibration procedure.
- Immediately after calibration: Baseline T1 and TOFr.
- After 20 min of TOF stimulation (prior to rocuronium administration): Post-stimulation T1 and TOFr values.
- Onset and recovery from neuromuscular block times (see Fig. 1 for full details).
- T1 amplitude at maximum recovery (the highest amplitude reached by T1 during recovery).

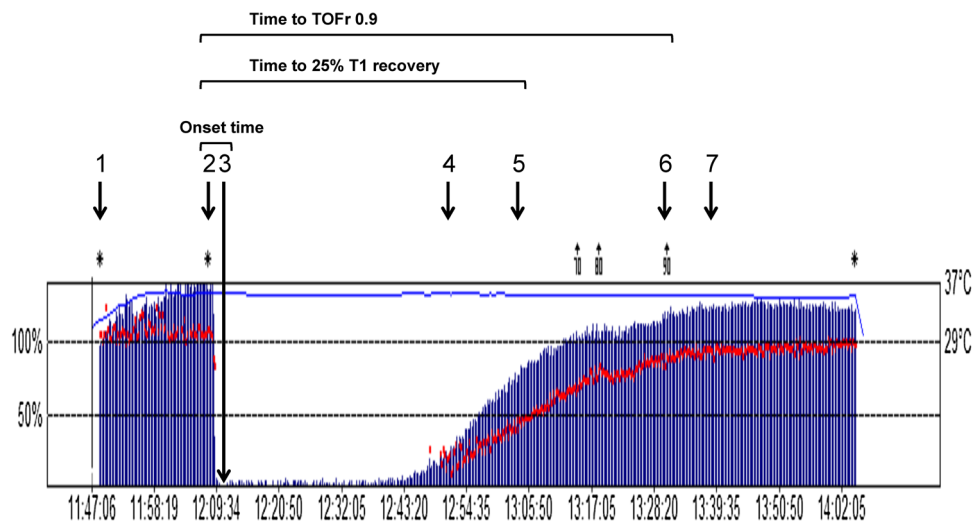


Fig. 1 Measured twitch values and neuromuscular block time-course as recorded on a patient's chart from C group. Blue columns: twitch values (scale left Y-axis). Red dots: Train of Four ratio (TOFr) values. Blue continuous line: patient's temperature (scale left Y-axis). 1: Baseline T1 and TOFr. 2: Post-Stabilization period T1 and TOFr. 3: Maximum T1 depression. 4: 25% T1 recovery. 5: 75% T1 recovery.

6: TOFr 0.9. 7: Maximum T1 recovery. Horizontal square brackets represent time intervals: Onset time (time from rocuronium injection to $\geq 95\%$ T1 depression in seconds, primary endpoint); Time to T1 recovery to 25% (normalized to maximum recovery T1 value, in minutes); Time to TOFr 0.9 (in minutes)

In addition two calculated variables were obtained:

- Normalized TOFr 0.9 and Time to normalized TOFr 0.9 ($\text{TOFr}0.9N = 0.9 \times \text{baseline TOFr}$).
- The ratio between T1 and TOFr (T1/TOFr) after calibration, post-stimulation and at T1 maximum recovery.

If the procedure was completed prior to spontaneous recovery of neuromuscular function, a reversal drug was administered, and the data following reversal were excluded from analysis. Patients were blinded to the allocation group. Due to the characteristics of the procedure, no other blinding was possible.

2.4 Statistical analysis

Analysis was performed with SPSS version 19 (IBM, Armonk, New York, USA) and Stata version 13 (Statacorp, College Station, Texas, USA) and R statistical software version 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria). A priori sample size calculation determined that 21 patients in each group were required to detect a difference in onset time (primary objective) of 20% with an α error of 5% and β of 10%, with a standard deviation (SD) of 30 s (from a healthy patients' cohort in a previous study with the same NMBA) [17]. In anticipation of possible losses 50 patients were finally recruited.

Analysis was performed on an intention to treat approach. Parametric and nonparametric data were compared between groups with unpaired Student's *t* tests or Mann–Whitney *U*

test respectively. Categorical data were compared with Fisher's exact test. To assess the relationship between T1 amplitude at maximum recovery and baseline T1 amplitude, and T1 amplitude at maximum recovery and post-stimulation T1 amplitude, Spearman's rank test was used. Bonferroni–Holm step-down method correction for multiple comparisons was used. For recovery values significance threshold was set at $P < 0.05$. A posthoc analysis to control for monitor sensitivity was performed by fitting a proportional odds model for T1 amplitude post stabilization period, and T1 amplitude at maximum recovery (in this case significance threshold for the variable in the model was set at 0.01).

3 Results

The flow chart of patient's enrollment is showed in Fig. 2. There were no differences in demographics between groups as showed in Table 1.

3.1 Clinical results

Onset time [median (25–75 percentile)] was significantly shorter in group S [75 (28–60) s] than in group C [90 (29–77) s], $P = 0.002$.

Recovery times from neuromuscular block are show in Table 2. There were no differences between groups in time of recovery of T1 to 25%, time to TOFr 0.9, and time to normalized TOFr 0.9.

Fig. 2 CONSORT diagram showing patient recruitment and flow. *CONSORT* Consolidated Standards of Reporting Trials

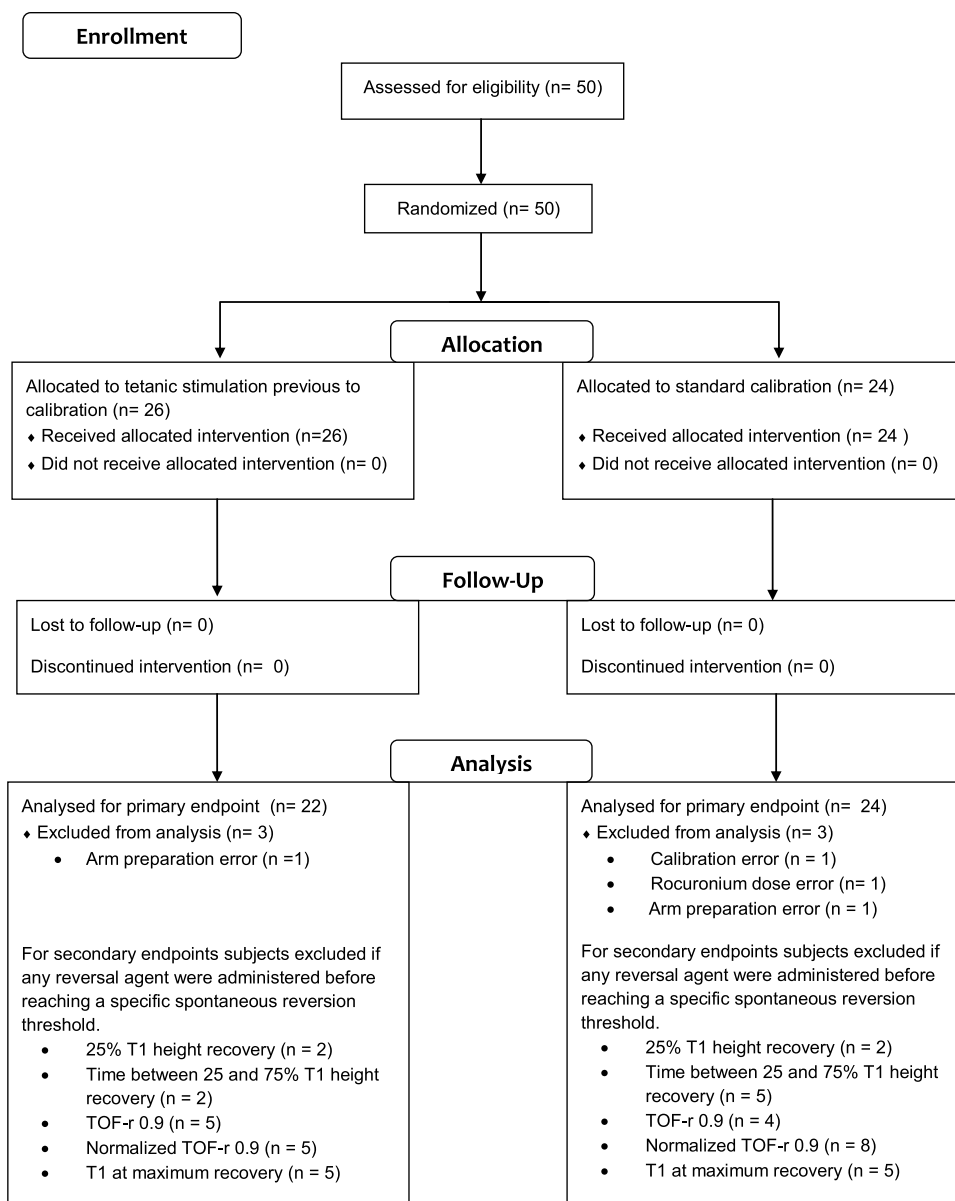


Table 1 Patients' characteristics

	C group (n=24)	S group (n=22)	P value
Age (years)	45.9 (12.6)	47.9 (10.5)	0.553
Sex (M/F)	17/7	17/5	0.742
BMI (kg/m ²)	25.6 (3.2)	25.1 (2.9)	0.594
Surgery time (min)	119 (46)	98 (41)	0.115
ASA			1.000
I	9	9	
II	15	13	

Values are mean (SD) or counts

BMI body mass index, *M* male, *F* female, *ASA* American Society of Anesthesiologists physical status

3.2 Accelerometer registration results

T1 amplitude and TOFr at baseline, 20 min after TOF stimulation (post-stimulation period), and at maximum recovery are summarized in Table 2. T1 was higher in group C than in group S post-stimulation and at T1 maximum recovery while TOFr showed no significant differences between groups (Table 2).

AMG sensitivity [mean (SD)] was significantly higher in group C [214.6 (63.2)] than in group S [160.6 (76.1)] ($P=0.014$). Supramaximal current was 55 (10) mA and 60 (10) mA in groups C and S, respectively ($P=0.407$).

Table 2 T1 and TOFr ratio values and T1/TOFr values at baseline, after 20 min stimulation period, and at maximum recovery and recovery times

	C group (N=24)	S group (N=22)	Difference 95% CI	P value
T1B	93 [87–98]	94 [78–97]	3 [–4 to 14]	0.42
T1ES	123 [105–139]	79 [70–92]	41 [24–54]	0.0001
T1MAX	105 [83–135]	55 [42–75]	49 (20–67)	0.001
TOFrB	113 [107–119]	110 [107–119]	3 [–3 to 7]	0.27
TOFrES	112 [103–116]	111 [103–115]	0 [–4 to 4]	0.95
TOFrMAX	84 [70–93]	90 [79–95]	–5 [–14 to 6]	0.28
Time to T1 25%	44.0 (2.45) N=22	38 (2.29) N=20	6.1 (–0.7 to 12.9)	0.081
Time to TOFr 0.9	65.0 (12.3) N=20	57.5 (17.5) N=17	7.5 (–2.5 to 17.5)	0.138
Time to normalized TOFr 0.9	70.3 (14.9) N=16	62.1 (21.2) N=17	8.0 (–4.6 to 20.7)	0.204
T1/TOFrB	82 [74–87]	84 [63–91]	0.01 [–8.6 to 10]	0.5
T1/TOFrES	104 (33)*	74 (20)	30 [13 to 47]	0.0007
T1/TOFr MAX	128 (41.5)*	72 (28.4)	56.4 (32.0–80.8)	0.001

Values in percentages (as recorded by TOF-Watch SX monitor) or in minutes. Values are median [25th–75th percentile] or mean (SD). *T1* first stimulus of train of four, *CI* confidence interval, *N* number of subjects analyzed, *TOFr* train of four ratio, *T1B* T1 amplitude after calibration, *T1ES* T1 amplitude before rocuronium administration, *T1MAX* T1 amplitude at maximum recovery, *TOFrB* first TOFr after calibration, *TOFrES* last TOFr before rocuronium administration, *TOFrMAX* train of four ratio at T1 maximum recovery, Time to T1 25%—time to T1 recovery to 25% (minutes), Time to TOFr 0.9—time to TOFr to reach 0.9 (minutes), Time to normalized TOFr 0.9—time to reach TOFr of 0.9 normalized baseline value, *T1/TOFrB* T1/TOFr ratio after calibration, *T1/TOFrES* T1/TOFr ratio before rocuronium administration, *T1/TOFrMAX* T1/TOFr at T1 maximum recovery

*1 missing value in this group

3.3 Calculated parameters results

Proportional odds model with T1 amplitude post-stimulation period as the dependent variable, and study group and sensitivity as independent variables returned a beta coefficient of 0.086 (0.021–0.313, $P = 0.001$) and 1.007 (1–1.016, $P = 0.067$) for group S and for sensitivity respectively.

The proportional odds model built with T1 amplitude at maximum recovery as the dependent variable, and study group (with group C as reference category) and sensitivity as independent variables returned a beta coefficient of 0.14 (0.033–0.525, $P = 0.005$) and 1.009 (1–1.019, $P = 0.042$) for group S and for sensitivity respectively.

There was a moderate correlation in both groups between baseline T1 amplitude after calibration and T1 amplitude at maximum recovery, and a strong correlation between T1 amplitude post-stimulation period and T1 amplitude at maximum recovery.

T1/TOFr ratio after the stabilization period and at maximum recovery were significantly higher in group C (Table 2).

4 Discussion

The results of this investigation show that, using acceleromyography, the onset of a single bolus of rocuronium is shortened when a tetanic stimulation is used prior to TOF monitoring and calibration. Precise identification of the onset time may help optimize intubating conditions, which are associated with vocal cord injury [18]. In a recently published systematic review on laryngeal morbidity after tracheal intubation, the degree of neuromuscular block was identified as an anesthesia-related risk factor for this adverse event [19].

During recovery from NMB, both the time for T1 to reach 25% and the time to reach a TOFr 0.9 in the recovery phase showed a lag (both in raw and normalized value) in the standard calibration (control) group, albeit not reaching statistical significance. Additionally, we found that T1 amplitude increased significantly during a 20-min stabilization period in the control group and that T1/TOFr ratio was higher in control group both in the post-stabilization time and at recovery from neuromuscular block. Moreover T1 final amplitude showed greater correlation with the

post-stimulation value compared to the baseline values in both groups.

The perioperative monitoring responses' accuracy is crucial since NMB monitoring is intended to be a standard for patient's safety. In the present study, we observed a progressive increase in evoked muscle responses that reached a plateau approximately after 15–18 min of stimulation; these observations are in agreement with previous reports [4]. It has been shown in humans and animal species that if calibration is performed without allowing twitch stabilization, the magnitude of evoked responses can increase during the recovery phase of neuromuscular block [20, 21]. Under these circumstances, which may resemble routine practice, recovery times might be biased and the displayed AMG values may overestimate the true recovery of neuromuscular function. In the present study, we recorded a difference between groups in the time to reach TOFr 0.9 albeit not statistically different between groups. Several patients were excluded from further analysis of recovery time if NMB was reversed, hence it is possible that the sample size was insufficient to detect differences for this parameter.

Previous reports show that, despite some inter-individual variability, the average twitch amplitude (measured with AMG) remains stable during a period of TOF stimulation if this is preceded by a 5 s 50 Hz tetanus [4]. Our results from patients receiving a tetanic stimulus differ from those mentioned above: twitch amplitudes showed a decrease toward the end of the 20 min of TOF stimulation if this was preceded by a 5 s 50 Hz tetanus. This could be due to a difference in supramaximal stimulation setting (which was not reported in the cited paper) or that a progressive decline in twitch amplitude following a tetanus might be observed once post-tetanic facilitation has ended, or simply by lack of statistical power in the previous report (no sample size calculation nor power analysis were reported). Such a behavior in twitch amplitude during recovery from non-depolarizing neuromuscular block has been previously observed with AMG [12–22] and mechanomyography [23, 24].

In accordance with other reports [12–20], we did not observe differences in TOFr values at any time. This observation, and the cited lack of necessity for a control value, highlights the usefulness of TOFr as a clinical monitoring parameter.

The magnitude of T1 at maximal recovery was smaller for group S than for group C. The clinical importance of a low T1 when the TOFr has recovered to 0.9 is unclear, however, a similar observation has been described when the selective reversal binding agent sugammadex was evaluated [25]. We evaluated the ratio between T1 and TOFr as an additional normalization measure, however, its potential role in patients with no or faulty monitor calibration remains to be elucidated.

Monitor characteristics, performance and accuracy are important factors that can determine the assessment that is intended to guide clinical management. Sensitivity is automatically set by the calibration process of the monitor, and ranges between 1 and 512 [26]. Sensitivity is adjusted to amplify the signal, with smaller values meaning less signal amplification. Our results show that the sensitivity of the accelerometer was significantly lower in group S than in group C. It is possible that lower sensitivity (and hence less amplification) was assigned in those patient receiving tetanus, since post-tetanic facilitation produced twitches of larger magnitude. This has also been observed in pediatric patients [13] and proposed as a possible explanation of between group differences. This suggestion raises the question of whether calibration after a tetanus might result in sensitivity values that are inadequately low. With this in mind, we fitted a proportional odds model to control for the potential effect of sensitivity on single twitch amplitude [27]. Our results show that even after controlling for sensitivity, group C has higher twitch amplitudes.

This study has limitations. We included only patients with an ASA physical status I–II without major comorbidities and undergoing elective surgery. We did not recalibrate the monitor after the 20 min stabilization period as research guidelines recommends [11]. This could imply an overestimation of the SP effect. We used standard calibration practices with currents of 55–60 mA and pulse widths (0.2 ms) that, although accepted by current guidelines, cannot absolutely guarantee a supramaximal stimulation [28]. Nevertheless we consider more important to replicate normal practice calibration. As for secondary outcomes, loss of sample size was the crucial limitation.

In conclusion, a tetanic stimulation prior to acceleromyographic monitoring reduced the onset time of rocuronium as assessed at the *adductor pollicis* muscle in adult patients, and resulted in lower T1 values both after a period of stimulation and at recovery, compared with patients not receiving a tetanus prior to calibration. Further studies specifically designed to elucidate the impact of monitor sensitivity on neuromuscular block function are warranted.

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Compliance with ethical standards

Conflict of interest GM and MMF have no conflict of interest. CLE and OD received payment for educational talks and scientific conferences from MSD (Merck Sharp & Dohme, Inc.). No conflict of interests related with the present work.

Ethical approval Ethical approval was granted by the applicable Institutional Review Board as detailed in the text.

Annex 1

The staircase phenomenon is a phenomenon related to, but not entirely identical to posttetanic potentiation [29]. It has been showed in animals that the increment in evoked mechanical response, but not the compound action potential, is associated to the number of indirect stimuli administered (up to a plateau of 250) [30], due to the phosphorylation of the myosin regulatory light chain [31] which enhances cell's sensitivity to calcium and a subsequent increase in contraction force [32], a process likely mediated by calmodulin [33, 34].

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