REGULAR PAPER

ACTA PHYSIOLOGICA

Body temperature control in rats by muscle tone during rest or sleep

Terje Lømo[1] (ID) | Torsten Eken[2] | Erling Bekkestad Rein[1] | Arild Njå[1]

[1]Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway

[2]Department of Anaesthesiology, Oslo University Hospital, Oslo, Norway

Correspondence
Terje Lømo, Institute of Basic Medical Sciences, Department of Molecular Medicine, Domus Medica Gaustad, Sognsvannsveien 9, 0372 Oslo, Norway.
Email: terje.lomo@medisin.uio.no

Funding information
The Institute of Basic Medical Sciences, University of Oslo; Oslo University Hospital; Anders Jahres fond til vitenskapens fremme

Abstract

Aim: To explore the role of tonic motor unit activity in body temperature control.

Methods: Motor unit activity in soleus and several other skeletal muscles was recorded electromyographically from adult rats placed in a climate chamber on a load sensitive floor, which, together with video monitoring, allowed detection of every successive period of movement and no movement.

Results: In the absence of movements during rest or sleep, motor unit activity was exclusively tonic and therefore equivalent to muscle tone as defined here. The amount of tonic activity increased linearly in the soleus as the ambient temperature decreased from 32°C to below 7°C, owing to progressive recruitment and increased firing rate of individual units. Brief movements occurred randomly and frequently during rest or sleep in association with brief facilitation or inhibition of motor neurons that turned tonic motor unit activity on or off, partitioning the tonic activity among the available motor units. Shivering first appeared when a falling ambient temperature reached ≤7°C in several muscles except soleus, which was as active between shivering bursts as during them.

Conclusion: Muscle tone and overt shivering are strikingly different phenomena. Tonic motor unit activity in the absence of movements evokes isometric contractions and, therefore, generates heat. Accordingly, when the amount of tonic activity increases with falling ambient temperature, so must heat production. Consequently, graded muscle tone appears as an important and independent mechanism for thermogenesis during rest or sleep at ambient temperatures ranging from <7°C to at least 32°C.

KEYWORDS
body temperature, motor neuron, motor unit, muscle tone, plateau potentials, shivering, thermogenesis

1 | INTRODUCTION

In cold environments, birds and mammals increase their heat production through non-shivering and shivering thermogenesis. Non-shivering thermogenesis (NST) occurs in brown adipose tissue (BAT).[1] The extent to which it also occurs in electrically inactive skeletal muscles is debated, as evidence both for and against such thermogenesis exists.[2-5] Studies on birds, beginning with Steen and Enger,[6] have provided important information on the physiology of shivering. Based on

electromyographic (EMG) recordings from pectoral muscles, these studies have shown that the amount of shivering and metabolic heat production are linearly correlated[7] and that shivering and oxygen consumption increase linearly with falling ambient temperature.[8-10] Birds lack BAT[11] and in the cold may shiver continuously[12] or in bursts,[10] depending on the species.

Also in mammals, including humans, shivering of skeletal muscle is described as either bursting or continuous[13] and continuous thermoregulatory muscle tone is often presented as "pre-shivering tone"[14,15] or as prestage to overt shivering.[16-20] A representative statement is "more anaerobic muscles, high in type II fibers, tend to shiver in bursts whereas those relying more on aerobic metabolism, high in type I fibers, shiver more continuously".[21] Kleinebeckel and Klussmann[16] identified three different patterns of activity during shivering, one of which was "Continuous tonic activity…found mainly during the initial phase of shivering in man (Göpfert and Stufler, 1952) and in the pectoral muscle of the pigeon during all stages of shivering (George, 1984)". By being included in the term shivering, muscle tone often goes unmentioned as a possible separate independent heat producing mechanism, as in a recent review of central control of body temperature[22] or in studies of NST in skeletal muscle.[23-27]

Muscle tone has been described as resting mechanical and EMG activity and shown to increase significantly in resting human quadriceps muscles at ambient temperatures below 21.5°C by McKay et al,[28] who write: "An accepted model is that resting muscle is electrically and mechanically inactive at room temperature, that cooling provokes invisible microvibrations called thermoregulatory tonus, that severe cooling causes visible shivering, and that these are simply degrees of the same phenomenon (Blatteis et al, 2001; Haider and Lindsley 1964; Kleinebeckel and Klussman 1990)". But they also cite studies "showing that thermoregulatory muscle tone and cold shivering are independent forms of muscle activity with different regulating mechanisms (Konstantinov 1975; Meigal et al 1993; Petajan and Williams 1972)" and they postulate a model "whereby resting muscle mechanical activity is invoked first at ambient temperatures below thermoneutrality, whereas shivering is invoked as a last-resort response". They also refer to Block (1994) as "considering resting muscle mechanical activity to be nonshivering thermogenesis"[29] and conclude that "whether resting mechanical activity represents an instance of nonshivering thermogenesis depends on the semantics of the word shivering".

In a recent review, Dulloo et al discuss "a role for skeletal muscle minor tremor or microvibration—nowadays referred to as resting muscle mechanical activity (RMMA) in maintaining body temperature in response to mild cooling"[30] They further write: "While it is considered to be the counterpart to the motor unit's electrical activity as measured by electromyography, several workers consider mechanomyographic to be a more sensitive method for examining resting muscle activity than electromyography".

From such reading of the literature we conclude that publications on body temperature regulation generally describe both muscle tone and overt shivering as shivering with the implication that they are different expressions of the same phenomenon and further that muscle tone is rarely discussed as a possible independent source of heat production for body temperature regulation unrelated to overt shivering.

In this study we define muscle tone as the muscle tension caused by motor unit impulse activity under resting conditions and then explore its role in body temperature regulation by separating all periods of movements from periods of no movements in unrestrained rats monitored by video recording as they rest, sleep or move on a load sensitive floor in a climate chamber. By recording EMG activity in soleus (SOL) and other muscles through chronically implanted electrodes and restricting our analysis to periods of rest or sleep in the absence of movements, we could isolate many motor units in the same muscle and study directly their firing patterns at different ambient temperatures. By using a load sensitive floor, we could also detect microvibrations during periods of rest or sleep that resembled RMMA.

This experimental approach has several advantages. First, by excluding all movements from the analysis, we could show that motor unit firing in the absence of movements is exclusively tonic and therefore equivalent to muscle tone. Because the contractions underlying such muscle tone are isometric, all the consumed energy is converted to heat. Accordingly, when muscle tone increases, so must heat production. Thus, muscle tone and by inference heat production, could be studied in isolation and shown to increase linearly with falling ambient temperature from approximately 32°C to below about 7°C, when overt shivering first appeared. Second, by isolating every movement we could show that periods of rest or sleep are frequently interrupted by brief movements that turn tonically active units on or off, thus partitioning the activity and accompanying heat production among the participating units. Third, by studying the effects of ambient temperatures ranging from 2°C to 36°C, we could show that regulated heat producing muscle tone also occurs within the thermoneutral zone (TNZ) as described.[4,31]

2 | RESULTS

2.1 | Overview

Figure 1 introduces some main results. At 14°C, the rat in Figure 1A curled up with hairs erected and legs tucked under the body to conserve heat. The SOL displayed continuous
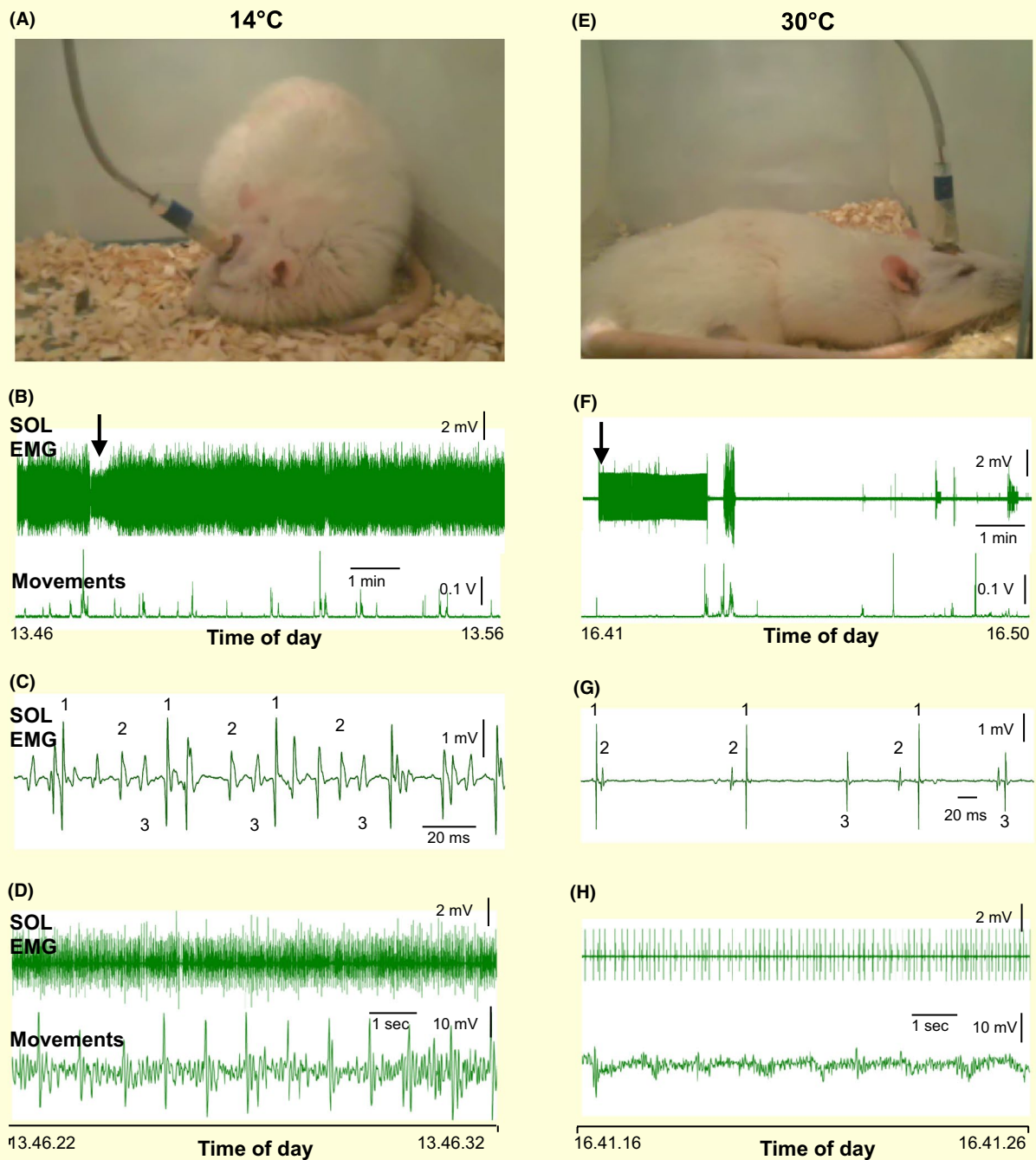
FIGURE 1 Ambient temperature affects posture and motor unit activity in SOL. A, Posture during sleep at 14°C. B, Raw EMG from SOL (upper trace) and movements (lower trace) with arrow indicating time of picture in (A) and expanded traces in (C) and (D). The lower trace in B is the signal from the force transducer under the floor after DC removal and processing as RMS amplitude. C, Three motor units (1,2,3) fired tonically at 24.6 Hz, 25.1 Hz and 24.2 Hz (mean values). D, Upper trace shows raw EMG. Lower trace shows amplified recording of movements (raw force signal after DC removal), the high amplitude signals representing cycles of respiration (mean frequency 1.3 Hz) and the high frequency signals in between "microvibrations" presumably from tonic motor unit activity. E-H, As in (A-D), but at 30°C. The three units in (G) fired at 7.1, 9.9 and 7.0 Hz (mean values) and mean respiration frequency in (H) was 0.9 Hz

motor unit activity and the rat made many brief movements throughout the recording period when it appeared to be sleeping (Figure 1B). The EMG activity consisted of single

motor units firing tonically at around 25 Hz (Figure 1C) and high amplification of the movement record (Figure 1D, lower trace) revealed respiratory movements (average rate

1.3 Hz) and high frequency "microvibrations" resembling mechanomyographic recordings[32] (see Introduction). In contrast, at 30°C the same rat lay flat on the floor (Figure 1E), the SOL generated much less tonic activity (Figure 1F) at lower motor unit firing rates (7-9 Hz for the units shown in Figure 1G). In addition, the average respiratory rate was lower (0.94 Hz) and the microvibrations less prominent as expected from the reduction in tonic motor unit firing (Figure 1H).

2.2 | Measuring tonic motor unit activity

The experiments were done during the day when the rats were mostly at sleep. Figure 2A (upper trace) shows raw EMG from SOL during one period of apparent sleep as assessed by video monitoring. It illustrates our method for separating periods of movements from periods of no movements using a load sensitive floor. During this period (14 minutes) several brief movements (lower trace) started or stopped the flat segments seen in the rectified integrated EMG (middle trace). Letters a, b, and c point to three segments, expanded in Figure 2B and again in Figure 2C to show the identity of individual motor units (1-7). These units fired tonically at rates shown in Figure 2B and produced the flat segments a, b and c in Figure 2A. Evidently, the brief movements turned tonic firing of many motor units on or off, resulting in mostly different motor units generating the flat segments between the movements (see below).

Off line on stored recordings, cursors were placed sequentially at the onset and termination of each movement (Figure 2A, 0-9). The average EMG activity during each move (M) and rest (R) period was then measured as the mean root mean square of the EMG activity between cursors (mean RMS EMG). The entire duration of every recording session that usually lasted 4-6 hours was analysed in this way.

Figure 2D from a different experiment further illustrates the relative ease with which several tonically active motor units can be isolated and their firing rate determined during rest or sleep using EMG electrodes. In addition, it shows the presence of such tonic activity at high ambient temperature (36°C).

2.3 | Tonic motor unit activity in SOL during rest or sleep

In a typical experiment, increasing the ambient temperature caused a marked decrease in SOL motor unit activity (Figure 3A). In Figure 3B, the decrease is shown for every consecutive rest or sleep period (no movement, middle trace), as described above and as moving averages (lower trace). The decrease in tonic motor unit activity followed closely the increase in temperature, suggesting a graded relationship with ambient temperature.

Simultaneous recordings from right and left SOL in a different rat exposed to three different ambient temperatures (13°C, 23°C and 30°C) on three different days confirmed this result. Both SOL muscles were much more active at 13°C (Figure 3C) than at 30°C (Figure 3D). The distributions of the amounts of motor unit activity in the absence of movements (rest or sleep) at the three temperatures appear in Figure 3E,F with arrows pointing to median values. These values decreased linearly with rising ambient temperature, reaching zero at approximately 32°C in both the right and the left SOL (Figure 4A, triangles). Similar linear decreases occurred in two other rats (red and blue symbols), in which the amount of tonic motor unit activity during rest or sleep was measured at nine or five different ambient temperatures on different days. The slopes are different because different EMG electrodes resulted in different amplitudes of the EMG signal. Similar decreases in SOL tonic motor unit activity in the absence of movements occurred in four other rats (Figure 4B).

2.4 | Brief movements turn tonic activity on or off

The rats were resting or sleeping (no movements) between 75% and 90% of the time at temperatures above 15°C. Below 15°C they became more active (Figure 5A). The movements that interrupted periods of rest or sleep occurred approximately 60 times per hour at temperatures above 15°C (Figure 5B) and were mostly brief (Figure 5C, median durations 2-3 s). They were usually associated with brief inhibitions or facilitations of tonic motor unit activity (Figure 6A-C). The distributions of the time intervals between movements were strongly skewed (Figure 6D,E). Since the time intervals between adjacent events in a Poisson point process have a negative exponential distribution with equal mean and standard deviation,[33] we examined the distributions of time intervals between movements in several recording sessions from a single animal at one temperature on one day. The mean and standard deviation varied between individual recording sessions but were only 5-10% different from each other within any single recording session, indicating that brief movements occurred at random time points.

Figure 7A shows one movement at an ambient temperature of 23°C that in the right SOL turned one unit off (1) and in the left SOL turned three units off (1-3) and five units on (5-9). More generally, when measured just before and after each move over one hour of recording, the amount of tonic activity usually either increased or decreased, reflecting respectively, recruitment or de-recruitment (Figure 7B, blue and grey lines). The total number of recruitments or de-recruitments after movements was similar in right and left SOL during this time period (Figure 7B). However, for a given movement, recruitment on one side frequently
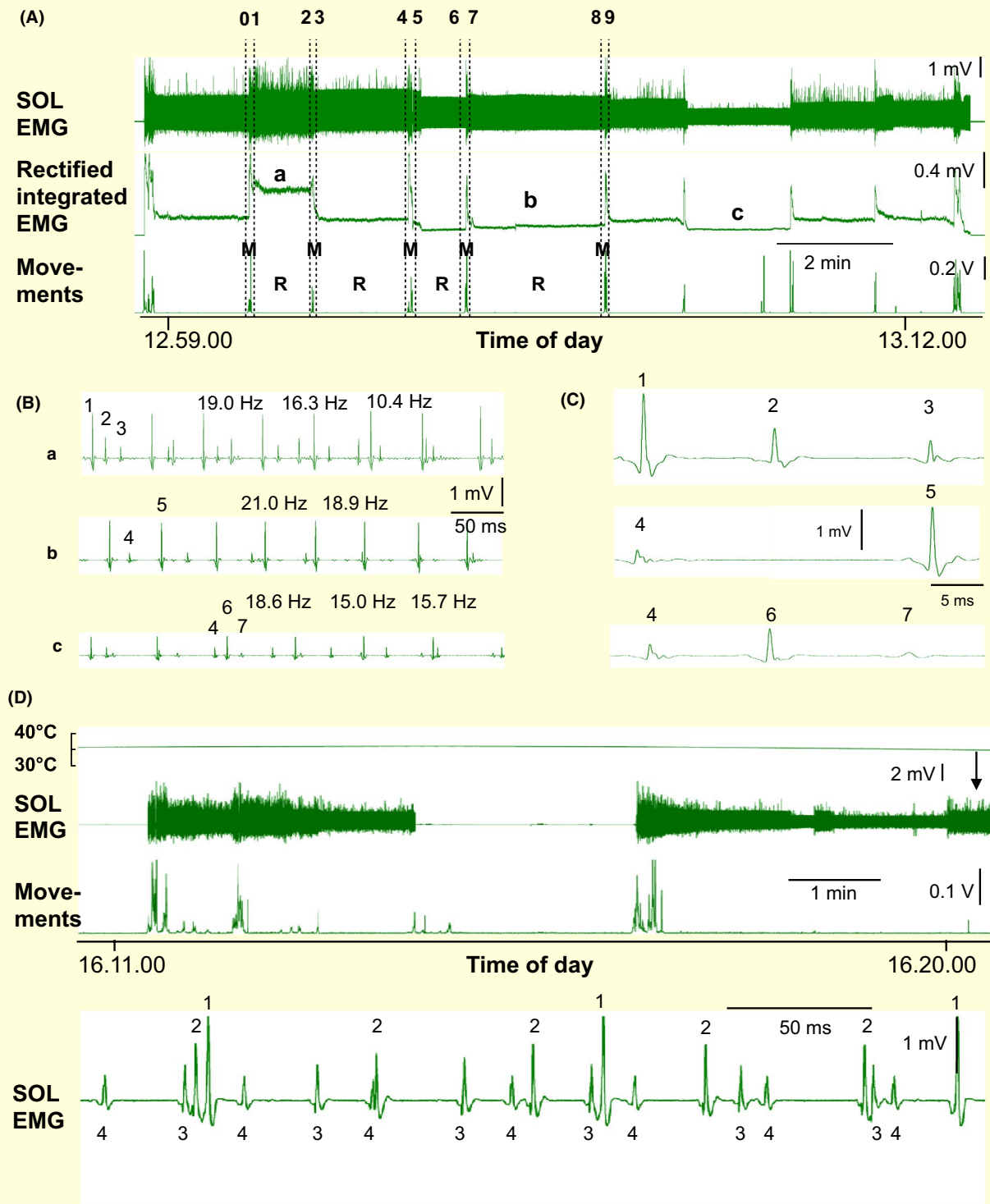
FIGURE 2 Detecting tonic motor unit activity in absence of movements. A, Raw EMG from SOL (upper trace), rectified and integrated EMG (middle trace, see Materials and methods). Ambient temperature was 13°C. M and R indicate respectively, five movements separated by four rest periods. Ten cursors (0-9) were placed by visual inspection at the start and end of each movement. B, Tonic motor unit activity during the flat EMG segments indicated by a, b and c in (A), numbers point to different motor units with firing rates as shown. C, Motor units in (B), expanded to show their identity. D, Raw EMG (upper trace) and associated movements (2rd trace) in SOL at 36°C in a different rat. Lower trace is an expansion of the EMG signal at time of arrow showing four motor units firing tonically at 10.0 Hz (unit 1), 18.2 Hz, (unit 2), 21.3 Hz (unit 3) and 22 Hz (unit 4)
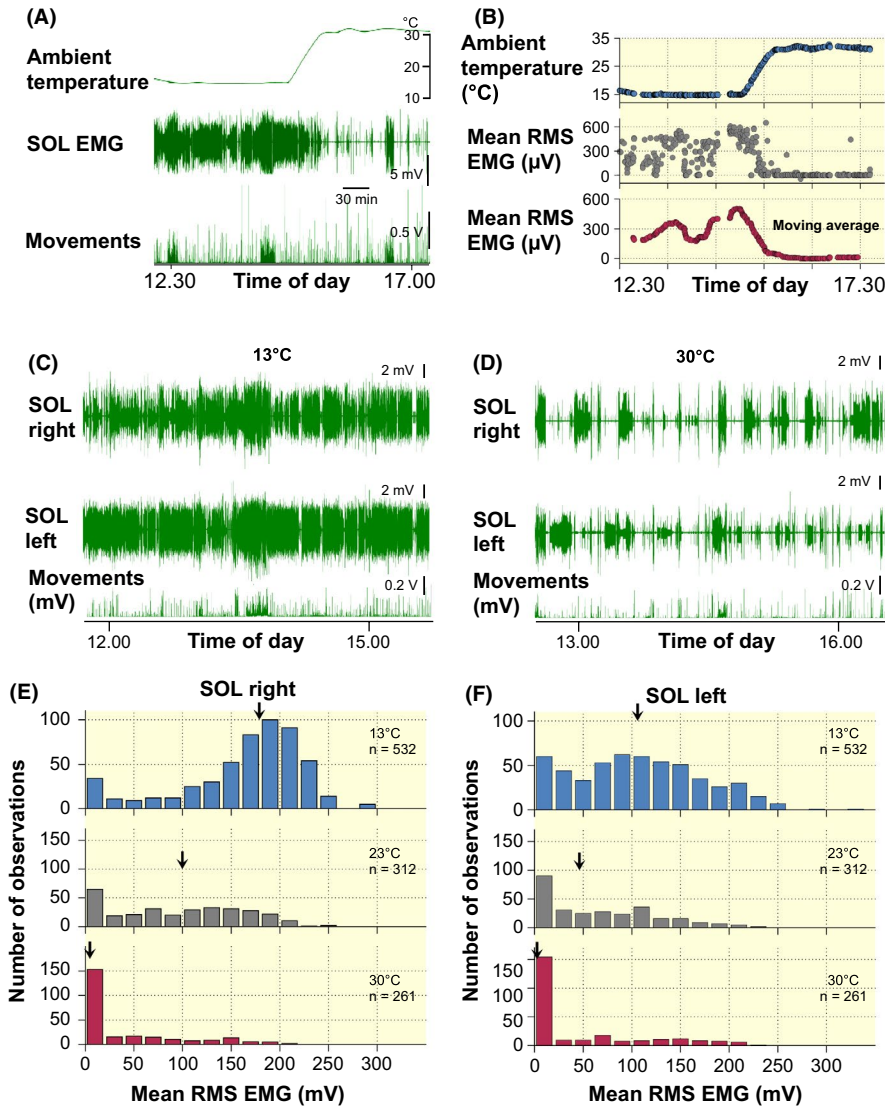
FIGURE 3 Tonic motor unit activity in SOL at different ambient temperatures. A, Rise in ambient temperature (upper trace) accompanied by reduced EMG activity in SOL (middle trace). B, Data from each consecutive rest or sleep period in (A) showing ambient temperature (upper trace) and motor unit activity (middle trace). Lower trace shows moving average of data in middle trace (20 consecutive rest or sleep periods). C, EMG recorded at 13°C from right and left SOL with associated movements (lower trace). D, As in (C), but at 30°C on a different day in the same rat. E and F, Distributions of motor unit activity during consecutive rest or sleep periods in right and left SOL of the same rat on three different days at the indicated temperatures, arrows point to median values

occurred together with de-recruitment on the other side (Figure 7C), suggesting a high degree of independence between the two sides.

Brief movements turned tonically active motor units on or off also at low (13°C) and high (30°C) ambient temperatures (Figure 8; see also Figure 2, which shows data from another rat). Thus, the power of brief movements to turn tonic motor unit firing on or off appears robust and reproducible.

2.5 | Duration and number of periods with tonic motor unit activity

Changes in ambient temperature also affected duration and number of periods with tonic motor unit activity in SOL. This was studied in an earlier separate series where 4-5 hours of continuous EMG recordings were obtained during spontaneous behaviour in a climate chamber without force platform and video recording. The recordings were obtained at four

different temperatures (Figure 9), one temperature per day in strict increasing or decreasing order for every other animal. The integrated rectified EMG signal revealed several nearly flat segments (cf. red arrows in Figure 9A), interpreted as resting periods with tonic motor neuron firing and briefer higher-amplitude episodes interpreted as movements. Periods with tonic firing appeared less prevalent with rising temperatures (Figure 9A). The start and stop of each flat EMG segment during each recording period was identified using a custom-developed computer algorithm and the effect of ambient temperature on their number, individual and summed duration evaluated in linear mixed models with animal as random effect. The summed duration of individual flat segments increased linearly with falling ambient temperature (Figure 9B), as did the number and duration of individual segments (see legend to Figure 9B).

Several of the flat EMG segments were studied in detail. An expansion of the flat segment underneath the black arrow in Figure 9A is shown in Figure 9C, upper trace. It was caused
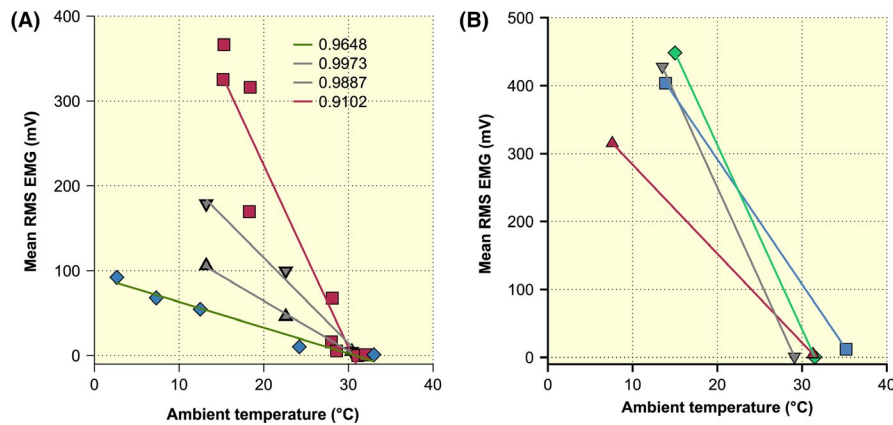
FIGURE 4 Tonic motor unit activity in SOL increases linearly with decreasing ambient temperature. A, Tonic motor unit activity (median values) during rest or sleep at different ambient temperatures in four SOL muscles from three different rats recorded at three or more different temperatures, insets show the associated correlation coefficients. Triangles represent the right and left SOL in Figure 3C-F. B, Median values of tonic motor unit activity in SOL muscles from four other rats recorded at two different temperatures

FIGURE 5 Rest or sleep dominates during daytime and is frequently interrupted by brief movements. A, Summed duration of all rest or sleep periods as a percentage of total recording time. Each symbol represents one experiment and each colour represents one animal. B, Number of movements per hour at different ambient temperatures. Each symbol represents one experiment and each colour represents one animal (the same animal as in (A). C, Distribution of individual movement durations at different ambient temperatures, excluding 31, 67 and 80 movements (top to bottom) lasting longer than 50 s. Arrows point to median durations. The data are based on 16, 15 and 10 recording sessions in four rats. Insets show ambient temperatures and number of movements (n)
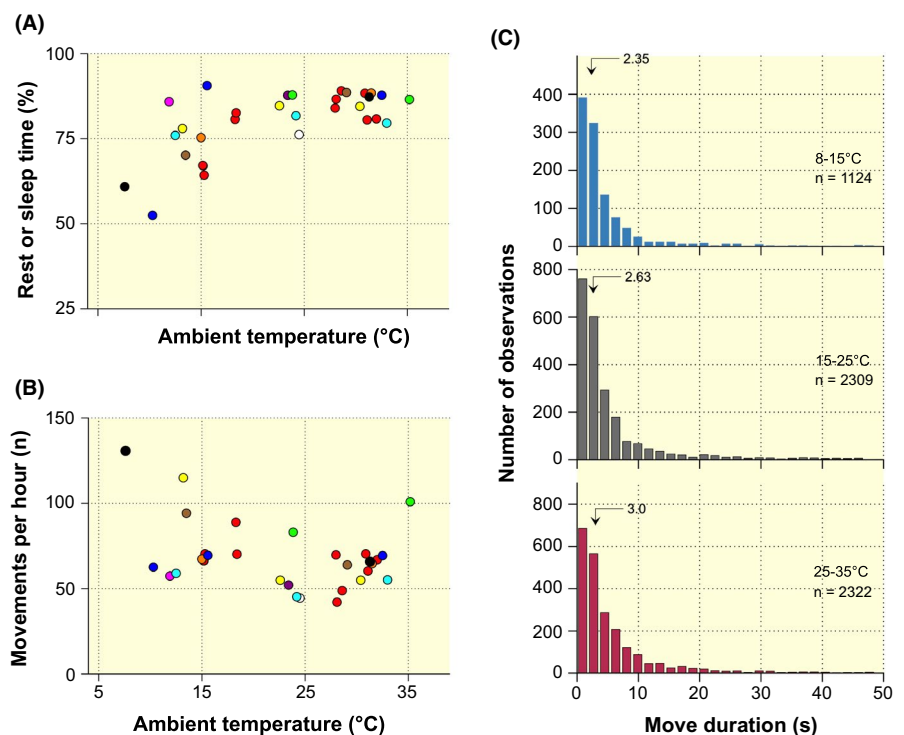
by three motor units, which were recruited in rapid sequence to fire tonically at 20-25 Hz (Figure 9C, coloured symbols represent individual action potentials). Similar recruitment to tonic firing resulted in other flat segments (not shown).

2.6 | Motor unit activity in muscles other than SOL

SOL is rarely studied in relation to body temperature regulation. We therefore compared its motor unit activity with that in other muscles. Recording simultaneously from SOL and extensor digitorum longus (EDL), we observed substantially more

tonic activity in both muscles in cold environments compared to warm environments (Figure 10A,B). Detecting tonic activity in EDL required much higher amplification than in SOL, as expected because slow fatigue resistant motor units in EDL have relatively few, small sized type 1 muscle fibres.[34,35] In EDL, as in SOL, the amount of tonic motor unit activity at rest or sleep changed in parallel with the ambient temperature (Figure 10C), confirming that tonic motor unit activity responds to changes in ambient temperature in a graded manner.

Figure 10D,E shows multiple EDL motor units firing tonically at high ambient temperature. Figure 10E is an expansion of the traces in (D) at the time of the red arrow. At this temperature
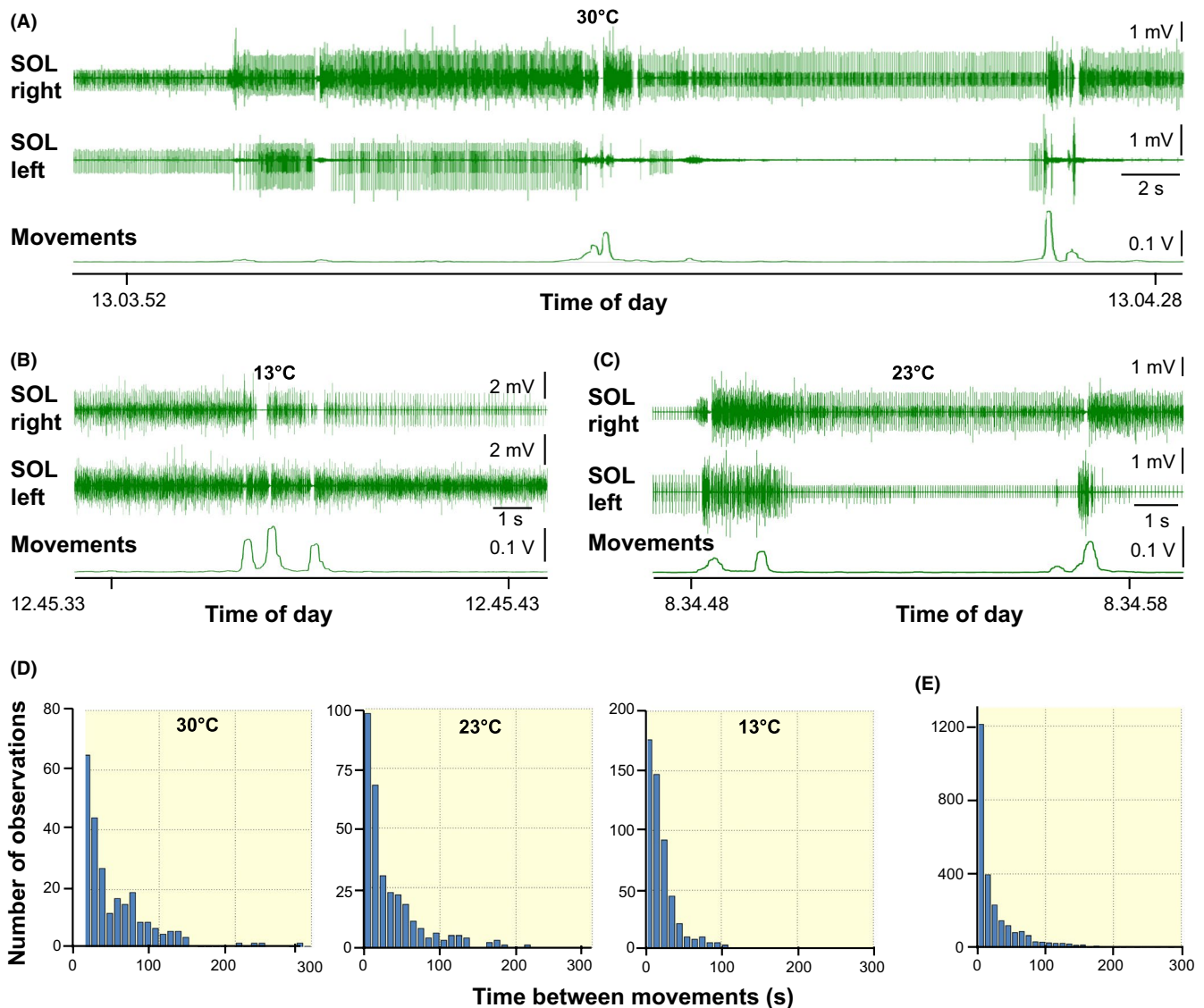
FIGURE 6 Brief movements briefly inhibit or facilitate motor unit activity in SOL. A,B,C, EMGs from right and left SOL during rest or sleep in a single rat on different days and at different ambient temperatures showing tonic firing in several motor units. At the time of brief movements (lower traces), motor unit activity was briefly inhibited or facilitated and many units started or stopped firing tonically. D, Histograms showing distributions of intervals between movements for the entire length of experiments partially represented in (A,B,C). E, As in (D) but based on nine experiments in seven rats irrespective of ambient temperature

the trains of tonic firing were brief and often started or stopped by brief movements or they stopped spontaneously without any relation to movements. Lower trace in Figure 10E displays movements arising from respiration (mean rate 0.9 Hz) and microvibrations resembling mechanomyographic recordings.[32]

Deep parts of tibialis anterior (TIBd) also displayed tonically firing motor units at different ambient temperatures ranging from below 10°C to at least 32°C. The units were often turned on or off by brief movements (Figure 11A,B). In contrast, we never recorded tonic motor unit activity in superficial parts of TIB (TIBs, Figure 11A, n = 3), nor in bilateral recordings from lower superficial parts of trapezius (not shown).

Like EDL and TIBd, deep neck muscle (Neck) displayed large amounts of tonic motor unit activity at low ambient

temperatures which decreased as the temperature increased (Figure 11C). In this case, overt shivering at temperatures below approximately 7°C (red line in Figure 11C) obscured any concomitant tonic activity. As in other deep muscles, brief movements briefly facilitated or inhibited motor unit activity and turned tonic motor unit activity on or off, also at high ambient temperatures (Figure 11D,E).

2.7 | Firing rates of tonic motor units increase in cold environment

Increases in the ambient temperatures, as in Figure 12A, decreased tonic motor unit firing rates, as shown for a SOL muscle in Figure 12B. Similar decreases in firing rates were
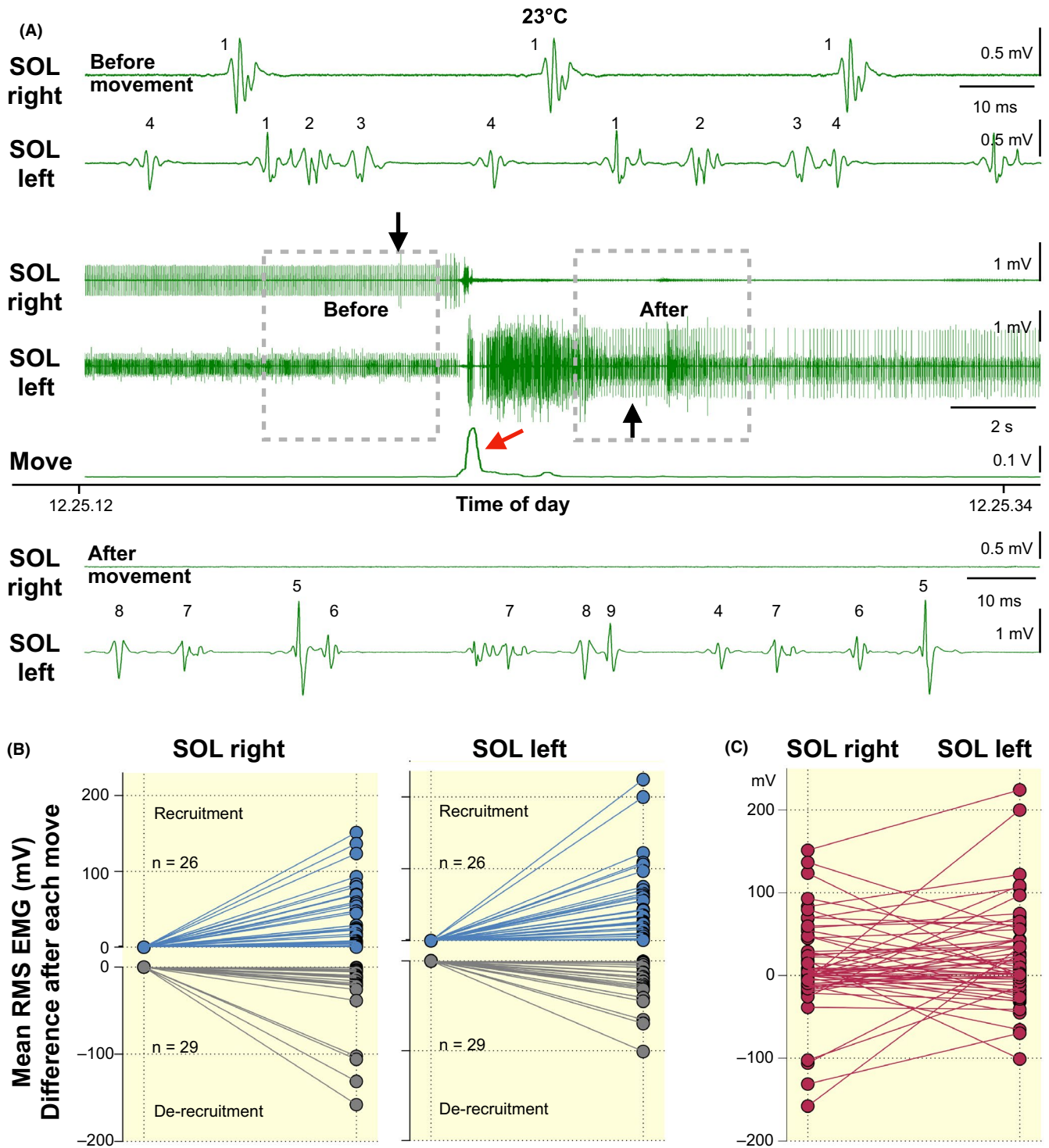
FIGURE 7 Brief movements turn tonic motor unit activity in SOL on or off. A, Tonic motor unit activity in right and left SOL before, during and after a brief movement (red arrow), upper and lower panels showing respectively, expanded traces before and after the movement at times of black arrows. In SOL right, the movement turned one unit (1) off. In SOL left, the movement turned three units off (1, 2, 3) and five units on (5, 6, 7, 8, 9), whereas one unit (4) fired both before and after the movement. B, Blue and grey lines connect respectively, the increase or decrease in motor unit activity after each movement in right and left SOL during one hour of recording, all measurements scaled to zero before each movement. The motor unit activity (mean RMS EMG) was measured during 4 sec of recording before and after each movement (dotted rectangles in [A]). Each line represents recruitment (blue lines), de-recruitment (grey lines) or no clear difference (horizontal or near horizontal lines) either because the movement had no effect or because it turned one or more units off and others on, resulting, by chance, in nearly the same amount of activity before and after the movement. Total number of movements was 59, with only one movement resulting in zero difference (SOL right). C, Red lines connect the effect of each movement (recruitment or de-recruitment) on right and left SOL
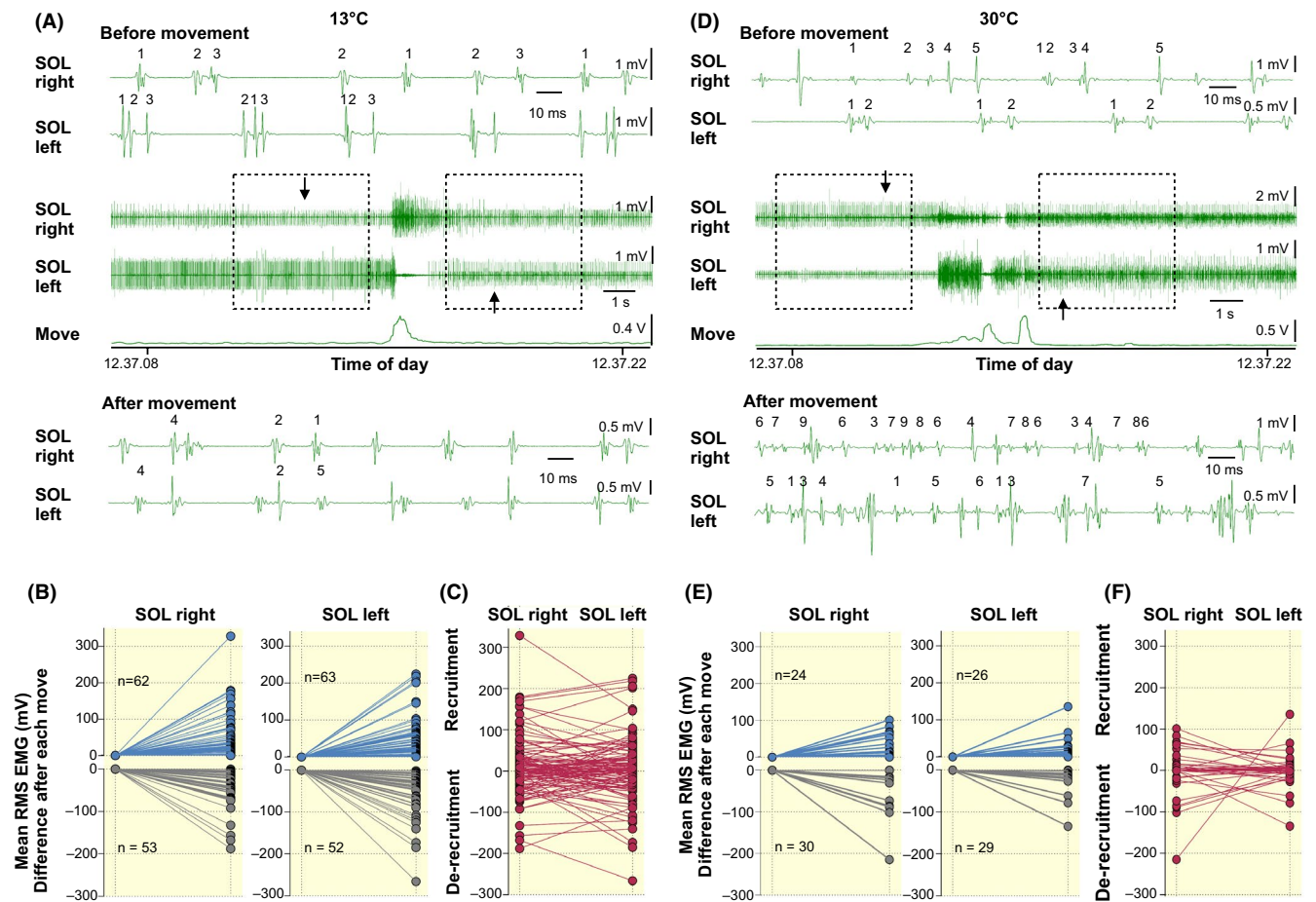
FIGURE 8 Brief movements turn tonic motor unit activity on or off at both 14°C and 30°C. A, Recordings from the same rat as in Figure 7 but at 13°C on a different day, showing tonic motor unit activity in right and left SOL before, during, and after a brief movement. Upper and lower panels show respectively, expanded traces before and after the movement at time of black arrows. In right SOL, the movement turned one unit off (3) and another unit on (4), while two units (1, 2) fired both before and after the movement. In left SOL, the movement turned two units off (1, 3) and two units on (4, 5), while one unit (2) fired tonically both before and after the movement. B, Blue and grey lines connect respectively, the increase or decrease in motor unit activity after each movement in right and left SOL during one hour of recording, all measurements scaled to zero before each movement (see legend to Figure 7 for further explanations). Total number of movements was 115. C, Red lines connect the effect of each movement (recruitment or de-recruitment) on right and left SOL. D,E,F, As in (A,B,C) but on a different day and ambient temperature 30°C. In right SOL in this case, the movement turned three units off (1, 2, 5) and four units on (6, 7, 8, 9), while two units continued firing tonically after the movement (3, 4). In left SOL, the movement turned one unit off (2) and 5 units on (3, 4, 5, 6, 7), while one unit (1) continued firing tonically after the movement. Total number of movements was 55

obtained in six of seven SOL examined in this way, as well as in EDL (Figure 12C), Neck (Figure 12D) and TIBd (Figure 12E). The tonic firing rates of SOL, EDL, TIBd and Neck are summarized in Figure 12F irrespective of ambient temperature. The histograms show that SOL motor units fire tonically at lower average rates (19.9 Hz) than units in EDL, TIBd and Neck and confirm the large overlap in firing rates within and between muscles seen in the histograms in Figure 12B-E.

An unavoidable bias in sampling occurred in these experiments. At low ambient temperatures, the large number of simultaneously active motor units often made it impossible to identify individual units and the analysis had to be restricted to the relatively infrequent periods when the number of simultaneously active units stayed below at most six. If

massive motor unit activity in the cold results from a generalized increase in motor neuron plateau potential activity, for example by increased monoaminergic inputs to the relevant motor neurons (see Discussion), then the driving effect of a cold environment on motor unit firing rate would be underestimated because of the need to select periods with less such activity for analysis of single motor units.

2.8 | Shivering

The ambient temperature was lowered to below 10°C in five rats. Bouts of shivering appeared in all of them but not before the ambient temperature reached approximately 7°C. During shivering (red lines in Figure 13A-C) the rat stayed in one
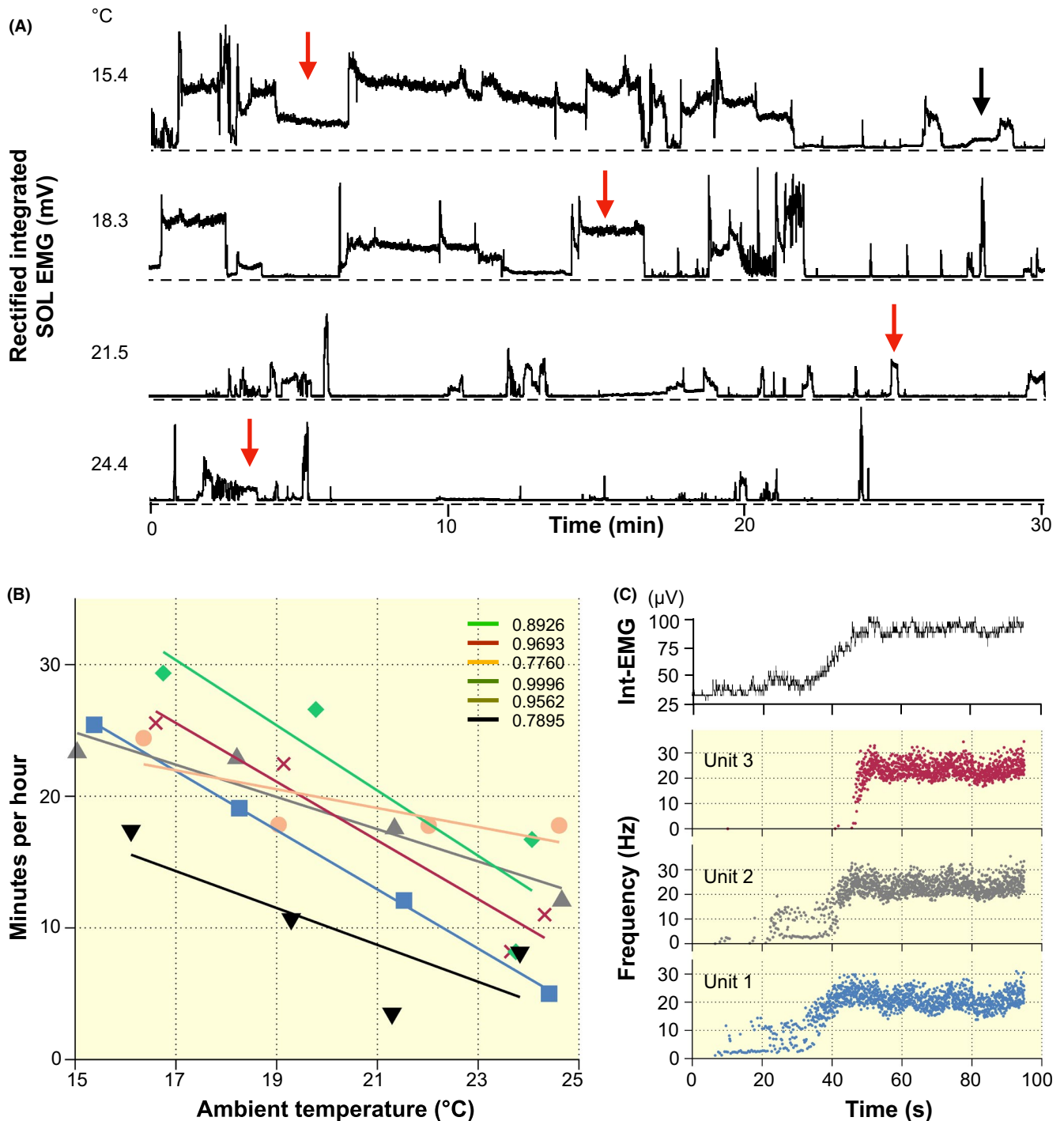
FIGURE 9 Periods of tonic motor unit activity decrease in duration and number with rising ambient temperature. A, Integrated rectified EMGs from SOL at different ambient temperatures on different days in the same rat, showing many nearly flat segments (red arrows point to one such segment at each temperature). B, Decrease in summed durations of flat EMG segments with rising ambient temperature, 102 s/h/°C (95% confidence interval 72-131), $P < .0001$, six different rats, 4-5 hours of recording per day, each rat subjected to four different temperatures on different days, insets show individual correlation coefficients. Total number of flat segments decreased 5.1/h/°C (95% CI 2.1 - 8.2), $P = .003$ and the 90 percentile duration by 2.0 s/h/°C (95% CI 1.2 - 2.9), $P < .0001$ (not shown). C, Three motor units as indicated begin firing tonically at 20-25 Hz, generating the flat segment (upper trace), which is an expansion of the segment seen under the black arrow in (A). Each coloured symbol represents one motor unit action potential

place, often with eyes closed as if sleeping, making no movements except for body heaves in synchrony with bursts of shivering motor unit activity (Figure 13G).

Shivering was commonly preceded by vigorous, often jerky movements from one corner of the cage to another (brown lines in Figure 13A-C), which correlated with high amplitude EMG
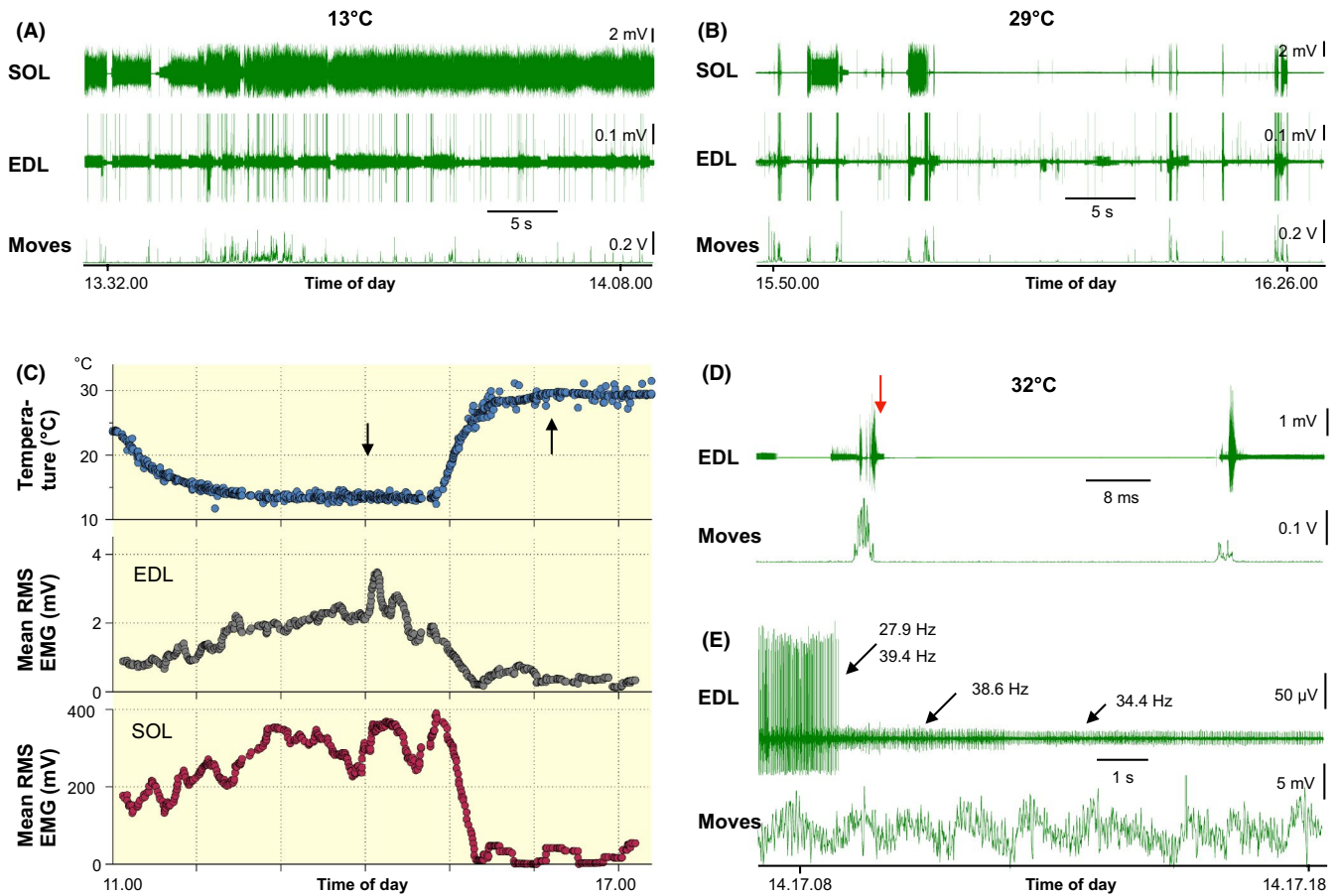
FIGURE 10 Tonic motor unit firing in EDL. A,B, Motor unit activity recorded simultaneously from SOL and EDL at 13°C and 29°C. C, Upper trace shows changes in ambient temperature. Arrows point to when the recordings in (A) and (B) were made. Middle and lower traces show motor unit activity for EDL and SOL as moving averages (20 consecutive rest or sleep periods) throughout the experiment. D, Raw EMG from EDL (upper trace) and associated movements (lower trace). E, Traces in (D) expanded at time of red arrow to show multiple units firing tonically at the indicated rates. Lower traces show respiratory movements and "microvibrations"

signals. The SOL contributed to such movements with bursts of high amplitude motor unit activity, but when shivering started, the activity of SOL motor units shifted to continuous tonic firing that lasted throughout the shivering bout (Figure 13A-C). The bursting pattern in all the shivering muscles appeared strikingly similar (Figure 13D-G). Simultaneous recordings from ipsilateral and contralateral muscles (Figure 13D,E) showed that the bursts were synchronous between muscles on one and the other side of the body. Moreover, large or small burst amplitudes in one muscle generally occurred together with correspondingly large or small amplitudes in the other muscle recorded from at the same time (Figure 13D,E), as further documented in Figure 13H. In contrast, no such correlation appeared between pairs of shivering TIBs and non-shivering SOL in three different rats (Figure 13I).

In these experiments the ambient temperature was lowered from room temperature to 7°C or below within 1-2 hours, kept at those temperatures from 30 minutes to 4 hours and then raised towards room temperature within 1-2 hours. A slower decline in room temperature may well have raised the threshold for overt shivering, perhaps by some form of cold

acclimatization depending on the rate of decline. We note, however, that in one experiment at an ambient temperature of 10°C that lasted 4 hours we did not observe overt shivering.

During shivering, low amplitude tonic motor unit activity filled the "gaps" between the high amplitude shivering bursts in all the deep muscles recorded from (Neck, EDL, and TIBd, Figure 14A). In contrast, no such tonic activity between shivering bursts was observed in superficial muscles (TIBs, Figure 14A lower trace) and caudal parts of the trapezius (not shown). This finding is further documented in Figure 14B,C. The mean RMS EMG activity is high during shivering bursts (violet symbols), intermediate between bursts (green symbols) in TIBd and EDL, but essentially absent in TIBs. When shivering started, the rate of high amplitude motor unit bursts first increased and then decreased before it stopped. In the present experiments, however, we let the temperature rise after only short periods of time below 7°C. Consequently, rising temperature may explain, at least in part, the drop in burst rate towards the end (Figure 14B,C). For the same reason, we do not know the exact duration of the bouts of shivering at low constant temperatures.
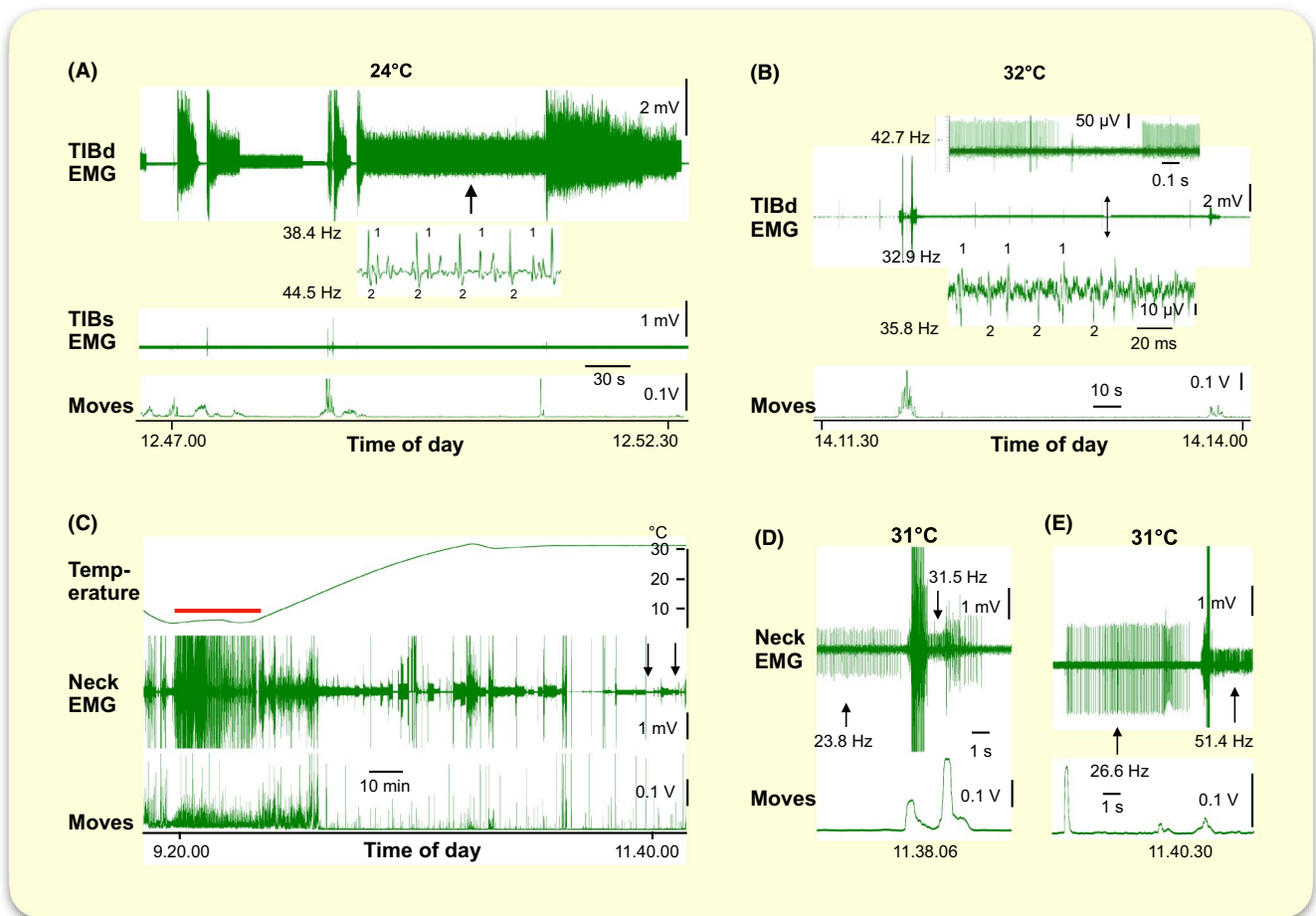
FIGURE 11 Tonic motor unit firing in TIBd and deep neck muscle but not in TIBs. A, EMG from TIBd (upper trace) and TIBs (middle trace) show tonic motor unit activity in TIBd but not in TIBs. The expanded trace at the time of the arrow (inset) shows several units in TIBd firing tonically at frequencies indicated for two of them (1, 2). B, EMG from TIBd in a different rat at 32°C shows several motor units firing tonically at frequencies shown in the insets, which are expanded traces at the time of the arrows (note different timescales and amplifications). C, Changes in ambient temperature (upper trace, red horizontal bar represents overt shivering), EMG activity in a deep neck muscle (middle trace) and associated movements (lower trace). D,E, Expanded traces at the time of the arrows in (C) show motor units firing tonically, arrows pointing to four periods of tonic activity by different motor units with mean firing rates as indicated

A different type of shivering appeared in two of the five rats examined for shivering. Two such periods of shivering are shown for the Trapezius in Figure 15 (below blue horizontal lines in Figure 15A). Each period contained multiple groups of motor unit discharges, each of which (below orange line in Figure 15B) consisted of brief individual bursts of motor unit activity at relatively high frequency (Figure 15C). During such shivering the rat made rapid masticatory movements, as if the teeth were chattering (visible in the video, not shown). Short observation periods at shivering temperatures (≤7°C) may explain their absence in the other three rats.

3 | DISCUSSION

This study deals primarily with motor unit activity in the absence of movements when rats are either resting or sleeping. Muscle fibre contractions are then essentially isometric and all utilized energy is converted to heat. The motor unit activity turned out to be exclusively tonic and therefore equivalent to muscle tone as defined here (see Introduction). It was present at ambient temperatures as high as 36°C and increased linearly when the temperature fell from approximately 32°C to 2°C. In contrast, overt shivering, which was also studied, appeared only when a falling temperature reached approximately 7°C.

3.1 | What drives tonic motor unit activity (muscle tone)?

Persistent inward currents (PICs) that arise from complex interactions among several ionotropic and neuromodulatory inputs generate plateau potentials in motor neurons.[36] PICs are self-sustained depolarizations that lead to regular action potential discharges and, consequently, to prolonged and stable motor unit firings, as observed here. The
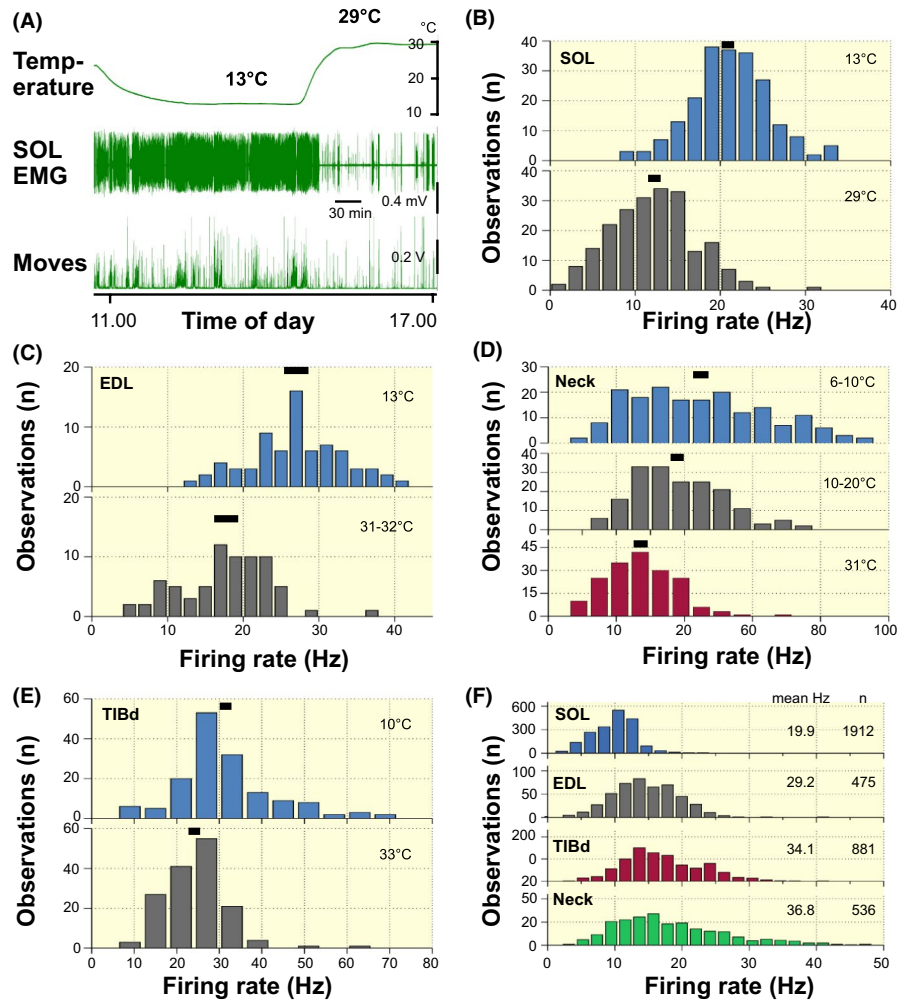
FIGURE 12 Tonic motor unit firing rates increase in cold environments. A, Changes in ambient temperature (upper trace) and accompanying changes in EMG in SOL (middle trace) and movements (lower trace). B, Distribution of motor unit firing rates during different episodes of tonic firing at 13°C or 29°C, as shown in the upper trace of (A), horizontal bars showing 95% confidence intervals. Ten consecutive unit action potentials from each of 22 motor units sampled at each temperature. Some of the sampled units may have been the same, but were always from different episodes of tonic activity. C, As in (B) but sampled from EDL in a different rat at 13°C or 31-32°C, as indicated, 16 units sampled at each temperature. D, As in (B) but sampled from deep neck (Neck) muscle in a different rat at temperatures as indicated, 20 units sampled at each temperature. E, As in (B) but sampled in TIBd in a different rat at the indicated temperatures, 17 units sampled at each temperature. F, Histograms showing the distribution of firing rates in SOL (seven muscles), EDL (three muscles), TIBd (three muscles) and Neck (one muscle), with mean rates and number of spike intervals as indicated

mechanisms that generate the plateau potentials are intrinsic to each motor neuron. Therefore, one expects the tonic firing pattern of any given motor unit to be largely independent of the firing patterns in other motor units, as also observed here, with the exception of the small changes in firing rate that affect multiple motor units in phase with the respiration, as described elsewhere[37] and also seen here. During rest or sleep, brief movements were accompanied by brief inhibitions or facilitations of EMG activity that turned some tonically active motor units off and others on. Similarly, brief excitatory or inhibitory inputs to motor neurons in reduced preparations turn plateau potentials and accompanying tonic firing on or off,[38] as do brief excitatory or inhibitory inputs to motor neurons in unrestrained

behaving rats.[39] From such observations we conclude that only plateau potentials can plausibly account for the tonic activity demonstrated here.

One modulator of plateau potentials, serotonin, is of particular interest. Serotonergic neurons in brainstem raphe nuclei are central to body temperature regulation.[40,41] Such neurons project to the spinal ventral horn, where their axon terminals innervate motor neurons extensively.[42,43] Serotonin strongly facilitates PICs,[36] stimulation in raphe nucleus inferior causes synaptic release of serotonin that promotes plateau potentials in spinal motor neurons[44] and monoamine receptor activation by serotonin and noradrenaline is essential for enabling persistent sodium currents and repetitive firing in rat spinal motor neurons.[45] Cold exposure increases
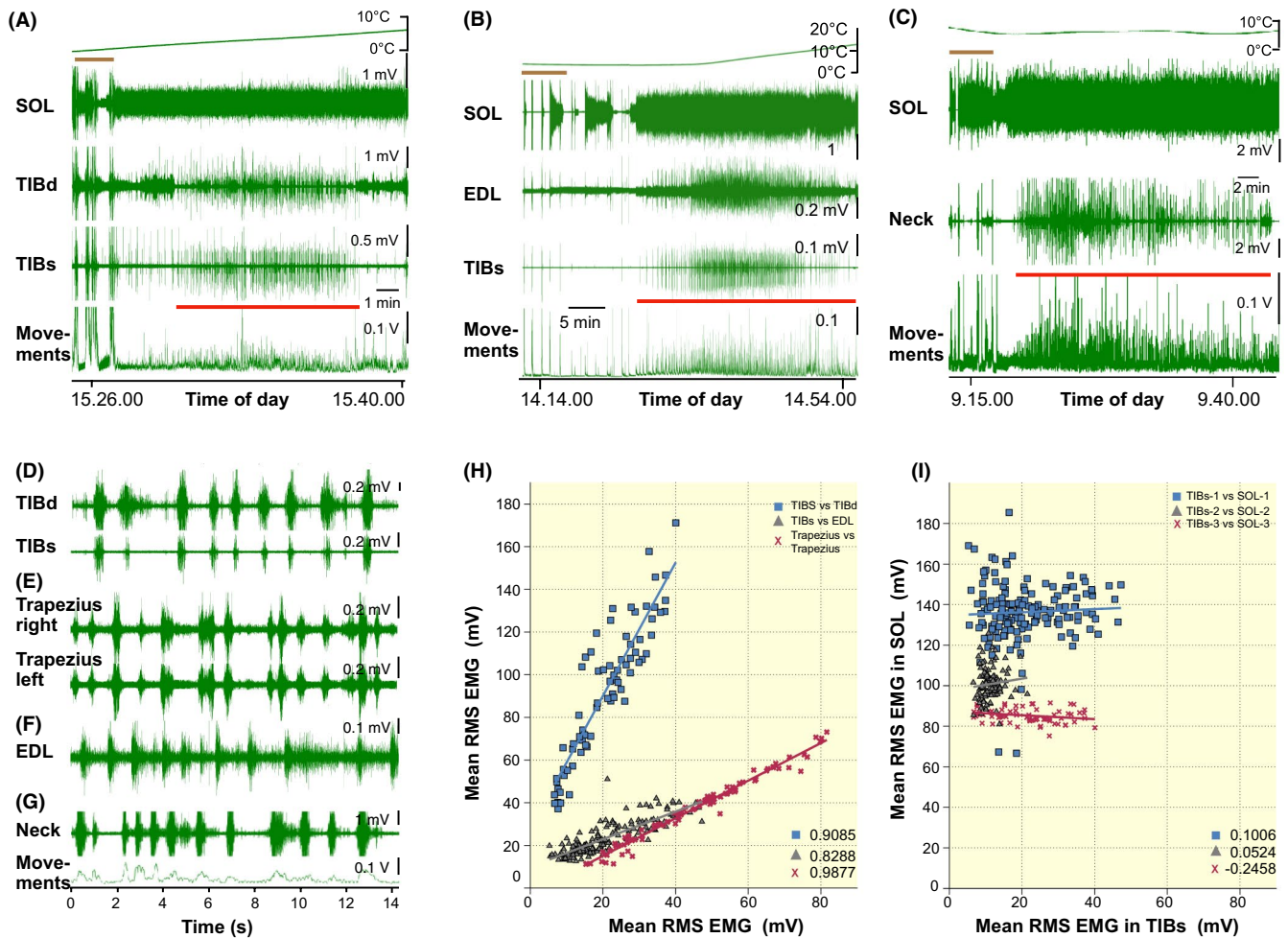
FIGURE 13 Shivering activity in TIBs, EDL and Neck but not SOL. A, Ambient temperature (upper trace) and EMG signals (three middle traces), showing a bout of shivering bursts (above red line) in TIBd and TIBs but not in SOL. Bursts of motor unit activity in SOL, TIBd and TIBs (brown line) correspond to vigorous movements (lower trace) that preceded shivering. B, A bout of shivering in a different rat showing bursts of shivering in EDL and TIBs, but not SOL (other explanations as for [A]). C, A bout of shivering in a different rat showing bursts of shivering in Neck but not SOL (other explanations as for (A)). D, Simultaneous EMG recordings from TIBd and TIBs in the same leg during shivering. E, Simultaneous bilateral EMG recordings from superficial caudal regions of Trapezius. F, EMG recordings during shivering from EDL in a different rat. G, EMG recording during shivering from deep neck muscle (upper trace) in another rat showing that shivering bursts and body movements (lower trace) occurred together. H, Relationships between shivering activity (mean RMS EMG) for each burst in simultaneous bursts in pairs of muscles as indicated, insets show correlation coefficients. I, Similar to (H) but with shivering activity during bursts in TIBs vs. SOL during each TIBs burst for three different rats

the firing rate of serotonergic neurons in medullary raphe nuclei[46] and acute silencing of such neurons causes the body temperature to fall within minutes to near the room temperature.[47] In addition, depletion of spinal monoamines greatly reduces tonic motor unit activity.[48] We therefore propose that falling ambient temperature would increase the amount of heat producing muscle tone by augmenting the release of serotonin onto motor neurons and facilitating plateau potentials. Interestingly, propofol, a commonly used general anaesthetic during surgery, suppresses plateau potential generation in motor neurons by blocking L-type calcium channels,[49] which may contribute to the fall in body temperature during such anaesthesia.

3.2 | Function of tonic motor unit activity in body temperature regulation

Falling ambient temperatures caused increased tonic motor unit activity, owing to motor unit recruitment and increased firing rates in SOL and other deep muscles (EDL, TIBd, Neck). Thus, recruitment and firing rate of tonically active motor units appear to control heat production during rest or sleep, as recruitment and firing rate control force output during voluntary contractions. However, whereas recruitment of motor neurons in voluntary contractions follows the so-called size principle,[50] tonically active, heat producing motor units appeared to be recruited or de-recruited at random.
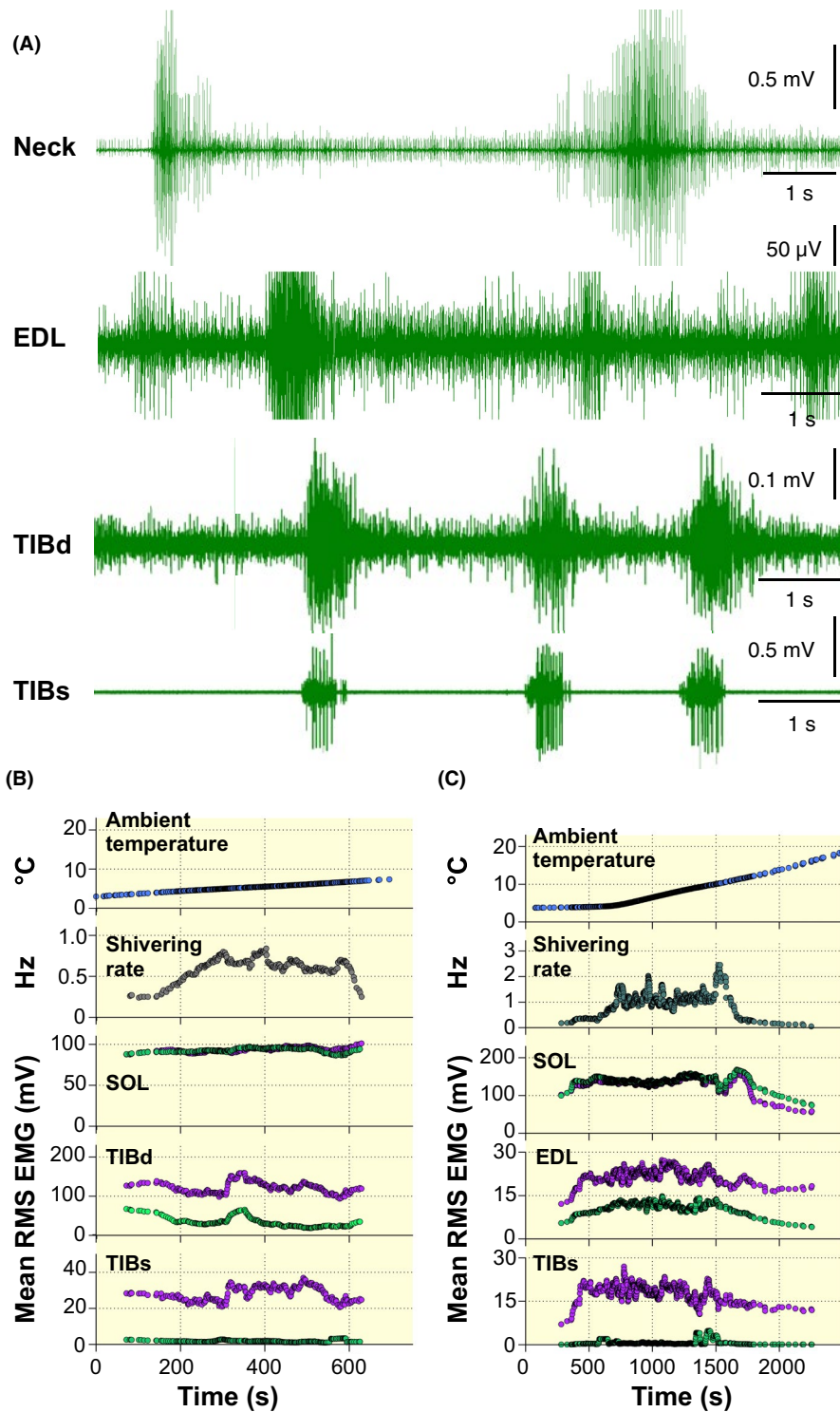
FIGURE 14 Deep but not superficial muscles display tonic activity during shivering. A, Low amplitude motor unit action potentials fill in the gaps between high amplitude shivering bursts in three deep muscles (Neck, EDL, TIBd) but not in superficial TIBs. B, Ambient temperature (upper panel), rate of shivering bursts (2nd panel), mean RMS EMG activity in SOL (3rd panel), TIBd (4th panel) and TIBs (lower panel) during a bout of shivering, violet and green symbols representing respectively, the activity during and between bursts, each symbol presented as moving averages during 20 consecutive bursts. The rate of shivering (2nd panel) was based on the shivering bursts in TIBs but was essentially the same when based on recording from either of the other two muscles. C, As in (B) but from a different rat in which EMG was recorded simultaneously from SOL, EDL and TIBs

3.3 | Function of brief movements during rest or sleep

The brief movements during rest or sleep that commonly turned tonically active units on or off appeared at random by unknown mechanisms. The movements appeared sufficiently frequent for partitioning the tonic activity among the available motor units, such that they can all participate and share in the production of heat and in this way better avoid neuromuscular fatigue. The phenomenon appears similar to the "rotation of motoneurons" that occurs in humans during prolonged isometric contractions, possibly also to "offset neuromuscular fatigue".[51]

3.4 | SOL differs from other muscles

In adult rats, SOL muscles are essentially homogeneous, consisting almost exclusively of type 1 fibres that differ from type 1 fibres in heterogeneous muscles by generating slower

FIGURE 15 A second type of shivering in deep muscles. A, Brief bouts of shivering (below blue lines) in right and left trapezius muscles. B, EMG traces below blue line b in (A) expanded to show that the brief bouts consisted of repetitive synchronous groups of discharges in both muscles. C, EMG traces below grey line c in (B) expanded to show that each group of discharges in (B) consisted of subgroups of repetitive synchronous discharges in both muscles (mean frequency 4.6 Hz)
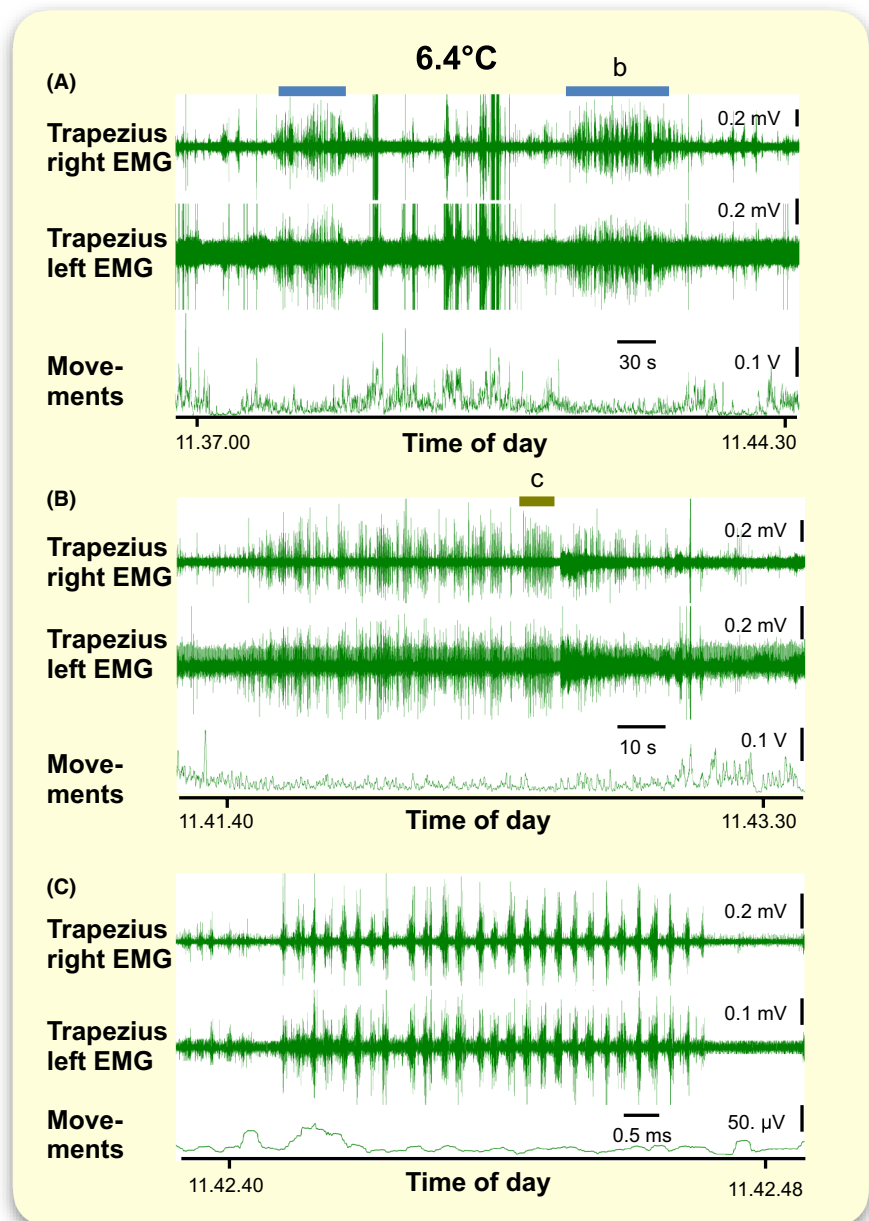
twitches, firing at slower rates and being particularly fatigue resistant.[34,35,52,53] Containing almost exclusively type 1 fibres, SOL did not generate the bursts of motor unit activity observed in EDL, TIBd, TIBs, Trapezius and Neck during overt shivering (see below). However, SOL and other muscles containing type 1 fibres are similar in that they generally lie deep in the leg and, as shown for the SOL, have a high number of capillaries around the muscle fibres and a high rate of blood perfusion.[54] Accordingly, we propose that muscle tone in SOL and other deep muscles will heat nearby venous blood from colder peripheral tissues before it returns to the body core. The striking linear relation between tonic motor unit activity and ambient temperature implies that small changes up or down in ambient temperature will lead to comparably small changes down or up in aggregate heat

producing slow motor unit activity. Thus, we see this relationship as a means to fine tune the body temperature around its set point across a large range of ambient temperatures.

Generally, muscle tone in extensor muscles such as the SOL is thought to be important for supporting postures of the body against the force of gravity. However, during rest or sleep such function seems of lesser importance in rats except in cold environments when muscle tone underpins postures that conserve heat, as in Figure 1A where the rat had curled up and brought its legs underneath the body both to support the body and avoid their exposure to cold air. In this situation, muscle tone has the double function of producing heat and supporting a heat conserving posture.

In medical practice, muscle tone is difficult to define and assess.[55] As argued below, tonic motor unit activity likely

plays similar roles in body temperature regulation in rats and humans. Accordingly, if patients are examined in situations when they feel cold, tonic activity and accompanying resistance to passive movements in SOL and other deep muscles is likely to be relatively high. Without knowing that muscle tone depends strongly on ambient temperature, as demonstrated here, increased tone in such muscles may be misjudged.

3.5 | Tonic motor unit activity in muscles other than SOL

All other deep muscles that we recorded from (TIBd, EDL, Neck) displayed tonic motor unit activity during rest or sleep. Low action potential amplitude and prolonged firing indicated that the motor units were of the slow and fatigue resistant type. Presence of tonic activity at ambient temperatures above 30°C and increased firing rate and amounts of activity with falling ambient temperature indicated participation in body temperature regulation across a large range of ambient temperatures. No tonic activity occurred in the two superficial muscles recorded from (TIBs, distal superficial parts of Trapezius), indicating that such activity is restricted to deep lying muscles or parts of muscles, assisting SOL and presumably other deep muscles in heat production as needed. Also, in these muscles, brief movements frequently turned tonic activity on or off and briefly excited/facilitated or inhibited motor unit activity.

3.6 | Contribution to heat production by tonic motor unit activity

Tonic motor unit activity during rest or sleep evokes isometric contractions that produce heat. We show here that when the ambient temperature falls, the amount of tonic activity increases progressively. Consequently, heat production also increases progressively. We find it likely that this heat production contributes substantially to body temperature regulation for the following reasons. First, the tonic activity occurred not only in the soleus and the three other deep muscles that we recorded from, but surely also in other heterogeneous deep muscles with type 1 fibres. Second, in cold environment, the recorded tonic motor unit activity was massive in the SOL muscle as a whole and nearly continuous for tonically active units in the other deep muscles. Third, because these experiments were done on adult rats acclimatized to room temperature (~23°C), the contribution by BAT thermogenesis was probably relatively small in view of the large amount of muscle tissue in question.

Quantitative data on the relative contributions of BAT and muscle tissue to cold-induced metabolic responses have been obtained in humans.[56] In this work, total glucose uptake is reported to increase 42-fold in skeletal muscle compared to BAT under conditions of minimal whole body shivering and

with most of the EMG activity occurring in deep lying muscles in the neck, back and inner thigh.[56] Thus, approximately 47% of glucose turnover occurred in muscle compared to 1% in BAT, skeletal muscle representing ~42% of total body wt and BAT ~1%.[56] In shivering humans, "burst shivering represents only <10% of total shivering activity", the remaining 90% representing "continuous low-intensity shivering"[57] or what we here call muscle tone.

Studies in humans reveal striking similarities in tonic motor unit behaviour to those described here for rats. In human gastrocnemius muscles, brief excitatory inputs to their motor neurons induce prolonged tonic firing apparently from recruitment of motor neuron plateau potentials.[58] During sleep in humans, the rectified integrated EMG from upper trapezius muscles shows multiple flat segments, like those observed here in rat (Figures 2 and 9) and also in humans such segments appear to be turned on or off by brief movements[59] (see their Figure 1A). In humans exposed to 0°C for 30 minutes, EMG recordings from SOL show much tonic activity between shivering bursts[15] (see their Figure 2), as seen here in rats. Thus, it appears that mucle tone may contribute substantially to body temperature regulation also in humans.

3.7 | Shivering

We only observed shivering when a falling ambient temperature reached approximately 7°C. Bouts of shivering occurred in all deep and superficial muscles recorded from except SOL, which was as active between the shivering bursts as during them, suggesting continuous tonic activity throughout the bouts. In contrast, in the other deep muscles recorded from (TIBd, EDL, Neck), each shivering burst coincided with high amplitude motor unit discharges, whereas in between the bursts, the activity consisted of low amplitude tonically firing motor units. In further contrast, in both superficial muscles recorded from (TIBs, caudal part of Trapezius), motor unit activity only occurred during the shivering bursts as high amplitude bursts of activity. The patterns of motor unit activity recorded here during overt shivering are strikingly similar to the patterns recorded by surface EMG electrodes over multiple muscles in humans exposed to 0°C for 30 minutes.[15] The burst rates, however, were higher in rats (0.6-1.0 per second) compared to humans (0.1-0.2 per second). Although most SOL motor units in these experiments in humans displayed both shivering burst activity and tonic activity in between the bursts, burst activity was lacking in two of 11 subjects, as in the rats observed here.

Reflex mechanisms are often described as central to shivering. Instead, we propose that it might be fruitful to look for central pattern generators in the spinal cord that turn tonic inputs into rhythmic outputs similar to those that drive locomotion, but with synchronous rather than reciprocal activation of motor neurons on each side of the body. Overt shivering in

our material occurred as simultaneous bouts of repetitive and synchronous bursts of motor unit activity of similar duration and correlated amplitudes in several muscles on one side of the body and on both sides of the body in the case of trapezius where bilateral recordings were made. In addition, during the bouts, the bursts in each muscle underwent parallel changes in both rate and amplitude of activity. Such observations strongly suggest that a central pattern generator drives the shivering. A "shivering pathway" running from the hypothalamus to the spinal cord has been described in cats based on lesions, electrical stimulation and single unit recordings from relevant regions in the hypothalamus and brain stem.[60-62] The neuronal activity was tonic and ranged in frequency from 6 to 26 Hz, increased with cooling and decreased towards zero with warming, resembling the descending tonic activity generated by serotonergic neurons in brain stem raphe nuclei that controls plateau potential generation in motor neurons (see above).

3.8 | NST in skeletal muscles

NST in skeletal muscle has generally been attributed to "leakage" of H^+ ions across the inner membrane of muscle mitochondria and/or "leakage" of Ca^{2+} ions across the membrane of SR, leading to "futile" heat producing ion transport. The importance of mitochondrial thermogenesis is uncertain because evidence has been reported both for[63-65] and against such thermogenesis.[27,66] The role of thermogenesis by "slippage" of Ca^{2+} back into the cytosol at the site of Ca^{2+} transport into SR by the ATPase Serca[25] also appears uncertain.[67] The main evidence is impaired thermogenesis in mice with sarcolipin (Sln) knocked out,[24,25] Sln being a regulator of Serca. Thus, $Sln^{-/-}$ mice die within a few hours of exposure to 4°C if their interscapular BAT (iBAT) is also ablated, whereas iBAT ablation alone has no such effect.[24,25] But the interpretation of this result may be questioned. For example, in these experiments the $Sln^{-/-}$ mice had been pre-acclimatized to 30°C[24] and were therefore without any training effect of preceding cold-acclimatization (see below). Moreover, mice without BAT (UCP-1$^{-/-}$) also die within hours at 4°C after preceding acclimatization to 30°C despite having intact Sln, their body temperature dropping by 10°C or more within 2 hours.[68] In contrast, after cold-acclimatization to 18°C such mice maintain their normal body temperature,[68] confirming the training effect of cold-acclimatization. Finally, mice that lack both Sln and BAT (double knock out of Sln and uncoupling protein-1) survive at least 9-10 days (end of experiment) at 4°C after gradual cold challenge, a finding described as paradoxical.[3] This last finding means that at least in cold-acclimatized mice alternatives to BAT- and Sln-dependent thermogenesis must exist, such as the muscle tone demonstrated here. Interestingly, Sln may contribute to body temperature control by modulating the capacity

for tonic motor unit activity. Thus, in mice that overexpress Sln, muscle fibres express increased succinyl dehydrogenase (SDH) activity, mitochondrial biogenesis and oxidative capacity, whereas in Sln knockouts they all decrease.[69]

In the cold, muscles undergo a form of training as they become redder, gain more slow-twitch fibres and capillaries around fibres and increase their oxidative metabolism and fatigue resistance.[70-73] Thus, cold-acclimatization results in increased capacity for muscle contractile activity and accompanying heat production. Yet, after cold acclimatization to 18°C, mice that lack BAT (UCP-1$^{-/-}$) "shiver incessantly" when exposed to 4°C[66] and begin to die after 5 weeks.[68] In such mice the ryanodine receptor 1 (RyR1) channel complex in sarcoplasmic reticulum (SR) becomes leaky for Ca^{2+} and the release of Ca^{2+} from SR and tetanic force output are markedly impaired.[26,68] Similar leaks in RyR1 and accompanying defective Ca^{2+} signalling, muscle damage and impaired exercise capacity occur in mice and humans after extensive endurance exercise.[74] Evidently, excessive muscle activity, particularly in untrained subjects and in the cold without preceding acclimatization, may overload the muscle and reduce the capacity for heat production by both overt shivering and muscle tone.

Bal et al. examined the role of electrical muscle activity in body temperature regulation by reducing visible shivering by ~50% in iBAT ablated mice injected with a low dose of curare ip.[24] Because this reduction did not affect the body temperature during exposure to 4°C, the authors suggested that "skeletal-muscle-based NST is an important mechanism for thermoregulation." But this experiment and its interpretations related only to overt shivering and provided no data on tonic motor unit activity. Given that overt shivering was only partially reduced and that slow muscle is less sensitive to curare than fast muscle,[75,76] our interpretation is that unexamined muscle tone rather than NST was responsible for the observed maintenance of body temperature.

3.9 | Regulated heat production occurs in the TNZ as presently described

The present results bear on the concept of the TNZ. TNZ is defined as "The range of ambient temperature at which temperature regulation is achieved only by control of sensible heat loss, ie, without regulatory changes in metabolic heat production (H) or evaporative heat loss".[77] It is influenced by environmental, methodological and other factors and is difficult to determine.[31,78] For the rat, ranges of TNZ are reported to lie between 28 and 34°C, most data suggesting that it is narrow (2-4°C) and centred at 29-30°C (see[31,79] and references therein). Under home cage conditions, however, its lower critical temperature is thought to approach the mid twenties.[4,31]

Here we show that regulated muscle tonus in SOL and by inference regulated heat production, occurs in a range of ambient temperatures that extends from less than 7°C to at least 32°C, which is well into the range that is generally ascribed to the TNZ. Motor units in other muscles, such as EDL, TIBd, and Neck, were likewise tonically active in this zone. We suggest that this fact has been missed, first because the SOL, in which such activity is relatively easy to detect, has not been studied in this context, second, because the low amplitude of such activity in muscles other than the SOL makes its detection difficult and third, because such activity to our knowledge has not been studied at high temperatures. Moreover, at high temperatures the amount of heat producing tonic activity is low and may have been missed in metabolic studies.

Could the tonic motor unit activity that we recorded within the TNZ, as presently defined, be just postural activity? Although it is difficult to distinguish between tonic activity in the service of either posture or body temperature regulation,[80] we think we are primarily recording thermogenic activity for the following reasons. The relationship between tonic activity and ambient temperature is linear also between 23°C and 32°C, 23°C representing the lower critical temperature of TNZ for rats under housing conditions, as reported in the literature.[4,31] If the tonic activity reflected only postural activity, we would not expect such linear dependence on ambient temperature. In this range of temperatures rats are usually lying flat on the floor with no signs of leg bearing postures. In one such case at 32°C, the video showed a rat apparently asleep and with the right leg containing the implanted EMG electrodes in full view. Close inspection of that video revealed no detectable movement of the body or leg during a period of time when several motor units were tonically active, individual units turning on or off apparently at random, in this case four units in EDL and three units in TIBd. In the absence of any detectable changes in posture, primary dependence on ambient temperature seems the more likely explanation. Nevertheless, we have no data that rigorously exclude postural rather than thermogenic mechanisms.

3.10 | Notes on terminology

In the literature on body temperature regulation, muscle tone is variously described as shivering, pre-shivering tone, invisible shivering, microvibrations, thermoregulatory tonus or resting muscle mechanical activity (RMMA). An accepted model is that muscle tone and overt shivering are "simply degrees of the same phenomenon" (see Introduction). But we show here that muscle tone and overt shivering are strikingly different phenomena, engaging different motor units and muscles, displaying different motor unit firing patterns and covering entirely different temperature ranges. The underlying mechanisms are also basically different, as overt shivering is produced by synchronous bursts of activity, arising perhaps in central pattern generators in the spinal cord, whereas muscle tone is produced by tonic impulse activity resulting almost certainly from intrinsic plateau potential generation in individual motor neurons. Consequently, to describe muscle tone as shivering is a misnomer that, in our view, causes unnecessary confusion.

For example, cold exposure-induced motor unit firing in a neck muscle was described as shivering in a recent study of central efferent pathways for shivering in anaesthetized rats.[17] But the motor unit activity shown in that paper was not bursting but regular at 37 Hz and therefore better described as tonic and in agreement with our observations of neck motor units in the absence of movements (average frequency 36.8 Hz, Figure 12F) at ambient temperatures extending from 6°C to 31°C. Interestingly, Nakamura and Morrison[17] noted: "this central command pathway for shivering parallels that for sympathetically regulated non-shivering thermogenesis in brown adipose tissue". In our view, however, "this central command pathway for shivering" may involve two commanding pathways, one for muscle tone and the other for overt shivering, which, as shown here, are separate phenomena. Similarly, when Cannon, Nedergaard, and co-workers discuss the detrimental effects of "incessant shivering" during cold acclimatization in UCP1^{-/-} mice (see above), it is unclear whether the resulting breakdown in muscle contractility results from disrupted muscle tone generation, overt shivering or both. That continuous low-intensity shivering or what we call muscle tone, is reported to represent more than 90% of total "shivering" activity in humans,[57] underlines the importance of distinguishing between shivering and muscle tone and strengthens our belief that the two processes should be kept conceptually and experimentally separate.

Interestingly, birds differ from mammals in that they generally do not display overt shivering but rather a smooth crescendo of muscle tone as it gets colder. Perhaps overt shivering has not evolved in birds partly because it might compromise brooding in the cold and partly because the massive pectoral muscle used for flying might provide sufficient heat by tonic mechanisms alone, whereas in mammals additional heat production by overt shivering is needed when it gets sufficiently cold.

Including muscle tone and overt shivering in the same term has a long and well-established history that will be difficult to change. The conflation dates back at least to 1955,[81] when it was stated that "Both "apparent" (observed visually) and "inapparent" (observed only with the aid of the electromyograph) shivering are degrees of the same phenomenon". Nevertheless, regardless of the choice of terminology, we believe that in the long run, the field will benefit from treating muscle tone and overt shivering as separate mechanisms.

There is no easy resolution to the problem of separating overt shivering from muscle tone given established usage. For example, one might call muscle tone NST, but this would contradict the commonly accepted definition of NST, which excludes electrical muscle activity. Alternatively, one might distinguish between muscular and non-muscular thermogenesis rather than between shivering and NST. Muscular thermogenesis would then include overt shivering, muscle tone and futile ion shuttle in mitochondria and SR, while non-muscular thermogenesis, would include BAT and enhanced metabolic rates because of increased levels of thyroid hormones. But, again, this would break with common usage and cause confusion. Or one may continue to conflate overt shivering and muscle tone under the same term, while hoping that the field will eventually agree on a more appropriate terminology.

A separate issue relates to using mechanomyography as a more sensitive method than EMG and resting muscle mechanical activity (RMMA) as a descriptor for minor tremor or microvibrations in skeletal muscles (see Introduction). We show here that by isolating periods of rest or sleep in the absence of movements, it is possible to study single motor unit activity by chronically implanted EMG electrodes because under such conditions the number of tonically active units at any particular time is often sufficiently low for single unit identification. Hence, this seems a more direct and better approach to the study of muscle tone in body temperature regulation than mechanomyography.

3.11 | General conclusions

This work shows (a) that muscle tone likely plays an essential and substantial, but so far generally unrecognized, role in body temperature regulation during rest or sleep, (b) that muscle tone and shivering are strikingly different phenomena and, therefore, should not both be described as shivering with the implication that they are "different degrees of the same phenomenon", and (c) that regulated muscle tone occurs in the TNZ as described in the literature, questioning the present description of this zone in rats. The scheme presented in Figure 16 illustrates (a) that falling ambient temperature increases the number of tonically active motor units, which generate trains of muscle impulses of increasing firing rates and duration (Figure 16A,B), and (b) that frequent brief random movements occur during rest or sleep, which may turn such trains on or off (Figure 16C). We propose that these effects arise because falling ambient temperatures increase the serotonergic inputs to the relevant motor neurons, thereby promoting and enhancing the generation of plateau potentials in them (Figure 16D,E). In addition, we propose that the random brief movements frequently made during rest or sleep reflect central mechanisms that briefly excite or inhibit the motor neurons, thereby turning plateau potentials on or off (Figure 16F). Thus, tonic motor unit activity is also turned on or off but in a random manner such that the units can share the task of heat production. We believe that the role of muscle tone in body temperature regulation described here has been missed so far because few, if any, have studied it in the absence of movement during rest or sleep, particularly in SOL where it is most prominent and all, or nearly all, the motor units may participate in muscle tone thermogenesis.

4 | MATERIALS AND METHODS

4.1 | Ethical approval

All experimental procedures were carried out in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123) and the Directive 2010/63/EU, implemented into Norwegian law and regulations 01.07.2015. The experiments were approved prior to execution by the Norwegian Animal Research Authority which is a national committee organized under the Norwegian Food Safety Authority. The Animal Care and Use Committee at the Institute of Basic Medical Sciences at the University of Oslo (animal research facility 016) and the Norwegian Food Safety Authority have also inspected the execution of the experiments.

4.2 | Animal experiments

The experiments were performed on male Wistar rats weighing 200-450 g, accommodated in the animal house on a 12 hour light-dark cycle with food and water ad libitum. Each rat was brought to the lab for chronic implantation of EMG electrodes. After the operation, the rat was returned to the animal house and, after 2-3 days of recovery, brought back to the lab and placed in a climate chamber for EMG recording. The recordings started in the morning, 1-2 hours after the rat had been placed in the chamber and lasted 4-6 hours. The rat was then returned to the animal house. On different days during the next 1-2 weeks the rats were brought back to the lab for additional recordings. At the conclusion of electrophysiological recordings animals were killed with an overdose of sodium pentobarbitone (200 mg kg^{-1}) via intraperitoneal injection.

4.3 | Electrodes for chronic EMG recording

Two types of electrodes were made. Type 1 was made from 50 μm diameter Teflon-insulated platinum/iridium wire (A-M Systems) or from 25 μm diameter polyimide-insulated Platinum 10% Iridium wire (California Fine Wire). The
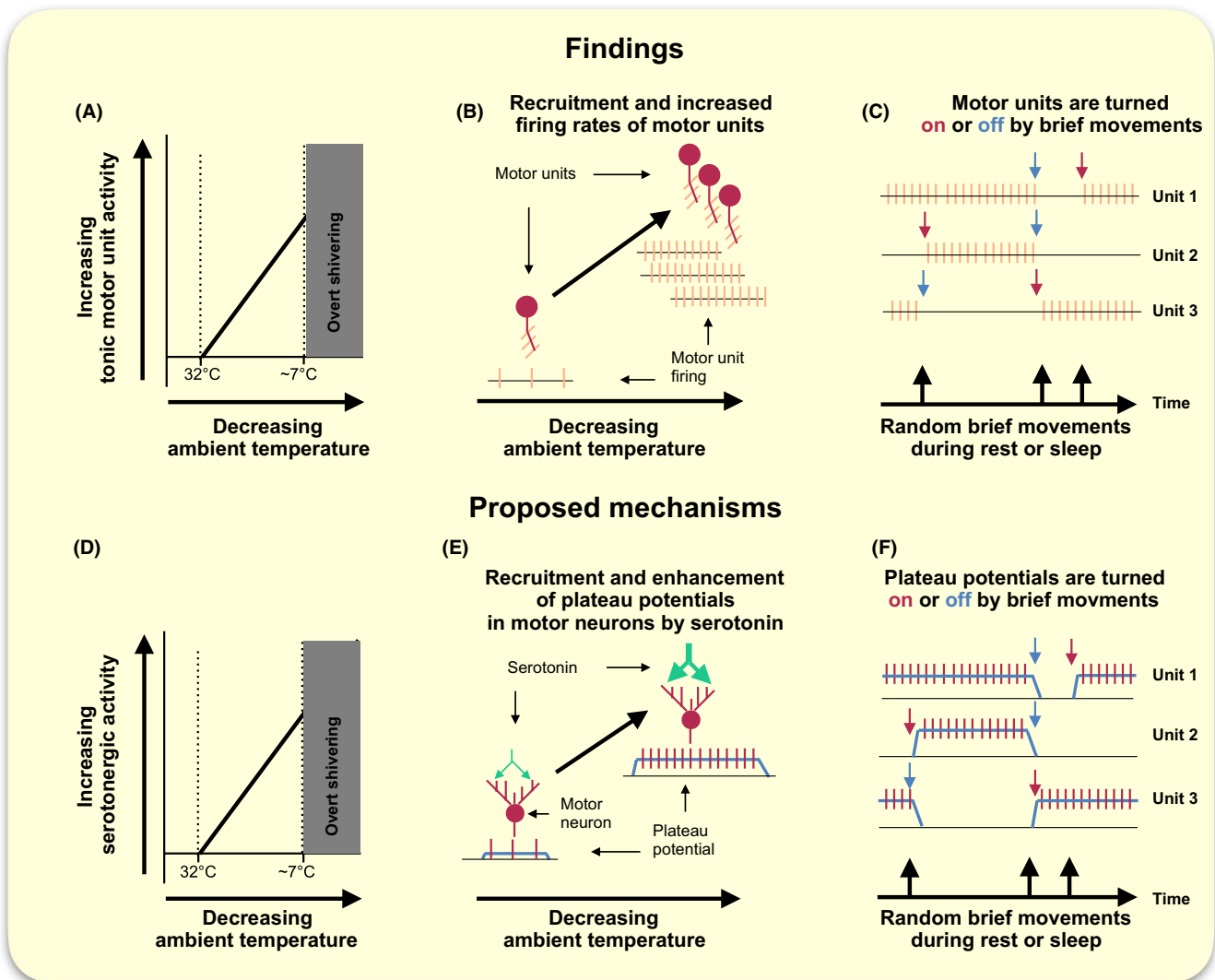
FIGURE 16 Summary of main findings and proposed mechanisms. A, Amount of tonic motor unit activity during rest or sleep (muscle tone) increased linearly with falling ambient temperature between 32°C and <7°C. Overt shivering began when the temperature reached approximately 7°C. Significant amounts of tonic motor unit activity was recorded also at temperatures above 32°C (up to 36°C), but the linear relationship disappeared. B, Recruitment of tonically active motor units and increased tonic firing rates contributed to the observed increase in total motor unit activity and therefore and necessarily, to an accompanying increased heat production. C, During periods of rest or sleep, brief movements occurred frequently and randomly, and at the time of such movements tonically active units were often turned on or off. Thus, many motor units could share the task of heat production in cold environments. D, Studies reported in the literature show that brain stem serotonergic neurons with axons descending in the spinal cord to make synaptic contacts with motor neurons increase their firing rate when the ambient temperature falls. E, Serotonin has been shown to promote plateau potential generation in motor neurons. Consequently, we propose that when the ambient temperature falls, increasing activity in serotonergic inputs cause progressively more motor neurons to generate plateau potentials that increase in duration and amplitude and result in trains of motor neuron action potentials of increasing duration and firing rates, ie tonic motor unit impulse activity. F, Brief excitatory or inhibitory inputs have been shown to elicit or terminate plateau potentials in motor neurons. Because the brief random movements observed here during rest or sleep similarly turn tonically active motor units on or off, we propose that such brief movements reflect intraspinal processes in unknown neuronal circuits that turn motor neuron plateau potentials on or off

insulation was removed from one end of the wire (~1.5 mm), which was bent into a hook. The other end of the 2-3 cm wire was soldered to a multifilament stainless steel wire (AS632, Cooner Wire) and the soldering site covered with a 0.5 cm silicone tube (OD 0.62 mm, ID 0.33 mm) filled with a silyl modified polymer (Trans7 Clear, Novatech International). Type 2 electrode, a pair, was made from 25 μm diameter polyimide-insulated Platinum 10% Iridium wires in which the insulation was removed for 0.5-1 mm at some distance from one end of each of two wires by a small flame through a metal slit. One wire was then placed along the other such that a 1 mm gap separated the two exposed segments. The ends of the two wires were then twisted and glued to a small suture needle. At the other end each wire was soldered to

multifilament stainless steel wires as described for Type 1 electrode.

4.4 | Surgical preparation for chronic EMG recording

The surgery was performed under deep anaesthesia obtained with Equithesin (4 ml kg^{-1} ip) in early experiments and a custom Zoletil mix (2.0 ml kg^{-1} ip) in later experiments. Equithesin contains pentobarbital sodium 9.7, magnesium sulphate (heptahydrate) 21.0, chloral hydrate 42.5, ethanol 76.0 and propylene glycol 428.0 mg mL^{-1}. The Zoletin mix contains 14.7 mg zolazepam, 14.7 mg tiletamine, 1.77 mg xylazine and 10 µg fentanyl per ml 0.9% NaCl. Temgesic (buprenorphine 10-20 µg kg^{-1} s.c.) was given at the end of surgery and in the morning and evening on the following day for post-operative analgesia. In some rats, SOL was exposed, the hook of a type 1 electrode inserted by a syringe needle in a distal-to-proximal direction into the proximal half of the muscle and a second electrode similarly into the distal half. Alternatively, the needle at the end of a type 2 paired electrode was inserted into, along and out of the SOL until the exposed recording segments reached a position within the proximal half of the SOL. The twisted end was cut and bent back to hold the electrode pair in place. Outside the SOL, the two thin wires with their attached multifilament stainless steel wires were bent 180°, secured with sutures to the subcutaneous fascia and run under the skin to the top of the head, where their ends were soldered to a socket contact (6-position E363/0, Plastics One) in early experiments and a 12-position micro plastic circular contact (Omnetics) in later experiments. The soldering sites in the socket were covered with a silyl modified polymer (Trans7 Clear) and the socket contact was then fixed to the exposed skull by dental cement (Simplex Rapid, Austenal Dental) around two screws in the bone. A wire loop under the skin in the back or thigh region prevented pull on the electrodes. In other rats, type 1 or 2 electrodes were inserted into EDL, deep parts of tibialis anterior (TIBd), superficial part of tibialis anterior (TIBs), lower superficial part of trapezius or deep neck muscles (biventer cervicis) with a procedure similar to those described for SOL. In many cases, electrode pairs were inserted into two or three muscles of the same rat for simultaneous recordings.

4.5 | Climate chamber

The EMG recordings were made in one of two custom made climate chambers. A flexible cable connected the contact on the rat's head (Figure 1A,E) to a custom made rotating contact above the chamber. In some rats, brief isoflurane anaesthesia was used when connecting the cable to the head contact. The inside temperature of Chamber 1 was kept nearly constant at 26, 23, 20 or 17°C by water from an adjacent temperature-controlled water bath circulating in three of the chamber's four walls. This chamber was only used in an early series of experiments (results in Figure 9). All other results were obtained in Chamber 2, which measured 23 × 23 × 23 cm on the inside. The walls were made from plexiglass and 2 cm polystyrene boards for insulation. The lid had one hole in the centre for a cylinder containing the rotating contacts, one hole near one corner for a metal cylinder protecting the probe that measured ambient temperature at the level of the rat and three holes along one wall containing metal cylinders into which dry ice could be poured to lower the temperature below 10°C. Between the lid and a metal grid 10 cm below, zigzagging copper tubes carried liquid (ethylene glycol in water) at different temperatures from an adjacent water bath and a small fan mixed the air in the chamber to an even temperature. A floor made of plexiglass and covered with cage bedding (filled into a tray made from overhead foil to prevent pieces of bedding from falling off the side of the floor and restrict its free movement) rested on four springs, one in each corner. A force transducer just touching the centre of the floor from underneath *via* a small spring recorded every movement of the rat. A double pane removable transparent front door allowed access to the chamber as well as video monitoring of the rat's behaviour inside the chamber.

4.6 | Data acquisition and analysis

Via the rotating contacts above the climate chamber the recording electrodes were connected to amplifiers (WPI) set as follows: differential AC, amplification 1000 X, band-pass filter 10 Hz-10 kHz. The EMG, ambient temperature and force transducer signals (movements) were fed into a data acquisition interface (Power1401-3, Cambridge Electronic Design) and stored on a desktop computer running Spike2 software (Cambridge Electronic Design). The EMG signals were sampled at 25 or 50 kHz. Spike2 simultaneously stored a complete video file of the rat's behaviour (recorded at 10 frames s^{-1} and 230 × 340 pixels per frame), using DivX real-time compression. Synchronized video, EMG and movement data were inspected on the PC monitor to confirm the reliability of the movement detection system. Even the smallest visible movements, including respiratory movements, were detected in the force transducer signals.

Movements were separated from periods of rest or sleep by placing cursors at the start and end of each movement (see Figure 2). Any two movements separated by less than 3 seconds were taken as belonging to the same movement. The "raw" EMG signal was subjected to DC removal and processed for mean RMS EMG (mV) in Spike 2. Results presented in Figure 9B were obtained using JMP 11.2.1 and 12.0.1 (SAS Institute). Influence of ambient temperature on

number, individual and total duration of flat EMG segments was evaluated in linear mixed models with temperature as fixed effect and animal as random effect.

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

None of the authors has any conflict of interest.

AUTHOR CONTRIBUTIONS

T.L. proposed the project. T.E. and E.B.R. performed the experiments and analysed the results presented in Figure 9. A.N. and T.L. performed the other experiments which were analysed by T.L. T.L. wrote the paper with important inputs from the other authors. All authors critically revised and approved the final version.

ORCID

Terje Lømo [ID] https://orcid.org/0000-0001-8609-4957

REFERENCES

1. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004;84:277-359.
2. Lichtenbelt W, Kingma B, van der Lans A, Schellen L. Cold exposure–an approach to increasing energy expenditure in humans. *Trends Endocrinol Metab*. 2014;25:165-167.
3. Rowland LA, Bal NC, Kozak LP, Periasamy M. Uncoupling protein 1 and sarcolipin are required to maintain optimal thermogenesis, and loss of both systems compromises survival of mice under cold stress. *J Biol Chem*. 2015;290:12282-12289.
4. Cannon B, Nedergaard J. Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J Exp Biol*. 2011;214:242-253.
5. Blondin DP, Daoud A, Taylor T, et al. Four-week cold acclimation in adult humans shifts uncoupling thermogenesis from skeletal muscles to BAT. *J Physiol*. 2016;595:2099-2113.
6. Steen J, Enger PS. Muscular heat production in pigeons during exposure to cold. *Am J Physiol*. 1957;191:157-158.
7. Hohtola E. Thermal and electromyographic correlates of shivering thermogenesis in the pigeon. *Comp Biochem Physiol A Comp Physiol*. 1982;73:159-166.
8. Tøien O, Aulie A, Steen JB. Thermoregulatory responses to egg cooling in incubating bantam hens. *J Comp Physiol B*. 1986;156:303-307.
9. West GC. Shivering and heat production in wild birds. *Physiol Zool*. 1965;38:111-120.
10. Hohtola E, Stevens ED. The relationship of muscle electrical activity, tremor and heat production to shivering thermogenesis in Japanese quail. *J Exp Biol*. 1986;125:119-135.
11. Saarela S, Keith JS, Hohtola E, Trayhurn P. Is the "mammalian" brown fat-specific mitochondrial uncoupling protein present in adipose tissues of birds? *Comp Biochem Physiol B*. 1991;100:45-49.
12. Hohtola E, Henderson RP, Rashotte ME. Shivering thermogenesis in the pigeon: the effects of activity, diurnal factors, and feeding state. *Am J Physiol*. 1998;275:R1553-R1562.
13. Haman F, Blondin DP. Shivering thermogenesis in humans: Origin, contribution and metabolic requirement. *Temperature*. 2017;4:217-226.
14. Petajan JH, Williams DD. Behavior of single motor units during pre-shivering tone and shivering tremor. *Am J Phys Med*. 1972;51:16-22.
15. Israel DJ, Pozos RS. Synchronized slow-amplitude modulations in the electromyograms of shivering muscles. *J Appl Physiol*. 1989;66:2358-2363.
16. Kleinebeckel D, Klussmann FW. Shivering. In: Schönbaum E, Lomax P, eds. *Thermoregulation: Physiology and Biochemistry*. New York: Pergamon Press; 1990:235-253.
17. Nakamura K, Morrison SF. Central efferent pathways for cold-defensive and febrile shivering. *J Physiol*. 2011;589:3641-3658.
18. McAllen RM, Tanaka M, Ootsuka Y, McKinley MJ. Multiple thermoregulatory effectors with independent central controls. *Eur. J. Appl. Physiol*. 2010;109:27-33.
19. Meigal A. Gross and fine neuromuscular performance at cold shivering. *Int J Circumpolar Health*. 2002;61:163-172.
20. Jessen C. *Temperature Regulation in Humans and Other Mammals*. Berlin Heidelberg, New York: Springer-Verlag; 2001.
21. Haman F, Blondin DP, Imbeault M-A, Maneshi A. Metabolic requirements of shivering humans. *Front Biosci*. 2010;2:1155-1168.
22. Morrison SF. Central control of body temperature. *F1000Research*. 2016;5:880.
23. Bal NC, Singh S, Reis F, et al. Both brown adipose tissue and skeletal muscle thermogenesis processes are activated during mild to severe cold adaptation in mice. *J Biol Chem*. 2017;292:16616-16625.
24. Bal NC, Maurya SK, Sopariwala DH, et al. Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. *Nat Med*. 2012;18:1575-1579.
25. Periasamy M, Maurya SK, Singh S, Sahoo SK, Reis F, Bal NC. Role of SERCA pump in muscle thermogenesis and metabolism. *Compr Physiol*. 2017;7:879-890.
26. Aydin J, Shabalina IG, Place N, et al. Nonshivering thermogenesis protects against defective calcium handling in muscle. *FASEB J*. 2008;22:3919-3924.
27. van der Lans A, Hoeks J, Brans B, et al. Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *J Clin Invest*. 2013;123:3395-3403.
28. McKay WP, Vargo M, Chilibeck PD, Daku BL. Effects of ambient temperature on mechanomyography of resting quadriceps muscle. *Appl Physiol Nutr Metab*. 2013;38:227-233.
29. Block BA. Thermogenesis in muscle. *Annu Rev Physiol*. 1994;56:535-577.

30. Dulloo AG, Miles-Chan JL, Montani J-P, Schutz Y. Isometric thermogenesis at rest and during movement: a neglected variable in energy expenditure and obesity predisposition. *Obes Rev*. 2017;18(Suppl 1):56-64.

31. Romanovsky AA, Ivanov AI, Shimansky YP. Selected contribution: ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality. *J Appl Physiol*. 2002;92:2667-2679.

32. McKay W, Chilibeck PD, Chad KE, Daku B. Resting mechanomyography after aerobic exercise. *Can J Appl Physiol*. 2004;29:743-757.

33. Cox DR, Miller HD. *The Theory of Stochastic Processes*. Boca Raton, FL: Chapman & Hall/CRC; 2001.

34. Close R. Properties of motor units in fast and slow skeletal muscles of the rat. *J Physiol*. 1967;193:45-55.

35. Ausoni S, Gorza L, Schiaffino S, Gundersen K, Lømo T. Expression of myosin heavy chain isoforms in stimulated fast and slow rat muscles. *J Neurosci*. 1990;10:153-160.

36. Heckman CJ, Enoka RM. Motor unit. *Compr Physiol*. 2012;2:2629-2682.

37. Eken T. Spontaneous electromyographic activity in adult rat soleus muscle. *J Neurophysiol*. 1998;80:365-376.

38. Hounsgaard J, Hultborn H, Jespersen B, Kiehn O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J Physiol*. 1988;405:345-367.

39. Eken T, Kiehn O. Bistable firing properties of soleus motor units in unrestrained rats. *Acta Physiol Scand*. 1989;136:383-394.

40. Morrison SF, Nakamura K, Madden CJ. Central control of thermogenesis in mammals. *Exp Physiol*. 2008;93:773-797.

41. Hodges MR, Tattersall GJ, Harris MB, et al. Defects in breathing and thermoregulation in mice with near-complete absence of central serotonin neurons. *J Neurosci*. 2008;28:2495-2505.

42. Steinbusch HW. Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience*. 1981;6:557-618.

43. Alvarez FJ, Pearson JC, Harrington D, Dewey D, Torbeck L, Fyffe RE. Distribution of 5-hydroxytryptamine-immunoreactive boutons on alpha-motoneurons in the lumbar spinal cord of adult cats. *J Comp Neurol*. 1998;393:69-83.

44. Perrier J-F, Delgado-Lezama R. Synaptic release of serotonin induced by stimulation of the raphe nucleus promotes plateau potentials in spinal motoneurons of the adult turtle. *J Neurosci*. 2005;25:7993-7999.

45. Harvey PJ, Li X, Li Y, Bennett DJ. Endogenous monoamine receptor activation is essential for enabling persistent sodium currents and repetitive firing in rat spinal motoneurons. *J Neurophysiol*. 2006;96:1171-1186.

46. Martín-Cora FJ, Fornal CA, Metzler CW, Jacobs BL. Single-unit responses of serotonergic medullary and pontine raphe neurons to environmental cooling in freely moving cats. *Neuroscience*. 2000;98:301-309.

47. Ray RS, Corcoran AE, Brust RD, et al. Impaired respiratory and body temperature control upon acute serotonergic neuron inhibition. *Science*. 2011;333:637-642.

48. Kiehn O, Erdal J, Eken T, Bruhn T. Selective depletion of spinal monoamines changes the rat soleus EMG from a tonic to a more phasic pattern. *J Physiol*. 1996;492(Pt 1):173-184.

49. Guertin PA, Hounsgaard J. L-type calcium channels but not N-methyl-D-aspartate receptor channels mediate rhythmic activity induced by cholinergic agonist in motoneurons from turtle spinal cord slices. *Neurosci Lett*. 1999;261:81-84.

50. Henneman E. Relation between size of neurons and their susceptibility to discharge. *Science*. 1957;126:1345-1347.

51. Bawa P, Pang MY, Olesen KA, Calancie B. Rotation of motoneurons during prolonged isometric contractions in humans. *J Neurophysiol*. 2006;96:1135-1140.

52. Kugelberg E. Adaptive transformation of rat soleus motor units during growth. *J Neurol Sci*. 1976;27:269-289.

53. Kugelberg E, Lindegren B. Transmission and contraction fatigue of rat motor units in relation to succinate dehydrogenase activity of motor unit fibres. *J Physiol*. 1979;288:285-300.

54. Hudlicka O. Microcirculation in skeletal muscle. *Muscles Ligaments Tendons J*. 2011;1:3-11.

55. Ward AB. Assessment of muscle tone. *Age Ageing*. 2000;29:385-386.

56. Blondin DP, Labbé SM, Phoenix S, et al. Contributions of white and brown adipose tissues and skeletal muscles to acute cold-induced metabolic responses in healthy men. *J Physiol*. 2015;593:701-714.

57. Haman F, Legault SR, Rakobowchuk M, DuCharme MB, Weber J-M. Effects of carbohydrate availability on sustained shivering II. Relating muscle recruitment to fuel selection. *J Appl Physiol*. 2004;96:41-49.

58. Kiehn O, Eken T. Prolonged firing in motor units: evidence of plateau potentials in human motoneurons? *J Neurophysiol*. 1997;78:3061-3068.

59. Westgaard RH, Bonato P, Holte KA. Low-frequency oscillations (<0.3 Hz) in the electromyographic (EMG) activity of the human trapezius muscle during sleep. *J Neurophysiol*. 2002;88:1177-1184.

60. Birzis L, Hemingway A. Descending brain stem connections controlling shivering in cat. *J Neurophysiol*. 1956;19:37-43.

61. Birzis L, Hemingway A. Efferent brain discharge during shivering. *J Neurophysiol*. 1957;20:156-166.

62. Birzis L, Hemingway A. Shivering as a result of brain stimulation. *J Neurophysiol*. 1957;20:91-99.

63. Simonyan RA, Jimenez M, Ceddia RB, Giacobino JP, Muzzin P, Skulachev VP. Cold-induced changes in the energy coupling and the UCP3 level in rodent skeletal muscles. *Biochim Biophys Acta*. 2001;1505:271-279.

64. Mollica MP, Lionetti L, Crescenzo R, et al. Cold exposure differently influences mitochondrial energy efficiency in rat liver and skeletal muscle. *FEBS Lett*. 2005;579:1978-1982.

65. Wijers S, Schrauwen P, Saris W, van Marken Lichtenbelt WD. Human skeletal muscle mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. *PLoS ONE*. 2008;3:e1777.

66. Shabalina IG, Hoeks J, Kramarova TV, Schrauwen P, Cannon B, Nedergaard J. Cold tolerance of UCP1-ablated mice: a skeletal muscle mitochondria switch toward lipid oxidation with marked UCP3 up-regulation not associated with increased basal, fatty acid- or ROS-induced uncoupling or enhanced GDP effects. *Biochim Biophys Acta*. 2010;1797:968-980.

67. Campbell KL, Dicke AA. Sarcolipin makes heat, but is it adaptive thermogenesis? *Front Physiol*. 2018;9:714.

68. Golozoubova V, Hohtola E, Matthias A, Jacobsson A, Cannon B, Nedergaard J. Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J*. 2001;15:2048-2050.

69. Maurya SK, Bal NC, Sopariwala DH, et al. Sarcolipin is a key determinant of the basal metabolic rate, and its overexpression

enhances energy expenditure and resistance against diet-induced obesity. *J Biol Chem*. 2015;290:10840-10849.

70. Hirabayashi M, Ijiri D, Kamei Y, Tajima A, Kanai Y. Transformation of skeletal muscle from fast- to slow-twitch during acquisition of cold tolerance in the chick. *Endocrinology*. 2005;146:399-405.

71. Arruda AP, Ketzer LA, Nigro M, Galina A, Carvalho DP, de Meis L. Cold tolerance in hypothyroid rabbits: role of skeletal muscle mitochondria and sarcoplasmic reticulum Ca2+ ATPase isoform 1 heat production. *Endocrinology*. 2008;149:6262-6271.

72. Suzuki J, Gao M, Ohinata H, Kuroshima A, Koyama T. Chronic cold exposure stimulates microvascular remodeling preferentially in oxidative muscles in rats. *Jpn. J. Physiol*. 1997;47:513-520.

73. Bruton JD, Aydin J, Yamada T, et al. Increased fatigue resistance linked to Ca2+-stimulated mitochondrial biogenesis in muscle fibres of cold-acclimated mice. *J Physiol*. 2010;588:4275-4288.

74. Bellinger AM, Reiken S, Dura M, et al. Remodeling of ryanodine receptor complex causes "leaky" channels: a molecular mechanism for decreased exercise capacity. *Proc Natl Acad Sci USA*. 2008;105:2198-2202.

75. Storella RJ, Baker T. Activity-dependent differences between rat fast and slow neuromuscular systems. *Eur J Pharmacol*. 1988;145:299-304.

76. Storella RJ, Riker WF, Baker T. d-Tubocurarine sensitivities of a fast and a slow neuromuscular system of the rat. *Eur J Pharmacol*. 1985;118:181-184.

77. Glossary of terms for thermal physiology. *J Therm Biol*. 2003;28:75-106.

78. Kingma B, Frijns A, van Marken LW. The thermoneutral zone: implications for metabolic studies. *Front Biosci*. 2012;4:1975-1985.

79. Gordon CJ. Thermal biology of the laboratory rat. *Physiol. Behav*. 1990;47:963-991.

80. Hohtola E. Tonic immobility and shivering in birds: evolutionary implications. *Physiol Behav*. 1981;27:475-480.

81. Davis TR, Mayer J. Nature of the physiological stimulus for shivering. *Am J Physiol*. 1955;181:669-674.